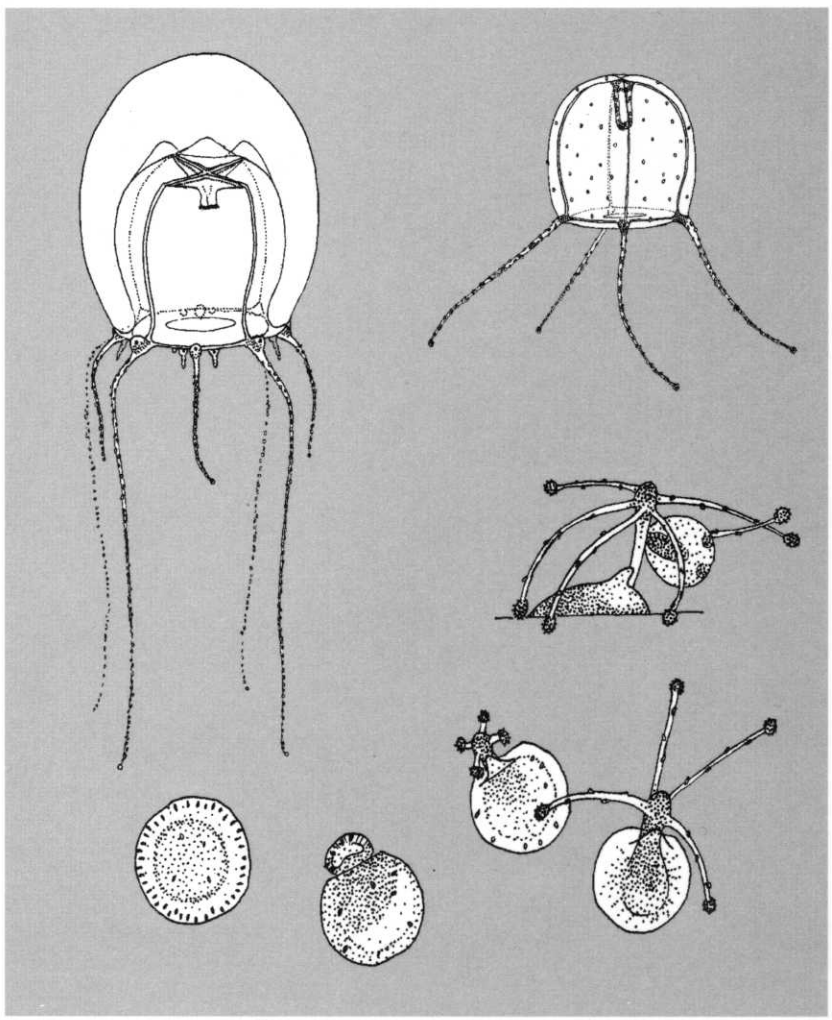
TRENDS IN HYDROZOAN BIOLOGY - IV

Edited by

C.E. Mills, F. Boero, A. Migotto and J.M. Gili



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This volume represents the fourth in a series published following workshops of the international Hydrozoan Society. The book is an outcome of the IV Workshop, held at the Bodega Marine Laboratory in Bodega Bay, California, from September 19 to October 3, 1998. Through a series of selected papers, this volume covers a wide range of topics comprising traditional systematics, zoogeography, life cycles, ecology, behaviour, and evolution as well as molecular and biochemical approaches. This collection of papers, written by experts in their fields, makes this book a valuable addition to the Cnidaria literature. At the same time, the papers provide a good sample of the different research fields now alive in marine invertebrate biology.

Front cover: *Halimedusa typus* life cycle (design by C.E. Mills), see Page 99 of this book.

FOREWORD

This volume represents the fourth in a series[1, 2, 3] published following workshops of the international Hydrozoan Society. Having previously met in Ischia, Italy (September 1985), Blanes, Spain (September 1991) and Roscoff, France (September 1994), this time the Society decided to venture into the New World, holding its Fourth Workshop at the Bodega Marine Laboratory in Bodega Bay, central California, from September 19 to October 3, 1998. Fifty participants, representing 16 countries and professional levels from advanced undergraduate students to professors emeritus, contributed to the two week workshop. This volume is composed of some of the presentations from that meeting. The Hydrozoan Society workshops provide a unique opportunity for those of us who study hydroids and hydromedusae, usually in comparative isolation, to really get to know each other at a personal level and to share ideas and promote future collaborations between people of similar interests, even if we come from different disciplines.

The Bodega Marine Laboratory, established in 1966, has a special place in the history of hydrozoan studies, as Cadet Hand, John Rees, Claudia Mills, and Nando Boero have all have worked there studying hydroids and medusae. When approached about hosting the Hydrozoan Society, both the Director James Clegg and Associate Director Paul Siri were enthusiastic, and thus the Bodega Marine Lab was selected as our venue. In addition to presenting original research papers and having daily topical round-table discussions, the Hydrozoan Society endeavors to do field-work during the course of the workshop. At the Bodega Marine Laboratory, we had a large teaching laboratory with running seawater tables and microscopes in addition to a conference room, projectors, library, dormitories and cafeteria. It was all very convenient and comfortable. We were surrounded by abundant wildlife, with large numbers of deer, songbirds and shorebirds, sea lions and even skunks. The lab residents were always smiling, willing to help and to do something for the "Hydrozoan people". This meant that our work was intense as usual, against a background of a happy environment. Being serious while smiling is the Bodega Bay formula. People work hard, but they are having fun; this is also the philosophy of the Hydrozoan Society. We gather not only to exchange our results and ideas, we get together to exchange our feelings. So Bodega Bay turned out to be a perfect place from every point of view. The success of the workshop resided in the number and diversity of attendees (this was the largest meeting in our short history) and in the quality of presentations and discussions. We saw unusual new live hydroid material, and are only sorry to report that a bloom of the freshwater jellyfish *Craspedacusta* occurred within a few miles of the meeting, but we did not learn about this unusual happening until after everyone had gone home; many of the attendees have never seen this species alive.

The Bodega Bay meeting occurred at a time of great change for international science, as the World Wide Web is coming into its own as a useful, authoritative venue. Within the last year, the essential and extensive hydrozoan bibliography compiled by Wim Vervoort[4] (who was bent over his computer working on this opus throughout our Third Workshop at Roscoff) has been made accessible over the Web (http://siba3.unile.it/ctle/mda/index.html) through the efforts of Cinzia Gravili and Ferdinando Boero and the expertise of the Library and Computer Services of the University of Lecce. The next step will be to scan these articles and put them up on the Web in their entirety, eventually leaving little excuse for nonfamiliarity with even the most obscure literature.

Some of the discussions at the Fourth Workshop of the Hydrozoan Society centered around the need to try to standardize data across a large number of species for future comparative work, requiring the collaborative efforts of a wide variety of scientists, including natural his-

torians, ecologists, developmental biologists, systematists, geneticists, molecular biologists and others. The concept of a giant matrix, available to all via the Web, including perhaps 100 species, was discussed – in which cells could be gradually filled in by any number of scientists, eventually yielding a much clearer picture of many kinds of patterns in the Hydrozoa. Such a matrix could guide future research towards filling in large gaps in our knowledge. In discussing our future needs as Hydrozoan scientists, the germ of a grand collaborative scheme was developed, which has now begun to blossom in the form of a Partnership for Enhancing Expertise in Taxonomy (PEET) grant from the American National Science Foundation. This effort to train new hydrozoan specialists stems directly from the Fourth Workshop and is continuing to link participants from all over the world, including senior taxonomists from the U.S. and Canada and students from Brazil and Italy, and has already resulted in a field workshop in Italy in the summer of 2000.

So we stand now looking forward to ever-more rapid advances in international science, as Web-accessible databases are beginning to be assembled on innumerable topics. No such database is yet in place for the Hydrozoa; we await the real work in building a useful tool. Scientists around the world are now connected electronically, so questions can be asked and answered overnight from even the most distant locations – the days of two to three week turnaround time for questions by mail are for the most part over. Still each scientist works in his or her own context, asking questions that arise from their own observations and interests. We present in this volume a wide variety of papers written by scientists living all over the world in highly different circumstances. The papers are all about Hydrozoa, but beyond that they represent a wide range of topics, and provide the reader with an overview of our knowledge and interests at the turn of the century and millenium.

THE EDITORS

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Towards understanding the phylogenetic history of Hydrozoa: Hypothesis testing with 18S gene sequence data*

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SUMMARY: Although systematic treatments of Hydrozoa have been notoriously difficult, a great deal of useful information on morphologies and life histories has steadily accumulated. From the assimilation of this information, numerous hypotheses of the phylogenetic relationships of the major groups of Hydrozoa have been offered. Here I evaluate these hypotheses using the complete sequence of the 18S gene for 35 hydrozoan species. New 18S sequences for 31 hydrozoans, 6 scyphozoans, one cubozoan, and one anthozoan are reported. Parsimony analyses of two datasets that include the new 18S sequences are used to assess the relative strengths and weaknesses of a list of phylogenetic hypotheses that deal with Hydrozoa. Alternative measures of tree optimality, minimum evolution and maximum likelihood, are used to evaluate the reliability of the parsimony analyses. Hydrozoa appears to be composed of two clades, herein called Trachylina and Hydroidolina. Trachylina consists of Limnomedusae, Narcomedusae, and Trachymedusae. Narcomedusae is not likely to be the basal group of Trachylina, but is instead derived directly from within Trachymedusae. This implies the secondary gain of a polyp stage. Hydroidolina consists of Capitata, Filifera, Hydridae, Leptomedusae, and Siphonophora. "Anthomedusae" may not form a monophyletic grouping. However, the relationships among the hydroidolinan groups are difficult to resolve with the present set of data. Finally, the monophyly of Hydrozoa is strongly supported.

Key words: Hydrozoa, Trachylina, Hydroidolina, Siphonophora, phylogeny, 18S, hypothesis testing.

INTRODUCTION

Hydrozoan classification and nomenclature have been infamous, posing difficulties for ecologists, taxonomists, biogeographers, as well as phylogeneticists who work with hydrozoans. This situation would appear to be an unfortunate backdrop as we move towards an understanding of the phylogenetic history of Hydrozoa because classification schemes, even those that were not explicitly aimed at grouping organisms based on common ancestry, often provide a first approximation of phylogeny. While by no means universal, many groups of organisms that were defined prior to the current trend toward phylogenetic classifications have held up as monophyletic clades. A pertinent example is presented by the present study, which strongly supports an assertion of monophyly for Hydrozoa, a finding in accordance with the conclusions of other students of cnidarian phylogeny (Schuchert, 1993; Bridge *et al*., 1995). Unless or until contradictory information is brought into view, it will be accepted that this hypothesis accurately represents true evolutionary history.

The difficult nature of hydrozoan classification is a consequence of separate treatment having been

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given the polyp and medusa stages of hydrozoan life cycles. In the absence of adequate life history information connecting medusae to polyps, separate taxonomies arose. Luckily, substantial attempts have been made to integrate older taxonomic schemes in light of our growing knowledge of complete life cycles. Naumov (1960) was the first to take on this onerous task. However, far from being daunted by the undertaking, Naumov remarked that his classification of hydrozoans would need only modest alteration, as it was based on phylogenetic relationships. Since then, taxonomically broad-based contributions have been made by Bouillon (1985), who proposed a revised classification for non-siphonophoran hydrozoans, and Petersen (1990), who offered a phylogenetic classification for the capitate hydroids.

Herein, I evaluate hypotheses of phylogenetic relationships of the major groups of hydrozoans that have been offered in the past. Specifically, I ask whether complete sequences of the 18S gene, which codes for the small subunit of the ribosome, are consistent with each of the hypotheses. The value of molecular sequence data lies in their capacity to provide relatively large sets of heritable and variable characters that can be used to evaluate prior phylogenetic hypotheses and generate new ones. Of course, anatomic features and other characters are also variable and inherited, making them equally useful for phylogenetic inference. Today, a great value is placed on molecular characters in phylogenetic studies. Part of this emphasis is pragmatic. Technological advances make it possible to gather numerous molecular characters relatively inexpensively. Another reason that molecules are emphasized is possibly that they are fashionable. Fortunately, the current wave of molecular phylogenies is spurring on phylogenetic analyses based on non-molecular characters. All types of data that have the potential to reveal phylogenetic history should be investigated.

To simplify the discussion, I have compiled a list of phylogenetic hypotheses, derived mostly from a few major works, as outlined below. The principal focus of this analysis will be to evaluate the monophyly of and the relationships among the following taxa: Anthomedusae, Capitata, Filifera, Hydridae, Leptomedusae, Limnomedusae, Narcomedusae, Siphonophora, and Trachymedusae. Many of these names have roughly equivalent appellations (Anthomedusae equals Athecata, Gymnoblastea, and Anthoathecata etc.). Choosing to use the above names (which are mostly descended from the medusae-based classifications) is not based on priority, as there is no rule of precedence for taxonomic groups above the family level, nor for any considerations of what phase of the typical hydrozoan life cycle represents the adult stage. Instead, I argue that the choice is largely arbitrary and should be recognized as such. Reference will also be made to Actinulidae and Laingiomedusae, but hypotheses involving these groups cannot be explicitly tested with the present molecular dataset since these taxa have not been sampled for the 18S gene.

To an extent, this highlights the tentative nature of phylogenetic analyses. All phylogenetic trees, with the somewhat obscure exception of experimental phylogenies (Hillis *et al*., 1992) are hypotheses of evolutionary relationships. Therefore, phylogenies are not final results. The analysis in this paper confirms that molecules and morphology often point to the same evolutionary relationships, but that there is not complete agreement. Therefore, the 18S data suggest some new phylogenetic hypotheses for hydrozoans. In turn, these hypotheses must be tested with other sets of data and additional analyses. The challenge of testing new possibilities forces us to look at old data in novel ways. 18S data will surely not reveal the complete truth about the evolutionary relationships among hydrozoans. However, through the process of testing, proposing, re-testing, and so forth, a coherent picture of hydrozoan phylogeny will emerge.

MATERIALS, METHODS, AND RESULTS

Compiling a list of phylogenetic hypotheses

Figure 1 shows three views of hydrozoan phylogeny that have been offered. The phylogeny of Hydrozoa that Hyman presented, stressing that it was “highly speculative”, is redrawn as Figure 1A (Hyman, 1940). From this conception we can begin to enumerate hypotheses, shown in Table 1. 1) Hydrozoa is not a monophyletic group, having given rise to the other cnidarians. 2) Anthomedusae and Leptomedusae form a clade. 3) Limnomedusae, Narcomedusae, and Trachymedusae form a clade. Hyman did not explicitly mention Limnomedusae, but her discussion of Trachymedusae includes direct references to limnomedusan species. 4) Siphonophora is the earliest diverging branch of hydrozoans; Anthomedusae, Leptomedusae, Lim-

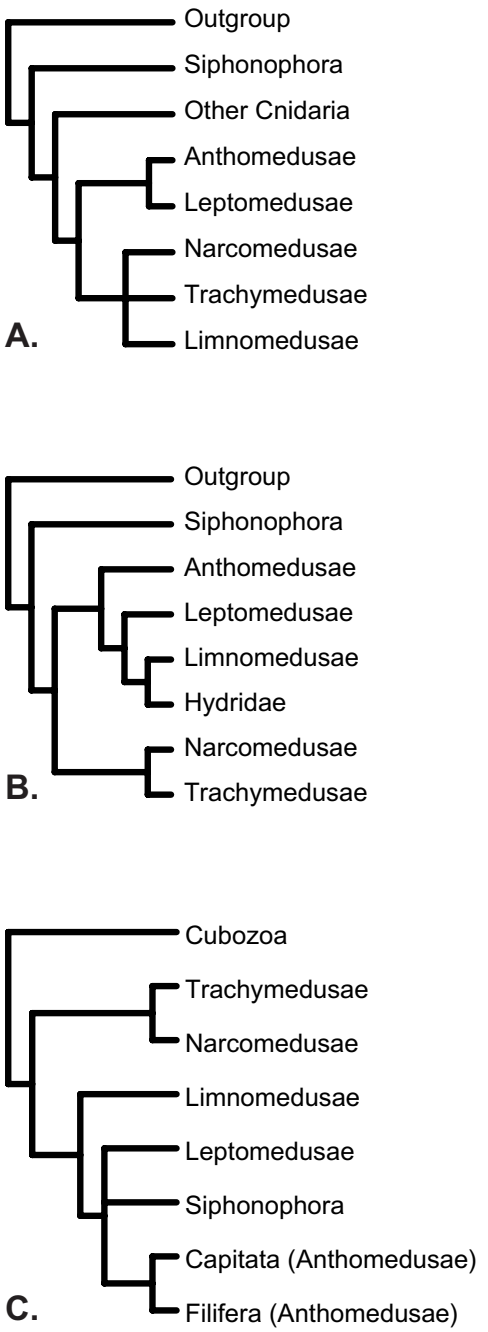

FIG. 1. – Three alternative views of the evolutionary relationships of Hydrozoa. A follows Hyman (1940), B follows Naumov (1960), and C follows Petersen (1979; 1990).

nomedusae, Narcomedusae, and Trachymedusae form a clade. In addition, Hyman's tree carries the implication that each of the major subgroups is monophyletic, augmenting the list of hypotheses. 5) Siphonophora is monophyletic. 6) Anthomedusae (containing Hydridae) is monophyletic. 7) Leptomedusae is monophyletic. 8) Narcomedusae is monophyletic. 9) Trachymedusae is monophyletic. 10) Limnomedusae is monophyletic (not argued by Hyman, but implied by Figure 1a). These hypotheses are mutually consistent, embodying a single view of hydrozoan evolutionary history. Entertaining alternative views of hydrozoan phylogeny expands the list greatly (Table 1).

TABLE 1. – List of phylogenetic hypotheses for hydrozoan groups.

Hypothesis Number	Description of Hypothesis
(1)	Hydrozoa is not a monophyletic group, having given rise to the other cnidarians.
(2)	Anthomedusae and Leptomedusae form a clade.
(3)	Limnomedusae, Narcomedusae, and Trachymedusae form a clade.
(4)	Siphonophora is the earliest diverging branch of hydrozoans
(5)	Siphonophora is monophyletic.
(6)	Anthomedusae (containing Hydridae) is monophyletic.
(7)	Leptomedusae is monophyletic.
(8)	Narcomedusae is monophyletic.
(9)	Trachymedusae is monophyletic.
(10)	Limnomedusae is monophyletic.
(11)	Hydrozoa is monophyletic, the converse of hypothesis 1.
(12)	Hydridae is monophyletic.
(13)	Anthomedusae excluding Hydridae is monophyletic.
(14)	Hydridae and Limnomedusae form a clade.
(15)	Hydridae, Leptomedusae, and Limnomedusae form a clade.
(16)	Anthomedusae, Hydridae, Leptomedusae, and Limnomedusae form a clade.
(17)	Narcomedusae and Trachymedusae form a clade.
(18)	Capitata (containing Hydridae) is monophyletic.
(19)	Filifera is monophyletic.
(20)	Anthomedusae (with Hydridae), Leptomedusae, and Siphonophora form a clade.
(21)	Anthomedusae, Leptomedusae, Limnomedusae and Siphonophora form a clade.
(22)	Cubozoa is the sister group to Hydrozoa.
(23)	Capitata is a monophyletic group that does not contain Hydridae.
(24)	Anthomedusae is not monophyletic, the converse of hypothesis 6.
(25)	Hydridae and Leptomedusae form a clade.
(26)	Hydridae, Leptomedusae, and Siphonophora form a clade.
(27)	Trachymedusae are not monophyletic, having given rise to Narcomedusae.
(28)	Hydrozoa, Scyphozoa, and Cubozoa form a clade.

The phylogeny of Hydrozoa according to Naumov is presented as Figure 1B (Naumov, 1960). Note that the position of Siphonophora is inferred. Naumov did not explicitly deal with siphonophores in his treatise on hydroids and hydromedusae of what is now the former Soviet Union. He considered them a separate subclass of Hydrozoa and thus they have been placed as the earliest branch of Hydrozoa. Some of the postulates of Naumov overlap with those already listed (4, 5, 7, 8, 9, and 10), but several are new. 11) Hydrozoa is monophyletic, the converse of hypothesis 1. 12) Hydridae is monophyletic. 13) Anthomedusae excluding Hydridae is monophyletic. 14) Hydridae and Limnomedusae form a clade. 15) Hydridae, Leptomedusae, and Limnome-

dusae form a clade. 16) Anthomedusae, Hydridae, Leptomedusae, and Limnomedusae form a clade. 17) Narcomedusae and Trachymedusae form a clade.

Petersen's account of the phylogeny of Hydrozoa is given in Figure 1C (Petersen, 1979). In addition to some conjectures already listed (5, 6, 7, 8, 9, 10, 11, and 17), several new hypotheses can be gleaned from Figure 1C. 18) Capitata (containing Hydridae) is monophyletic. 19) Filifera is monophyletic. 20) Anthomedusae (with Hydridae), Leptomedusae, and Siphonophora form a clade. 21) Anthomedusae, Leptomedusae, Limnomedusae and Siphonophora form a clade. 22) Cubozoa is the sister group to Hydrozoa, an assertion reiterated by Bouillon (Bouillon, 1985, 1987). Finally, hypotheses suggested by the 18S data, as detailed below, will complete this compilation of phylogenetic hypotheses of the major groups of Hydrozoa.

Accumulating molecular sequence data

All primers, sequences and molecular datasets used in this analysis are available upon request from the author. Genomic DNA was isolated from tissue samples of 23 hydrozoan species, seven scyphozoan species, and two anthozoan species. In addition, DNA samples from eight hydrozoan species and one cubozoan species were kindly provided by other researchers, as acknowledged below. Tissue samples were either fresh, preserved in 75 to 95 percent ethanol, or frozen (-80°). The extraction of high molecular weight genomic DNA was achieved by pulverization of tissue in the reagent DNAzol, followed by centrifugation and ethanol precipitation (Chomczynski *et al*., 1997). The complete sequence for the 18S coding region was amplified from genomic DNA preparations using eukaryotic-specific primers (Medlin *et al*., 1988) via PCR (30 cycles: 10s at 94°, 60s at 38° to 48°, and 180s at 72°, after an initial two minute 94°denaturation). The PCR products were directly sequenced with an ABI Prism 377 DNA Sequencer, with the exception of the 18S gene of *Aequorea aequorea*, which was sequenced with a Li-Cor model 4000L infrared automated DNA sequencer. The complete 18S sequences will be deposited in GenBank, as part of a publication that deals with the phylogeny of a broader taxonomic grouping, the medusa-bearing cnidarians, Medusozoa (Collins, in prep).

Sequences were entered into a data matrix that includes more than 150 other 18S gene sequences (derived from a wide array of metazoans and their allies). Sequences were aligned by eye using primary sequence similarity. Regions which were difficult to align were excluded from the analyses by using an alignment mask because putative homology of the sequence characters could not be asserted. Two subsets of the data matrix were used in the present analysis. The first dataset has 66 taxa, 56 cnidarians and a sample of 10 non-cnidarian metazoans to serve as outgroups (four poriferans, two ctenophores, two placozoans, and two bilaterians). Bilaterians are often excluded from phylogenetic analyses of lower metazoan groups (e.g. Bridge *et al*., 1995). This may be unwise in light of evidence that bilaterians and cnidarians are relatively closely related (Collins, 1998; Kim *et al*., 1999). Because of the inclusion of a wider diversity of outgroups, this 66-taxon dataset is more appropriate to address hypotheses that deal with Hydrozoa as a whole, e.g., whether Hydrozoa is or is not monophyletic and what group is the sister clade of Hydrozoa. The second dataset is limited to just the 56 cnidarian taxa (11 anthozoans, 8 scyphozoans, 2 cubozoans, and 35 hydrozoans). The 56-taxon dataset is used to address hypotheses concerning the various subgroups of Hydrozoa. In analyses carried out with this dataset, anthozoans are used as the outgroup, a hypothesis supported by prior phylogenetic investigations of morphological and molecular data (Bridge *et al*., 1995; Schuchert, 1993).

Finding optimal trees and completing the list of hypotheses

The first step to explicitly testing prior phylogenetic hypotheses is to find an "optimal" or "best" tree implied by the 18S data. The optimal tree depends on how optimality is measured. There are a number of commonly-used measures of tree optimality (Swofford *et al*., 1996). In this analysis, the primary optimality criterion is parsimony. The "best" tree obtained by a parsimony search is the one that minimizes the number of character changes or steps throughout a tree. PAUP* 4.0 (Swofford, 1998) was used for all phylogenetic analyses. A parsimony search (heuristic search option with 100 random replicates) with equally-weighted characters was performed. Ideally, the relative weight given a type of character change would reflect the relative likelihood of that type of change. That is, less likely character changes shared by two or more taxa should carry more weight than changes that occur more readily. Without any evidence that all

TABLE 2. – Maximum likelihood estimations of the ratio of transitions to transversions and the gamma shape parameter for most parsimonious trees with equally weighted characters and trees obtained by the neighbor-joining algorithm.

Description	T-Ratio	Gamma
66-Taxon Trees		
Most Pasimonious #2 of 10	1.61	0.273
Most Pasimonious #6 of 10	1.61	0.273
Neighbor-Joining	1.58	0.271
56-Taxon Trees		
Most Pasimonious #3 of 8	1.59	0.211
Most Pasimonious #7 of 8	1.60	0.213
Neighbor-Joining	1.58	0.212

changes in the 18S gene are equally likely, there is no reason to assume that all character changes are equally likely. In fact, there is a bias toward transitions in ribosomal genes, although the unequal rates of transitions and transversions is typically less than what is observed for other genes (Vawter and Brown, 1993). Fortunately, these rates can be estimated for a given set of taxa and molecular characters and appropriate weights can be implemented for subsequent analyses.

PAUP* 4.0 was used to make a maximum likelihood estimate of the relative difference in rates (T-Ratio) of transitions and transversions given the most parsimonious trees found in the search where character changes were weighted equally. The T-Ratio can then be used to weight transitions and transversions during subsequent parsimony analyses. The logic of such a method could be construed as circular. Is there a problem with taking parsimony trees, estimating the relative rates of transitions and transversions, and then building new parsimony trees with transitions and transversions weighted differently? In order to test this thought, an additional tree was obtained by the neighbor-joining algorithm and the T-Ratio was estimated with this tree. The results show that estimates using the unweighted parsimony trees are nearly identical to those made using the neighbor-joining tree. Table 2 reports the maximum likelihood estimates of the transition to transversion ratios for the 18S data given the 66-taxon and 56-taxon trees built by neighbor-joining and unweighted parsimony analyses. There is very little difference between the estimates; transitions are roughly 1.6 times as common as transversions. Thus, trees that serve as the "optimal" trees of this analysis are found by implementing a parsimony search where transitions were weighted 2/3 times (approximately 1/1.6) as heavily as transversions, according to their likelihood of occurrence.

A consensus of five most parsimonious trees (Fig. 2) was found using the 66-taxon dataset and weighted transitions and transversions (heuristic search option with 1000 random replicates). A single most parsimonious tree (Fig. 3) was detected using the 56-taxon dataset with weighted transitions and transversions (1000 random replicate searches). The relationships among the hydrozoans are similar in the two trees, but not exact. In fact, hydrozoan relationships revealed by the 18S data are not strongly influenced when different combinations of outgroups are used (results not shown). Several of the hypotheses enumerated in Table 1 are consistent with the most parsimonious trees (3, 5, 7, 8, 11, 12, 13, 17, 19, and 20). In addition, some novel hypotheses are suggested by these trees. 23) Capitata is a monophyletic group that does not contain Hydridae, in contrast with hypothesis 18. 24) Anthomedusae is not monophyletic, the converse of hypothesis 6. 25) Hydridae and Leptomedusae form a clade. 26) Hydridae, Leptomedusae, and Siphonophora form a clade. These last two hypotheses, drawn from the 56-taxon tree, are conflicted by the relationships shown in the 66-taxon tree. 27) Trachymedusae are not monophyletic, having given rise to Narcomedusae, in contrast to hypothesis 9. 28) Hydrozoa, Scyphozoa, and Cubozoa form a clade, sometimes referred to as Medusozoa.

Testing phylogenetic hypotheses

Each of the aforementioned hypotheses can be explicitly tested with the 18S data. However, it is difficult to devise a test of phylogenetic hypotheses that has a clear black-or-white result, e.g., pass versus fail. For instance, it is not sufficient to simply build trees with molecular data and to conclude that they are correct when different tree-building methodologies yield divergent results. Thus, concordance between a hypothesis and a given molecular analysis lends support to the hypothesis, but it is not conclusive. Similarly, discordance between a hypothesis and a molecular analysis casts some doubt on the hypothesis, but it does not completely falsify it. Knowing the extent to which a molecular analysis agrees or disagrees with a prior hypothesis would be useful. To this end, I follow a procedure that relies on imposing various topological constraints on tree-building analyses to determine the relative strengths of the hypotheses that are support-

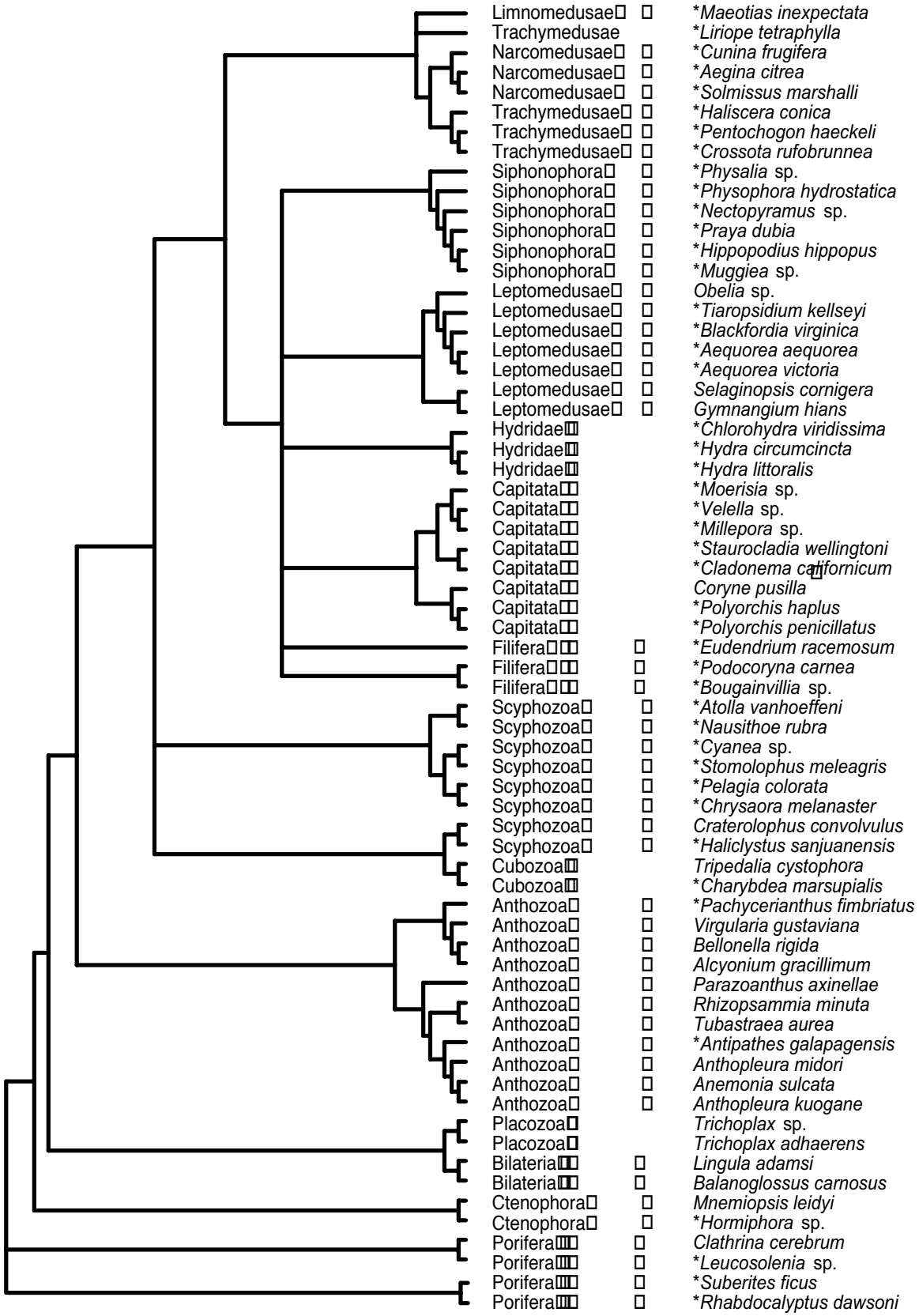

FIG. 2. – Consensus of five most parsimonious trees found by heuristic search (with 1000 random replicates) using the 66-taxon dataset, transitions are weighted 2/3 as heavily as transversions. The dataset consists of 1,807 characters, 635 of which are parsimony-informative. The five trees are 8,214 steps long, with consistency indices of .400, rescaled to .260, and retention indices of .650.

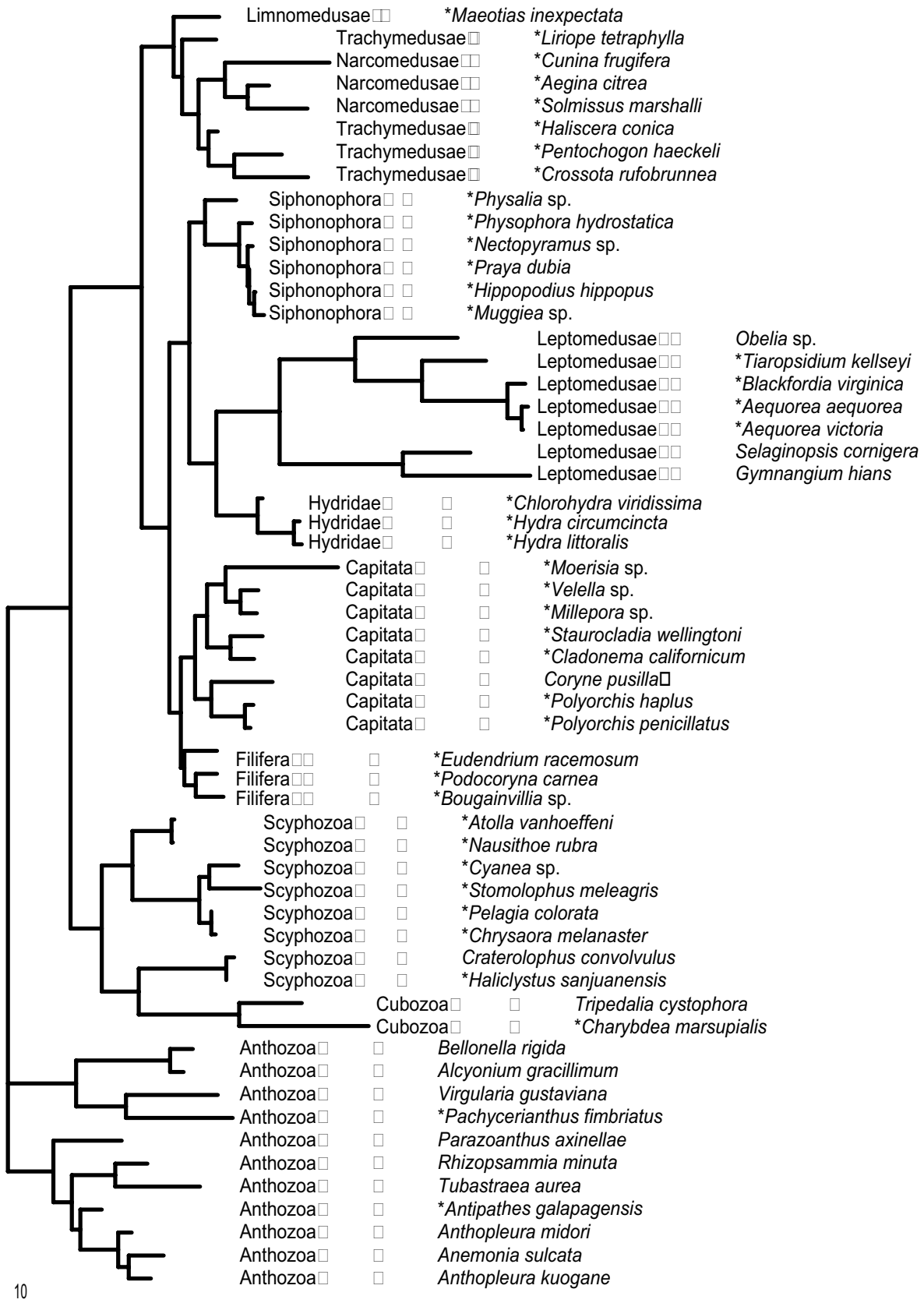

FIG. 3. – Most parsimonious tree found by heuristic search using the 56-taxon dataset, transitions are weighted 2/3 as heavily as transversions. The dataset consists of 1,807 characters, 531 of which are parsimony-informative. The tree is 5,472 steps long, with a consistency index of 0.446, rescaled to 0.306, and a retention index of 0.687.

TABLE 3. – List of hypotheses that are not consistent with the optimal parsimony trees. Column 1 reports the number of additional weighted-parsimony character changes it would take to accommodate the hypothesis. For instance, the most parsimonious tree that has Hydrozoa not monophyletic is 45 steps longer than the overall most parsimonious tree. The hypotheses are sorted by Column 2, which is Column 1 as a percent of the length of the most parsimonious tree (8,214 for 66 taxa and 5,472 for 56 taxa). Columns 3 and 4 show p-values for the Kishino-Hasegawa and Templeton tests. See text for interpretations of these values.

Hypothesis Number	Hypotheses not consistent with optimal trees	Number of steps to accommodate hypothesis (1)	As a percent of total number of steps (2)	Kishino Hasegawa Test P-value (3)	Templeton Test P-value (4)
(16)	Anthomedusae, Hydridae, Leptomedusae, and Limnomedusae form a clade	53	0.969%	0.001	0.001
(15)	Hydridae, Leptomedusae and Limnomedusae form a clade	46	0.841%	0.001	0.001
(14)	Hydridae and Limnomedusae form a clade	42	0.768%	0.001	0.001
(1)	*Hydrozoa is not monophyletic	45	0.548%	0.029	0.035
(21)	Anthomedusae, Leptomedusae, Limnomedusae and Siphonophora form a clade	24	0.439%	0.002	0.003
(4)	Siphonophora is the earliest diverging clade of Hydrozoa	23	0.420%	0.094	0.110
(18)	Capitata (including Hydridae) is monophyletic	12	0.219%	0.152	0.124
(9)	Trachymedusae is monophyletic	9	0.164%	0.170	0.119
(22)	*Cubozoa is the sister group of Hydrozoa	10	0.122%	0.551	0.593
(6)	Anthomedusae (containing Hydridae) is monophyletic	6	0.110%	0.396	0.314
(2)	Anthomedusae and Leptomedusae form a clade denotes . hypotheses addressed with the larger dataset	4	0.073%	0.157	0.157

ed by the 18S data, and the relative weaknesses of those that are contradicted by the 18S data.

With an optimal tree determined, it is now possible to divide the list of hypotheses listed in Table 1 into two groups, those that are consistent with the optimal parsimony trees and those that are not, shown in Tables 3 and 4 respectively. For each hypothesis that is inconsistent with the most parsimonious trees, an additional search (with 100 replicates and transitions and transversions weighted as before) was performed with the constraint that only trees that are consistent with the given hypothesis were considered. The length of the optimal tree that is consistent with the given hypothesis was then compared to the length of the optimal tree in the absence of constraints. Subtracting the two lengths yields a measure of the extent to which the hypothesis is controverted by the 18S data. A summary of hypotheses that are not consistent with the 18S data is presented as Table 3. For each hypothesis that is controverted by the 18S data, the number of extra weighted-character changes that it would take to accommodate the hypothesis is given in column 1. The hypotheses are sorted by column 2, which reports the number of steps (column 1) as a percent of the total number of steps in the most parsimonious trees.

In addition, PAUP* was used to implement two tests that aim to determine whether the optimal trees are significantly shorter in a statistical sense than the best trees that conform to each hypothesis. The first test (Kishino and Hasegawa, 1989) is a parametric test that compares the difference in length of the two trees to a distribution of differences whose mean is zero. The null hypothesis for this test is that there is no difference in the lengths of the phylogenetic arrangements derived from the molecular data, and so p-values can be interpreted as the probability of getting the observed difference in tree lengths if there is no true difference in tree lengths. The smaller the p-value, the lower the probability that the observed difference is due to chance alone, and consequently the higher the probability that the difference is due to phylogenetic signal. The second test (Templeton, 1983) is a non-parametric test that addresses the number of changes in each character implied by the two competing trees. In this test, randomness is expected to favor each of the competing trees equally. P-values from this test can be interpreted as the probability that the observed difference in character changes implied by the two trees is due to random error. Again, lower p-values should be associated with the most strongly controverted hypotheses. However, the validity of both the Kishino-Hasegawa and Templeton tests is somewhat suspect. First, an underlying assumption for these tests is that the data are randomly selected and independent. Phylogenetic history necessitates violation of independence of the data, while experimental design ensures that the choice of data, taxa and characters, is not random. Second, these two tests are two-tailed and should technically not be applied in a situation where one has an *a priori* expectation that one tree is shorter than the other, a situation which is true in

the present analysis. Nevertheless, results from these tests (p-values) are presented as columns 3 and 4 respectively on Table 3 in order to provide a sense of which hypotheses are most strongly contradicted by the 18S data. For instance, by any measure, the 18S data indicate that it is highly unlikely that Anthomedusae, Hydridae, Leptomedusae, and Limnomedusae form a clade.

Similarly, it is helpful to know the level of support for the hypotheses that are consistent with the optimal tree or trees. In order to achieve this, a search was performed (for each of the supported hypotheses) that was constrained to consider only those trees that are in violation of the given hypothesis. The difference in length between the unconstrained and constrained trees is equivalent to the number of extra character changes that are necessary to compromise the given hypothesis. Higher differences imply greater support for the hypotheses from the 18S data. The process of evaluating hypotheses consistent with the unconstrained trees is roughly equivalent to a Bremer analysis of clade support (Bremer, 1988; Bremer, 1994). Hypotheses that are concordant with the 18S data are presented in Table 4. For each hypothesis that is supported by the 18S data, the number of weighted-character changes necessary to compromise the hypothesis is shown in column 1. Column 2 shows the number of steps (column 1) as a percent of the total number of steps in the most parsimonious trees. Columns 3 and 4 contain p-values from Kishino-Hasegawa and Templeton tests that compare shortest constrained trees to the overall most parsimonious trees. These hypotheses are ordered from most to least support by sorting on column 2. The most strongly supported hypotheses are that Hydrozoa and Hydridae are each monophyletic. The hypothesis that Limnomedusae is monophyletic (10) cannot be tested in the current analysis because just a single representative limnomedusan taxon is included; monophyly of the group in these analyses is guaranteed.

Of course, this method begs the question of how to interpret the number of extra character changes needed to either compromise or accommodate a given hypothesis. The results of the Kishino-Hasegawa and Templeton tests are also difficult to understand given their limitations. It is largely arbitrary where the line is drawn. However, a comparison of results obtained using different tree-building methods may help. Phylogenetic relationships that are consistently inferred, regardless of the methodology used, should be considered the most robust results. Two methodologies that employ alternative measures of optimality were used to build trees in an attempt to determine the approximate number of steps (as a percent of the total number of steps) in the parsimony analyses that is indicative of support, or the lack thereof, irrespective of tree-building methodology.

TABLE 4. – List of hypotheses that are consistent with the optimal parsimony trees. Column 1 shows the number of additional weighted-parsimony character changes it would take to compromise the hypothesis. For instance, a tree that is just a single step longer than the most parsimonious tree contains an arrangement where Filifera is not monophyletic. The hypotheses are sorted by Column 2, which is Column 1 as a percent of the length of the most parsimonious tree (8,214 for 66 taxa and 5,472 for 56 taxa). Columns 3 and 4 show p-values for the Kishino-Hasegawa and Templeton tests. See text for interpretations of these values.

Hypothesis Number	Hypotheses consistent with optimal trees	Number of steps to compromise hypothesis (1)	As a percent of total number of steps (2)	Kishino Hasegawa Test P-value (3)	Templeton Test P-value (4)
(12)	Hydridae is monophyletic	45	0.822%	0.001	0.002
(11)	*Hydrozoa is monophyletic	45	0.548%	0.029	0.035
(3)	Limnomedusae, Narcomedusae, and Trachymedusae form a clade	22	0.402%	0.050	0.038
(7)	Leptomedusae is monophyletic	16	0.292%	0.312	0.295
(8)	Narcomedusae is monophyletic	14	0.256%	0.052	0.053
(20)	Anthomedusae (with Hydridae), Leptomedusae, and Siphonophora form a clade	13	0.238%	0.369	0.423
(5)	Siphonophora is monophyletic	10	0.183%	0.316	0.313
(27)	Trachymedusae is not monophyletic	9	0.164%	0.170	0.119
(24)	Anthomedusae is not monophyletic	6	0.110%	0.396	0.314
(17)	Narcomedusae and Trachymedusae form a clade	4	0.073%	0.520	0.491
(26)	Hydridae, Leptomedusae, and Siphonophora form a clade	4	0.073%	0.347	0.295
(25)	Hydridae and Leptomedusae form a clade form a clade	4	0.073%	0.556	0.449
(13)	Anthomedusae (excluding Hydridae) is monophyletic	2	0.037%	0.665	0.606
(23)	Capitata (excluding Hydridae) is monophyletic	2	0.037%	0.845	0.867
(19)	Filifera is monophyletic denotes hypotheses addressed with the larger dataset	1	0.018%	0.827	0.706

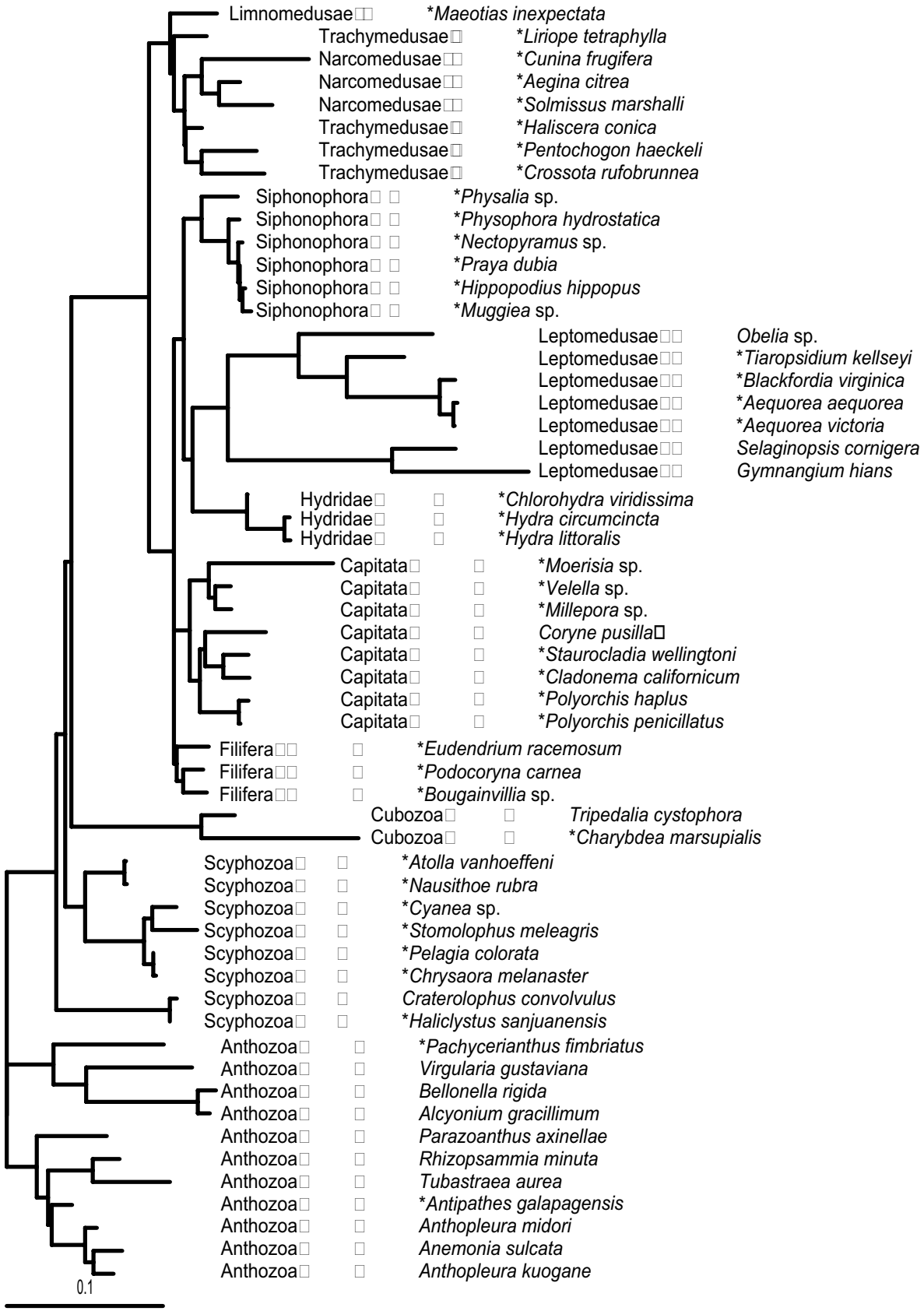

FIG. 4. – Maximum likelihood tree for the 56-taxon dataset. Model of nucleotide evolution is HKY85, with a T-Ratio assigned to be 1.59 and a gamma shape parameter of .212.

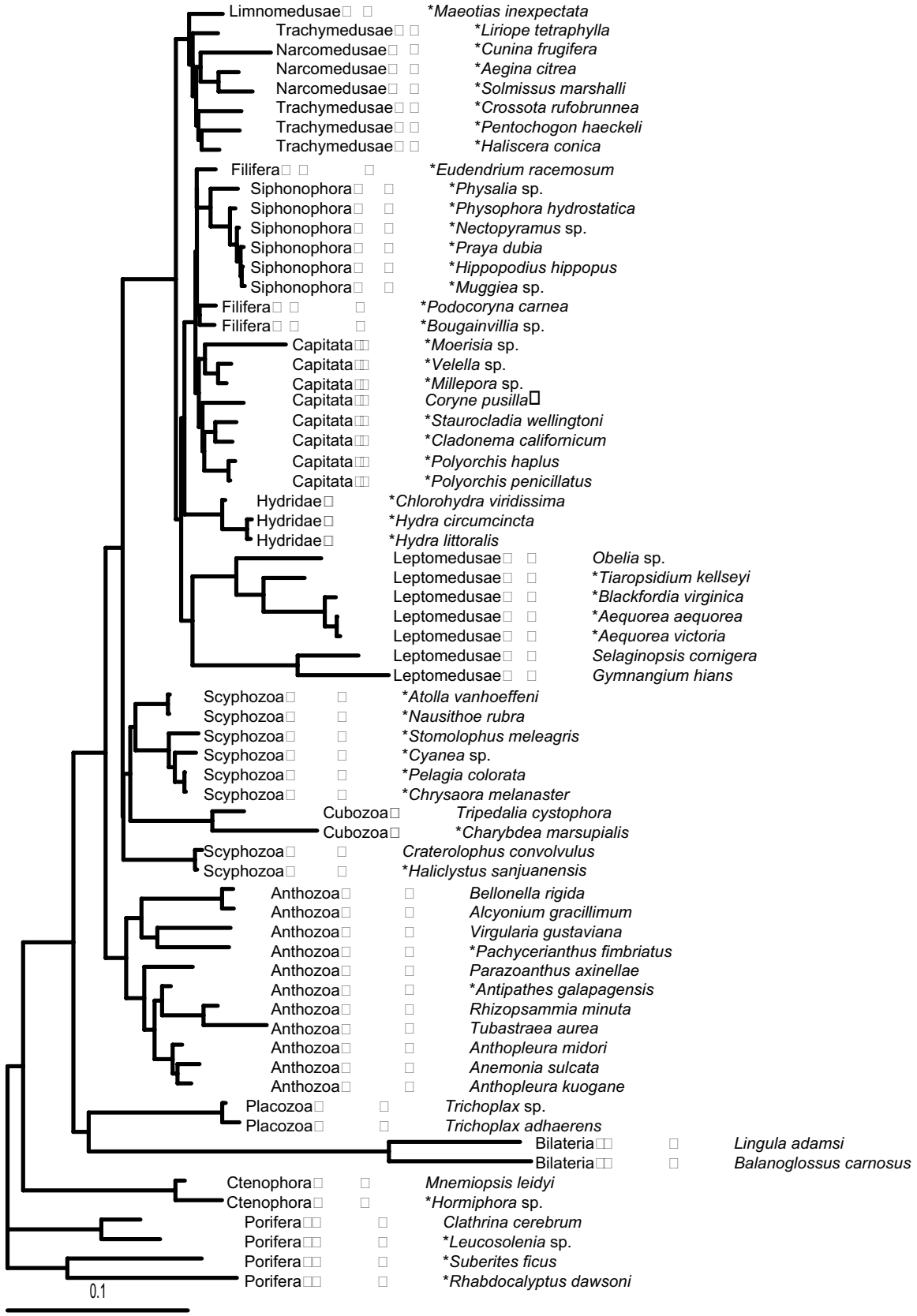

FIG. 5. – Minimum evolution tree for the 66-taxon dataset. The assumed model of nucleotide evolution is HKY85 with gamma shape parameter of .272.

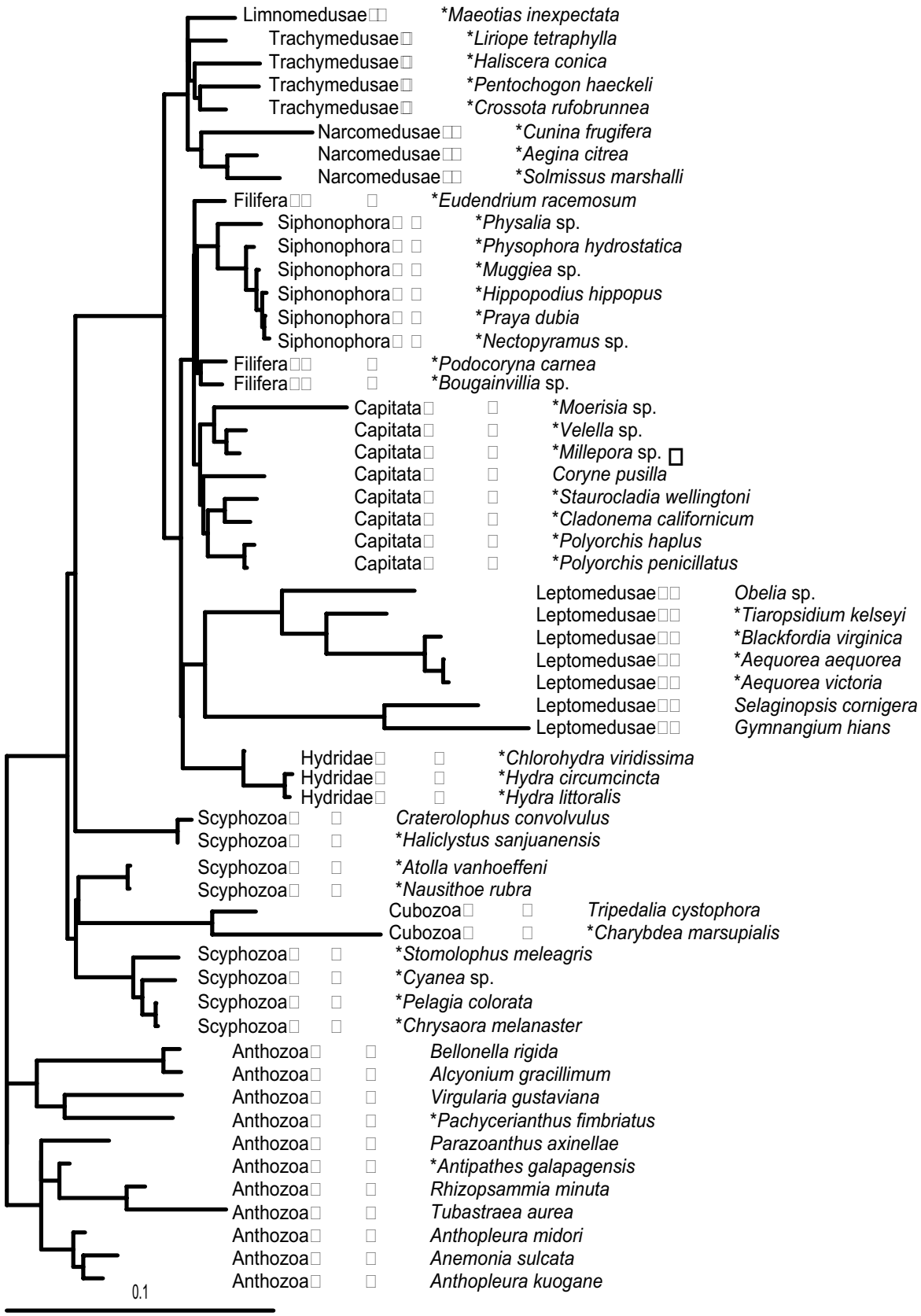

FIG. 6. – Minimum evolution tree for the 56-taxon dataset. The assumed model of nucleotide evolution is HKY85 with gamma shape parameter of .212.

The methods of maximum likelihood and minimum evolution take into account the possibility that a given nucleotide state may have evolved by character transformations from an identical state (e.g., from A to C to A again). The maximum likelihood method seeks the tree for which the data are most probable given an assumed model of nucleotide evolution. The model of nucleotide substitution used in this analysis (HKY85) allows for variation in the rate of evolution at different sites, unequal nucleotide frequencies, as well as different rates of transitions and transversions (Hasegawa et al., 1985). The two required parameters, the T-Ratio (as described above) and gamma (the shape of the distribution of substitution rates), were estimated using trees obtained by neighbor-joining and the unweighted parsimony analyses. These estimates are presented in Table 2. Due to computational difficulty, the maximum likelihood search was performed for just 10 replicates, and only on the smaller 56-taxon dataset. The maximum likelihood analysis performed on the 56-taxon dataset used a transition to transversion ratio of 1.59 and a gamma shape parameter of .212. The most probable tree given the 18S data and the specified model of nucleotide evolution is presented as Figure 4.

The minimum evolution method employs a distance-based optimality criterion (unweighted least-squares) and searches for the tree that minimizes the total sum of branch lengths given a model of nucleotide evolution. The HKY85 model was also assumed for minimum evolution searches, with gamma shape parameters of .272 used for the 66-taxon dataset and .212 for the 56-taxon dataset. 100 replicate searches were performed under the minimum evolution criterion. Negative branch lengths were disallowed. The trees with the smallest sum of unweighted least-squares distances between the taxa, given the specified models of evolution, are presented as Figures 5 and 6.

All of the hypotheses that are not consistent with the most parsimonious trees, listed in Table 3, are also not in harmony with trees built by the alternative methodologies. This increases the confidence one can have in the assertion that the 18S data contradict these hypotheses. Of the hypotheses consistent with the most parsimonious trees (Table 4), several (13, 17, 19, 25, and 26) are contradicted by trees that fit an alternative definition of optimal. However, contradictions occur only among those hypotheses for which four or fewer steps are required to be compromised. Thus, in this analysis 0.1 percent of the total number of weighted-character changes would appear to be an appropriate line of demarcation that is indicative of support, or the lack thereof, provided by the 18S data.

DISCUSSION

The optimal trees (Figs. 2, 3, 4, 5, and 6) inferred by 18S data reveal phylogenetic patterns that make sense in light of hydrozoan classifications and past phylogenetic hypotheses. Many well-defined groups are recognized as monophyletic, including Capitata (excluding Hydridae), Filifera (only Figs. 3 and 4), Hydridae, Leptomedusae, Narcomedusae, and Siphonophora. Some associations among these groups are expected, e.g., Trachymedusae with Narcomedusae, while others are more surprising, such as Leptomedusae or Siphonophora with Hydridae. However, just from looking at a tree, one cannot ascertain how much support there is for a given relationship. Nor does a tree indicate the extent to which an opposing view is contradicted by the tree. That is why this paper explicit tests competing hypotheses of hydrozoan relationships. Each of the groups, and the hypotheses associated with them, will be addressed in turn.

Anthomedusae

Anthomedusae are difficult to characterize; no synapomorphies for the group are known (Schuchert, 1996). The 18S data mirror this situation, indicating that Anthomedusae may not be a monophyletic group. The shortest 56-taxon trees that have a monophyletic Anthomedusae including Hydridae are six steps longer than the most parsimonious trees, which have Anthomedusae grouping with Siphonophora, Leptomedusae and Hydridae (Fig 3). A clade composed of these four groups is fairly strongly supported by the 18S data. Each of the "optimal" trees built by alternative methods reveal this grouping. Moreover, an additional 13 steps are required to find an alternative hypothesis in conflict with this arrangement (Table 4). Although, they appear to form a clade, the relationships among the following groups cannot be clearly distinguished with the present dataset: Capitata, Filifera, Hydridae, Leptomedusae, and Siphonophora.

Capitata and Filifera

The two recognized subgroups of the Anthomedusae, Capitata (excluding Hydridae) and Filifera may be monophyletic. In particular, a monophyletic Capitata is revealed in all "optimal" trees. Still, only two additional weighted-character changes are required to violate this relationship in the most parsimonious 56-taxon tree (Fig. 3). Two capitate families (Cladonematidae and Polyorchidae) are sampled more than once in this analysis. In all trees, the two families are revealed as monophyletic groupings. The relationships among the capitate families are unclear. The best supported result places the velellid (*Velella* sp.) and the milleporid (*Millepora* sp.) together, a situation anticipated by others (Bouillon, 1985; Petersen, 1979; Petersen, 1990). This relationship poses an interesting historical question, as both of these groups are derived morphologically and endowed with a fossil record. Fossil milleporids are known from the Cretaceous (Oliver and Coates, 1987). On the other hand, fossil velellids (or chondrophores as they are better known to the paleontological community) possibly date from the Neoproterozoic (Glaessner and Wade, 1966) and are probably known from the Cambrian (Waggoner and Collins, 1995). Of course, this phylogenetic relationship does not imply that one of these distinctive morphologies is necessarily derived from the other. If an excess of 500 million years has created the molecular divergence seen between these two species, then the 18S gene has evolved slowly in these lineages.

Even less support is provided for the hypothesis that Filifera is monophyletic. In all trees, the filiferan species branch somewhere near the base of Capitata. Interestingly, Naumov derived Capitata from Filifera (Naumov, 1960). This provides some support for the idea that filiform tentacles represent the ancestral condition for Capitata (Petersen, 1990). With just three filiferan species sampled and little resolution for the group as a whole, it is difficult to speculate on their relationships. However, the clavid species (*Podocoryna carnea*) and the bougainvilliid (*Bougainvillia* sp.) group together in these analyses. Filifera species appear to exhibit a relatively slow rate of 18S evolution. This may pose difficulties for using this gene to elucidate the more inclusive phylogenetic relationships among this group.

Hydridae

The finding that Hydridae is a monophyletic group has a great deal of support (Table 4). This is not too surprising in light of how different hydrids are from other hydrozoans. They completely lack the medusa stage and they are adapted to fresh water. The latter is presumably a difficult transition as it has only been made a few times in the history of cnidarians (e.g. Hydridae, Craspedacusta and Limnocnida). With just three species sampled (*Hydra littoralis*, *Hydra circumcincta*, and *Chlorohydra viridissima*), there is little one can say about the relationships among them. However, it has been unclear how distinctive the different species of the genera are. Some workers consider *Chlorohydra* to be a synonym of *Hydra* (Petersen, 1990). In all trees, the two *Hydra* species group together to the exclusion of *Chlorohydra viridissima* and so the two genera may delimit phylogenetically distinctive groups. Additional sampling is needed to investigate this issue further.

While the monophyly of the hydrids is likely, it is much less clear where the Hydridae fall within Hydrozoa. Petersen argued strongly that Hydridae and Moerisidae are closely related, based on similarities in early development including the presence of a resting stage, an embryo protected by periderm, and a planula without cilia (Petersen, 1990). This analysis includes just a single representative moerisid (*Moerisia* sp.), but it does not cluster with the hydrid species. In fact, an additional 20 weighted-character changes are required in order to bring the two groups together, a relatively convincing indication that they do not constitute a clade. Moerisid species are often associated with brackish waters that have a strong fresh-water influence (Naumov, 1960). The similarities between the two groups may have arisen convergently as adaptations to the rigors imposed by strong seasonal changes in fresh and very low-saline brackish waters.

At a broader taxonomic level, Hydridae is often included as part of Capitata and/or Anthomedusae (Bouillon, 1985; Hyman, 1940; Petersen, 1979; Petersen, 1990). These hypotheses are contradicted by the 18S data. The Hydridae probably have an independent origin from that of Capitata. The shortest 56-taxon tree containing Hydridae within Capitata is 12 steps longer than the most parsimonious tree. Hypothesizing that Anthomedusae contains Hydridae requires six additional character changes. The most parsimonious 56-taxon tree has Hydridae grouped with Leptomedusae. Such a grouping has not been anticipated by any worker in the past, and specific morphological connections to support the grouping are not readily apparent. In fact, support for an assertion that Hydridae and Leptomedusae

form a clade is rather limited. Four extra character changes are enough to remove the relationship between the two groups in the 56-taxon analysis. Neither of the 66-taxon trees (Figs. 2 and 5) contain this grouping. In the absence of additional indications of a close phylogenetic affinity between these two groups, it may be best to consider this grouping a questionable result with little support.

Leptomedusae

18S data suggest that Leptomedusae is monophyletic. Not surprisingly, they are a reasonably well-characterized group. The polyps in this group usually have thecae, the medusae bear their gonads on the radial canals, and the statocysts are of ectodermal origin. Among the leptomedusae sampled, all tree-building methodologies reveal the same relationships. Although Bouillon (1985) stated that his tree of Leptomedusae families should not be read as a phylogeny, the 18S data agree remarkably well with it. In these analyses, the aglaopheniid (*Gymnangium hians*) and the sertulariid (*Selaginopsis cornigera*) form a clade. The one difference is the placement of the campanulariid (*Obelia* sp.), which would branch basal to the other Leptomedusae included in this analysis if it followed Bouillon's scheme. Proboscoida (all Leptomedusae other than campanulariids) may not be monophyletic. Instead, *Obelia* is at the base of a clade that includes exemplars of Mitrocomidae (*Tiaropsidium kellseyi*), Blackfordiidae (*Blackfordia virginica*), and Aequoridae (*Aequorea aequorea* and *A. victoria*). Among these groups, Aequoridae and Blackfordiidae appear to be the most closely related. The rate of evolution of the 18S gene appears to be relatively high in this group, as evidenced by their longer branch lengths. Additional sampling of the 18S gene from leptomedusan species should continue to help resolve their relationships.

Siphonophora

Forming fantastic colonies of highly polymorphic zooids, siphonophores are a distinctive group of hydrozoans and their monophyly is supported by 18S data. All three major subgroups of the siphonophores that are typically recognized are present in this analysis, Calycophorae, Cystonectae, and Physonectae. *Physalia*, the Portuguese man-of-war, is the lone representative of Cystonectae, and it always branches basal to the calycophorans and physonects in the analysis. This is in contrast to Totton's view that the cystonects and physonects are most closely related based on a fairly lengthy list of similarities (Totton, 1965). In light of the data presented here, these similarities, which include the possession of gas-filled floats, can be considered as ancestral to Siphonophora. In agreement with Totton, these data suggest that gas-filled floats were lost in calycophores. The calycophores, which hold together strongly as a monophyletic group based on 18S data, appear to have a great number of autapomorphies beyond those derived features that characterize the siphonophores as a whole.

Siphonophores are so distinct from other hydrozoans that they are often excluded from discussions that deal with the rest of Hydrozoa. In fact, Bouillon *et al*. (1992) were prompted to ask "Non-siphonophoran Hydrozoa: what are we talking about?" In this scholarly discourse on hydrozoan nomenclature, the authors wrestle with the question of what to call non-siphonophoran hydrozoans and conclude that "Hydroidomedusae" is the best name. However, the present analysis suggests that no formal taxonomic name should be used for the non-siphonophoran hydrozoans since they are very unlikely to be monophyletic. Siphonophores probably did not branch basally to the other hydrozoan groups. Such a scenario requires 23 additional weighted-character changes (Table 3).

Instead, Siphonophora may be allied with Anthomedusae. Haeckel was the first to assert this affiliation, suggesting that the ancestors of Siphonophora should be looked for among the capitate groups Corymorphidae and Tubularidae (Haeckel, 1888). Since Haeckel, there have been a number of workers who have also supported the idea of an ancestral connection between Siphonophora and Capitata primarily based upon larval similarities (Daniel, 1985; Garstang, 1946; Leloup, 1955; Totton, 1965). Such an explicit connection between Siphonophora and Capitata is not suggested by the present analysis, requiring eight extra steps to be accommodated. Schuchert (Schuchert, 1996) hinted that Siphonophora may share affinities with Anthomedusae because both share gonads on the manubrium and desmonemes, characters that Petersen listed as synapomorphies for Anthomedusae (Petersen, 1990). Still, the most parsimonious 56-taxon tree that places Siphonophora and Anthomedusae in a single clade is four steps longer than the overall most parsimonious 56-taxon tree (Fig. 3). It is possible that the Actinulidae and/or the Laingiomedusae also belong among these groups. Sampling these taxa would be a logical extension of this analysis.

Limnomedusae, Narcomedusae, and Trachymedusae

18S data provide a substantial buttress for the assertion that Narcomedusae species in this analysis comprise a clade. Still, the three Narcomedusae species in this analysis are members of just two of the four Narcomedusae subgroups, Cuninidae and Aeginidae, that are typically recognized (Bouillon, 1987). Surprisingly, the two members of Cuninidae, *Cunina frugifera* and *Solmissus marshalli*, do not group in the analyses. Additional taxa need to be sampled in order to address the internal relationships of Narcomedusae. The same can be said for the Trachymedusae. Just three subgroups of Trachymedusae are sampled in the present analysis, Geryonidae, Halicreatidae, and Rhopalonematidae. The two rhopalonematids (*Crossota rufobrunnea* and *Pentochogon haeckeli*) group together in most of the "optimal" trees (but see Fig. 6). *Haliscera conica*, the representative halicreatid, groups with the rhopalonematids, while the geryonid, *Liriope tetraphylla*, tends to branch basal to the other Trachymedusae as well as the Narcomedusae. The hypothesis of Trachymedusae monophyly is contradicted by the 18S data, for it would take an additional nine weighted character changes to accommodate (Table 3). With just a single limnomedusan species sampled, it is impossible to make any statements concerning the hypotheses that the group is or is not monophyletic.

In a phylogenetically broader view, Narcomedusae and Trachymedusae form a clade in all analyses. Bouillon asserted that the Trachymedusae were most likely derived from the Narcomedusae (Bouillon, 1987). In contrast, the 18S data actually imply that Narcomedusae are derived from Trachymedusae. If this hypothesis is true, then the polyp stage that some Narcomedusae species possess has been secondarily re-gained. This follows because all Trachymedusae for which the complete life cycle is known are direct developers. Moreover, this placement of Narcomedusae implies that the similarities that appear evident between Cubozoa and Narcomedusae, such as the complete metamorphosis of polyp into medusa and the sculpted medusa bell margin (Bouillon, 1987; Petersen, 1979) are convergent characters.

There can be little doubt that the limnomedusan species *Maeotias inexspectata* is part of a clade that includes Trachymedusae and Narcomedusae. The 18S data strongly indicate that Narcomedusae are related to Trachymedusae and Limnomedusae (Table 4). This is not too surprising since Limnomedusae were considered to be part of Trachymedusae prior to the discovery that they possess a polyp stage. In addition, Limnomedusae, like Narcomedusae and Trachymedusae, have statocysts that are ecto-endodermally derived. Further, the position of the gonads of Limnomedusae is typically on the radial canals, a character also seen in Trachymedusae (Hyman, 1940). An exception is the group Proboscidactylidae, which has been considered part of Limnomedusae in the past. However, the Proboscidactylidae are no longer considered to be Limnomedusae, but instead Filifera (Petersen, 1990). Sampling more limnomedusan species may be necessary to determine the phylogenetic limits of the group. Also, the hypothesis that Laingiomedusae are allied with Limnomedusae (Bouillon, 1987) can only be tested by obtaining additional samples of these taxa.

Hydrozoa

Among the hypotheses that are best supported by 18S data is the assertion that hydrozoans all share a more recent common ancestor with each other than any do with any other cnidarian. It would appear then that the velum, the medusa ring canal, and gonads of epidermal origin were present in the most recent common ancestor of hydrozoans (Schuchert, 1993). An additional character supporting the monophyly of Hydrozoa is a loss of nematocysts in the gastric cavity (Bridge *et al.*, 1995). It is unclear from this analysis which cnidarians constitute the sister group to the Hydrozoa and there is only limited support for a monophyletic grouping of the medusa-bearing cnidarians. Additional taxa and/or characters should be brought to bear on this question.

CONCLUSION

Phylogenetic classifications provide very tangible advantages over character-based classifications (De Queiroz and Gauthier, 1990; 1992). Phylogeny provides a natural and useful scheme for organizing life. By giving organisms names that correspond to evolutionary history, then learning names is equivalent to learning history. It is nice to know, therefore, that a phylogeny-based classification of Hydrozoa, which does not greatly contradict older classifications, can be offered in light of the present discus-

sion. Hydrozoa appears to be composed of two clades. One includes Limnomedusae, Narcomedusae, and Trachymedusae. A reasonable name for this clade is Trachylina, as it has been used in the past to encompass these groups (Haeckel, 1880; Bouillon *et al*., 1992). The other main clade of Hydrozoa is comprised of Capitata, Filifera, Hydridae, Leptomedusae, and Siphonophora. This clade can be given a new name Hydroidolina (A. Marques, pers. comm.), because it is a novel grouping. In light of our present understanding, it is unclear where to place Actinulidae and Laingiomedusae.

Increasing the number of taxa in an analysis enhances phylogenetic accuracy (Graybeal, 1998; Hillis, 1996). Thus, future work should include sampling the 18S gene more widely. In addition, other sources of data need to be consulted. Alternative genes, life history information, nematocyst characters, other gene sequences etc. should all be used to test the results of this study. A combined data analysis would be particularly interesting and may hold the most hope for yielding a stable well-supported phylogeny of Hydrozoa.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Trends in hydroidomedusan research from 1911 to 1997*

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SUMMARY: The papers on hydroidomedusae published from 1911 to 1997 total 10,934. They have been assigned to the following categories: faunistics and systematics; sub-organismal biology; ecology; evolution; life cycles; paleontology. The general trend, comprising all papers, can be divided into four time intervals: the first (1911-1939) with an average of sixty papers/year and with a slight decrease due to First World War; the second one (1940-1947), with an average of 38 papers/year, marked by a dramatic decrease coinciding with Second World War; the period 1948-1991 shows a steady increase until the mid-Seventies, when a small decrease occurred, followed by an increasing trend reaching its apex in the late Eighties-early Ninenties with a record of 296 papers in 1991 and with an average of 175 papers/year; the period 1992-1997, with an average of 178 papers/year, is marked by a sharp decrease, reaching the values of the mid Sixties. The most important category in terms of number of papers is sub-organismal biology, followed by faunistics and systematics. Systematic studies dictated the trend in the first decades of the century, whereas sub-organismal ones are prevalent from the Sixties onwards. Faunistic and systematic-taxonomic papers have a steady trend of production, with just a slight decrease over these last years. The formerly leading countries in systematics (UK, USA, France) are now almost inactive in this discipline, whereas countries with little or no tradition in this field (such as Spain) are taking the leadership.

Key words: Cnidaria, Hydrozoa, history, bibliography.

INTRODUCTION

Literature data are important in all fields of science but, due to the law of priority, they are essential for taxonomic work. The law of priority, in fact, obliges taxonomists to be aware of every description throughout the literature and no paper can be ignored, no matter the language employed and the journal chosen for publication. The decline of taxonomy (Boero, 1994) is due to manifold causes, from low impact of tribunes for publication and related career opportunities, to enormous requirements in terms of bibliographic expertise.

Biodiversity assessment requires answering the basic question "How many species are there on Earth?" (May, 1988) and if, on the one hand, it is true that many species are still to be discovered, it is also true, on the other hand, that many "species" are just names which have been given autonomous rank often because of unavailability of previous descriptions of the same material. The task of modern taxonomy, thus, is not just to describe and name the still unknown species, but also to revise the known taxa (especially at generic level) getting rid of all the syn-

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onyms that burden the inventory of biodiversity. A serious taxonomic revision requires huge bibliographic resources and taxonomists routinely build up specialised libraries and make their inventory. As for hydroids and hydromedusae (from now on termed here hydroidomedusae, as suggested by Bouillon *et al.,* 1992), Bedot (1901; 1905; 1910; 1912; 1916; 1918; 1925) listed all the papers published in the period 1581-1910 and all the taxa mentioned therein, with nomenclatural updates. The same did Mayer (1910), providing description, synonymy and complete literature for every medusan species known at the time. The year 1910, thus, is a landmark in hydroidomedusan literature, dividing an almost completely surveyed production from a *terra incognita*. This lack in knowledge has been partly filled by Kramp (1961) with the Synopsis of the Medusae of the World, covering the period 1911-1959. In this case, as with the case of Mayer, however, just the medusae were dealt with, whereas hydroids were neglected.

Vervoort (1995) published a list of papers on hydroidomedusae from 1911 to 1995. No attempt was made to provide information on species, and the sole list required 432 pages!

Modern personal computers allow easy information retrival, and in a more efficient format than a simple list on paper. The scope of this work is to refer on a computerised data base of hydroidomedusan literature from 1911 to 1997, deriving from Vervoort (1995) and from subsequent updates. The data base was built up for easy access to systematic literature, but is also a tool to reconstruct the history of hydroidomedusan research as witnessed by the trends in the production of papers in different fields.

MATERIALS AND METHODS

The list of papers in Vervoort (1995) was transcribed into a single file using the software FileMaker Pro 4.0, a relational database (Mac/Windows) allowing the archiviation of information with the possibility of multithematic indexing and research.

Papers from 1985 to 1997 have been searched for through S.I.B.A. (Servizi Informatici Bibliotecari di Ateneo, of the University of Lecce) consulting the **'**Biological Abstracts**'** and the **'**Current Contents**'**. The last ten years of Vervoort's (1995) list were thus checked against commercially available data bases. The choice of key words was crucial since apparently "logical" choices such as "Hydrozoa" did not extract all papers on Hydrozoa present in the data bank. We finally chose "Cnidaria" as the sole keyword, then extracting relevant records from a longer list (including also Anthozoa, Scyphozoa, and Cubozoa).

A file with about 11,000 records was created. Each record comprises the following fields: Author(s), Title, Journal, Year, Volume, Issue, Pages, Keys.

Keys cover general subjects such as Ecology, Evolution, Faunistics and Systematics, Life Cycles, Paleontology, Sub-organismal biology, and other minor disciplines. Papers were ascribed to categories according to the information deriving from their title; a given paper can be ascribed to more than one category, but only the main one has been considered for the present analysis. Further information was obtained by consulting directly each paper, even though the library available to us includes just 6,000 papers. The Keys include also abbreviations of the genera mentioned, but this entry is present only for the papers available to us.

The papers have been ascribed to nations according to the nationality of the first author.

For the papers extracted from electronic data banks we added also the abstract. A "Notes" field is available to contain any other information on the paper (e.g., author's address) and might even contain the whole scanned paper. A last field informs whether or not the paper is present in our library.

The size of the file is 22 MB but, in spite of its size, searches lead to immediate results even with small computers and never caused problems.

Specific information on scientific production throughout the data base has been extracted by customised Macros (series of computer operations performed authomatically as a single command) which enabled to order references against time and to divide yearly records by main Subject. Yearly trends were smoothed with the technique of mobile averages over five-year periods.

RESULTS

General trends in hydroidomedusan research

The scientific production on hydroidomedusae in the period 1911-1997 sums up to 10,934 papers. The whole trend, expressed in number of papers per year, can be divided into four periods, marked by changes in the patterns of scientific production (Fig. 1):

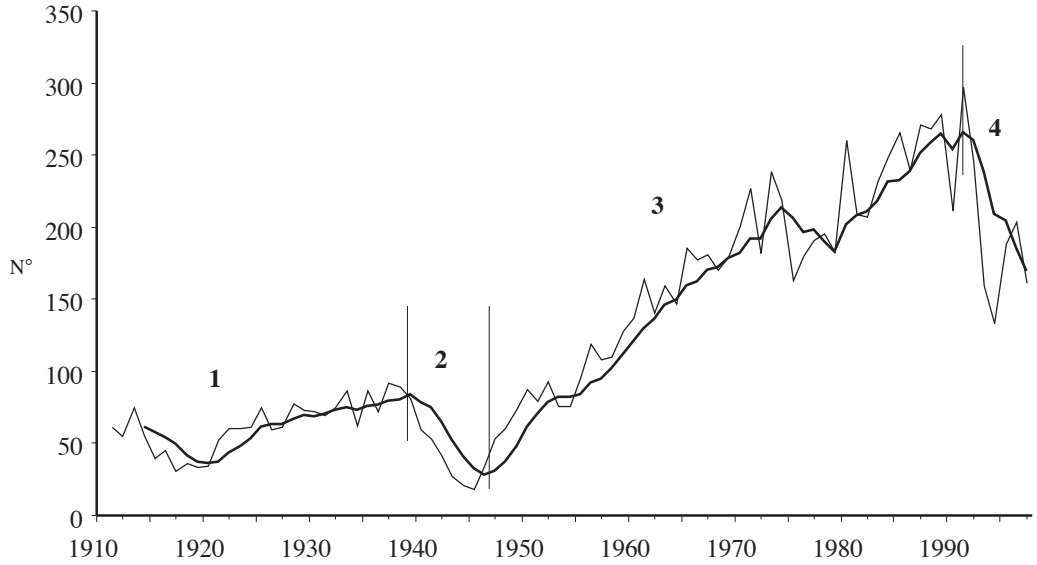

FIG. 1. – General trend in hydroidomedusan research (thin line) with mobile average over 5 year periods (thick line). Vertical lines separate four main periods within the trend.

- 1911-1939, with an average of 60 papers/year and a sharp decrease due to First World War;

- 1940-1947, with an average of 38 papers/year, almost coinciding with Second World War;

- 1948-1991, with an average of 175 papers/year: 125 papers/year in the sub-period 1948-1969, and 226 papers/year in 1970-1991, with a record of 296 papers in 1991;

- 1992-1997, with an average of 178 papers, marked by the inversion of the steady increase of the preceeding period.

In the period 1580-1910 (not covered in the present survey) 2942 papers have been published, with an average of about 9 papers per year.

Trends in the main scientific areas

A better understanding of what determined the general trend is possible by considering specific topics (Fig. 2).

Sub-organismal biology covers topics ranging from general physiology to histology, cell biology, molecular biology, and genetics. It is the most productive area of study, with 4,429 papers, representing 41% of the total. This topic contributed little to the production of the first decades of the period, whereas it dominated the production from the Sixties onwards (Fig. 3). The papers on *Hydra* (Fig.4a) alone comprise 23% of the total production. These works treat mainly sub-organismal biology and represent 56% of the papers on this topic, being the main topic responsible for this trend (Fig. 4b).

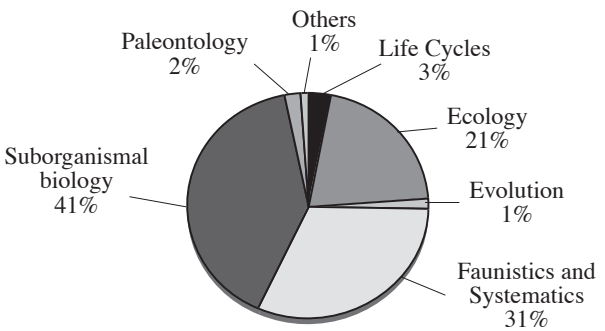

FIG. 2. – Percentages of papers treating the main topics in hydroidomedusan literature from 1911 to 1997.

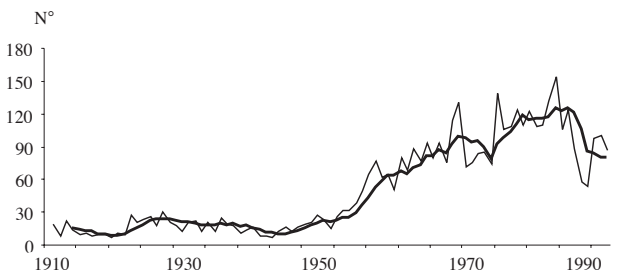

FIG. 3. – Trend of production on sub-organismal topics (thin line) with mobile average over 5 year periods (thick line).

Faunistics and systematics is the second most important subject, with 3,404 papers (31% of the total) and a stable trend of production, with just two sharp decreases coinciding with World Wars and a decrease over the last few years (Fig. 5). Faunistics and systematics was the first-ranked topic from the

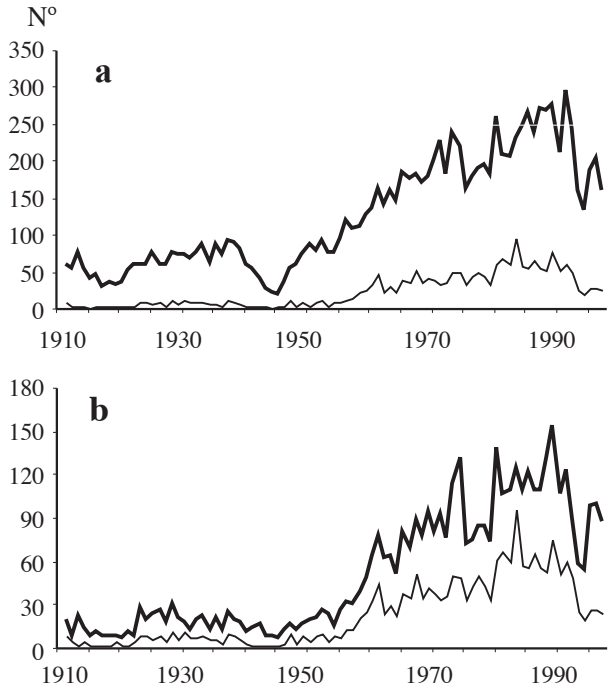

FIG. 4. – The contribution of papers devoted to research on *Hydra* (thin line) compared with: a) the whole trend (thick line); b) the trend of sub-organismal biology (thick line).

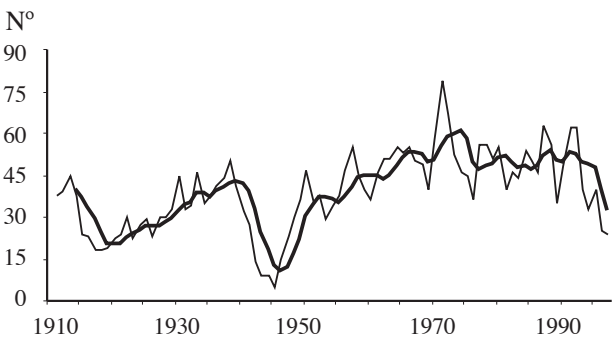

FIG. 5. – Trend of production on faunistics and systematics (thin line) with mobile average over 5 year periods (thick line).

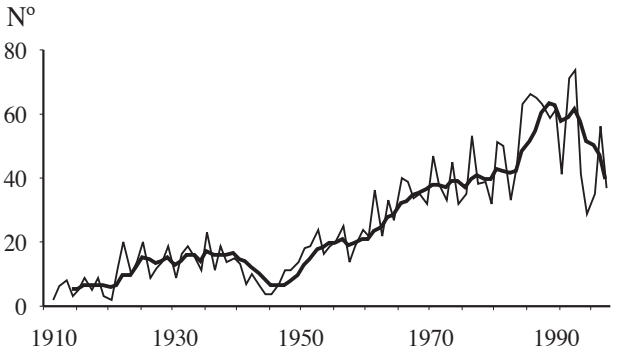

FIG. 6. – Trend of production on ecology (thin line) with mobile average over 5 year periods (thick line).

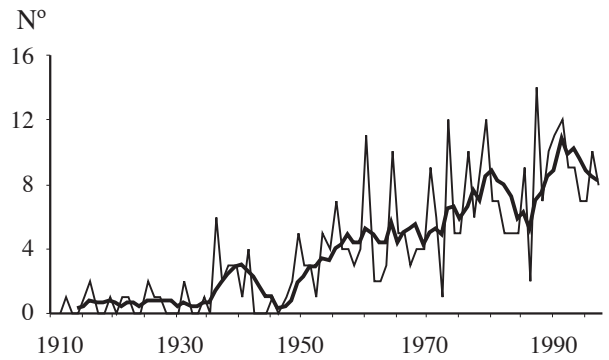

FIG. 7. – Trend of production on life cycles (thin line) with mobile average over 5 year periods (thick line).

beginning of the period to 1956, being responsible for the general trend, whereas it became the lowest-ranking topic in the last decades.

Ecology (2,270 papers, 21% of the total) was rather neglected in the first decades, but went through a steady increase after Second World War (Fig. 6). Ecology cannot be considered a trendy topic in hydrozoan literature as a whole (see Gili and Hughes, 1995, for a recent review).

Life cycle studies (348 papers, 3% of the total), after a boom in the mid-Thirties, and the usual decrease during Second World War, display a fluctuating increase after the mid-Forties (Fig. 7).

Paleontology (223 papers, 2% of the total) and Evolution (162 papers, 1% of the total) have almost no detectable trends, representing a negligible portion of the total production.

A small number of papers (98, 1% of the total) deal with various topics and cannot be ascribed to particular research areas.

Contribution of the main countries in hydrozoan systematics and faunistics

One of the main reasons for the decline of taxonomy is that taxonomic journals have low Impact Factor and that career opportunities in research are often based on the scores of Impact Factor of the applicants for a certain position. This system of evaluation of research is partial, since journals are also classified according to the time their articles continue to be cited. Taxonomic papers will be cited as long as there will be taxonomic work: descriptions of new species are “immortal”. A low immeditate impact is counterbalanced by a prolonged life of taxonomic production. We made a particular analysis of the trend of production in systematics and faunistics, so to have a test of the tendencies regarding this field of research not only on a global scale but also country by country.

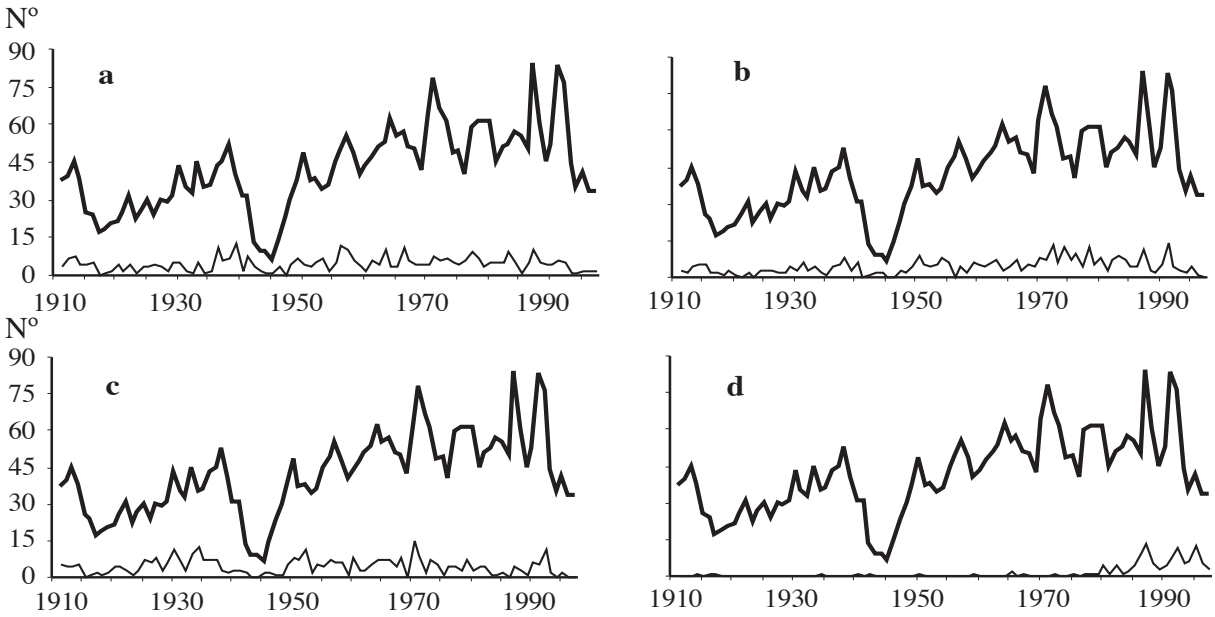

FIG. 8. – Trend of production on faunistics and systematics in the three Countries that more contributed to this field (thin line) against the total faunistics and systematics trend (thick line): a) United Kingdom; b) USA; c) France. The same for an "emerging" Country: d) Spain.

With the exception of the very last years, most taxonomic papers have a single author. Only recently a fine network of collaboration led to multi-authored papers, with authors from different countries. This new trend of international collaboration is the result of the institution of the Hydrozoan Society, established in 1985 and, since then, a major cataliser of international work. We tried to extract the contribution to systematics of single countries by considering the nationality of the first author. The main contributors to faunistics and systematics are United Kingdom, United States of America, and France (Fig. 8a, b, c), each going through a fall in scientific production in the last decades. Other countries are now flourishing in this field and the main example is Spain (Fig. 8d), with an almost non-existent production in most of the considered period and with a recent production, in the last 20 years, that parallels that of the leading countries in their best periods. The situation of Spain is the result of the influence of such eminent personalities as Ramon Margalef, one of the founders of modern ecology. Margalef advised his pupils to study taxonomy before carry out ecological research (J. M. Gili, pers. comm.).

Fig. 9 reports the yearly average production, with standard deviation, of the countries that contributed more than other ones to faunistic-systematic research on hydroidomedusae.

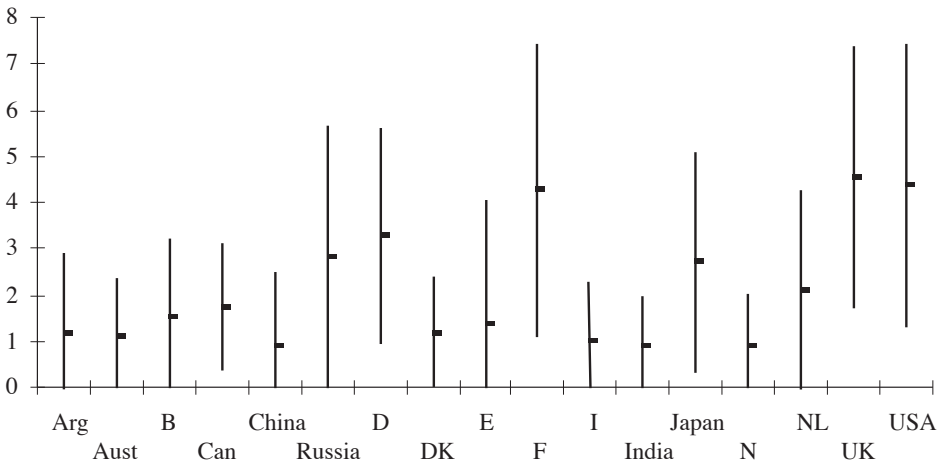

FIG. 9. – Average yearly production of papers, with standard deviation, of the main Countries in hydroidomedusan faunistics and systematics (Arg: Argentina; Aust: Australia; B: Belgium; Can: Canada; D: Germany; DK: Denmark; E: Spain; F: France; I: Italy; N: Norway; NL: The Netherlands).

DISCUSSION

Besides the negative influence of the World Wars, the interest in hydroidomedusae saw a continuous increase until the Nineties, when a tendency towards a decrease occurred. It is not certain whether this is due to an actual decrease in production or to an artefact due to our inability to find all records. The decrease was sharper in Vervoort's survey since many papers published in the Nineties (especially 1993-94) are not reported, but the trend towards a decrease is evident even after the addition of many papers to the last considered years. One reason advanced by Volker Schmid (personal communication) is that recent focusing of sub-organismal biology on model animals other than hydrozoans has distracted many researchers from working on the group.

The main discipline from 1911 to 1959 was systematics and faunistics, whereas sub-organismal biology dominated the production from 1960 to date. Systematics and faunistics was the least category in the last two years of the survey. This change in scientific trends reflects a general attitude in biology, with a shift of interest from organisms to cells and molecules.

The decline of taxonomy is due to manifold reasons:

1. The introduction of the Impact Factor to evaluate the scientific content of publications. Taxonomists publish mainly in journals that are not covered by the Institute for Scientific Information, such as those issued by Museums and scientific Societies. Monographs are ignored by the Impact Factor system. The publication score of taxonomists is usually low and not competitive. The "life" of publications (almost "eternal" due to the priority law) is never considered to evaluate the level of publications.
2. Difficulties in locating and/or getting type material for revisions.
3. Difficulties in tracing and consulting a huge body of specialised literature.

A scientific paper in taxonomy requires a high investment in terms of time and expertise, whereas the reward in terms of impact and career possibilities is low. The tendency towards the decrease of interest in systematics and faunistics is more evident in the leading scientific countries, since they have abandoned this type of research almost completely. This is paradoxical if the recent concern on biodiversity is considered. The leading countries in the knowledge of biodiversity are now relinquishing their expertise in this field in favour of newcomers like Spain and Italy, with the eclipse of an important scientific tradition. Worldwide political choices orient young researchers towards sub- or supra-organismal topics (Boero, 1994).

Ecology is the third-ranking category throughout the whole period, but is less important than the former two areas and, in fact, does not deeply influence the general trend in any period. Life cycle studies are not important from a quantitative point of view, but also acquire a relevant role in systematics. The building of a single classification for hydroids and hydromedusae started to be perceived as a strategy in the mid-Thirties and continues to date. The number of papers dedicated to this topic is small (with a maximum of 14 in 1987) but the trend towards increase contrasts the general trend in faunistics and systematics. Life cycles should be much more relevant in ecological papers, but these are mostly one-stage oriented (in fact only a small percentage of ecological papers consider life cycles) in spite of ecology being labelled as the science of connections and interactions. Most hydroidomedusae have complex life cycles, and the existence of polyps is dictated by the success of the medusae and vice versa, this being relevant to ecological approaches (see Boero *et al.,* 1996 for a discussion of the importance of life cycles in marine ecology).

Palaeontology gives a scant contribution of hydroidomedusan science: only few groups fossilise easily and only Milleporids, Stylasterids (e.g., Cairns, 1983) and a host of supposed *Velella* and *Porpita* (e. g., Stanley, 1982) are usually considered.

The study of evolution is rather neglected in the hydroidomedusae; most evolutionary papers, furthermore, refer to the internal evolution of supraspecific taxa, whereas the importance of the Hydrozoa, and of the Cnidaria as a whole, in the evolution of the Metazoa is mostly neglected. This is surprising since the Cnidaria are diploblastic, as were the ancestors of the triploblasts that dominate the animal kingdom today. The importance of the last existing diploblasts in the understanding of metazoan evolution should be greater than it actually is (Boero *et al*., 1998). The reason for this illogical tendency is possibly that scientists that argue about metazoan evolution do not care for Cnidaria and that cnidarian specialists are not attracted by evolutionary speculations. Recent molecular techniques are now calling attention to Cnidaria and it is possible that this trend will be inverted (Bridge *et al.,* 1995; Schierwater *et al.,* 1991; Schierwater and Kerstin, 1998).

Bibliographic information is paramount mainly in systematic and faunistic work, whereas the bearing of old literature on fastly developing fields such as molecular biology is almost negligible. Our database, thus, will be of great help mainly to systematists and we hope to make it available in electronic format as soon as possible.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

A guideline to nematocyst nomenclature and classification, and some notes on the systematic value of nematocysts*

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SUMMARY: Cnidocyst nomenclature is based on the structure of the tubule and its armature as viewed in the light microscopy (LM). Investigations utilising optically improved LM and scanning electron microscopy have revealed some errors in the interpretation of the fine structure of tubules and armature of nematocysts. Categories of nematocyst have been modified, therefore, to incorporate observations made with new visualising techniques. Isorhizas are defined as nematocysts whose tubule is of uniform or nearly uniform thickness proximal to the midpoint, while b-mastigophores are nematocysts with a rod-shaped encapsulated shaft and a prominent armature on the everted shaft. The category of amastigophores is retained but redefined as p-amastigophores for the V-shaped notch at the base of encapsulated shaft. The trirhopaloids are found to be similar to the birhopaloids. Mesobasic is redefined as an intermediate length of discharged shafts between micro- and macrobasic. Astomocnidae is redefined as entangling nematocysts and stomocnidae as penetrants.

Key words: Cnidaria, nematocyst nomenclature, cnidocysts, isorhizas, mastigophores, amastigophores

INTRODUCTION

Cnidocysts, or cnidae, are membrane-enclosed cellular organelles or secretions of the golgi apparatus, consisting of a capsule and an eversible tubule (Slautterback and Fawcett, 1959; Watson and Wood, 1988; Arai, 1997). They have been classified traditionally in two major categories, nematocysts and spirocysts (Bedot, 1896; Weill, 1934) to which a third category, the ptychocyst, has been added (Mariscal *et al*., 1977, see also Watson and Wood, 1988). Cnidarian systematics have considered types of cnidocysts an important systematic character and have defined the cnidome as the census of cnidocyst present in a species (Weill, 1934). Cnidomes and information on the size and distribution of cnidocysts are now considered an important part of a species's descriptions (Itô and Inoe, 1962; Kubota, 1976, 1978a,b; Gravier-Bonnet, 1987; Östman, 1983).

Spirocysts and ptychocysts are homogeneous categories, each comprising a single type of cnidocyst. Spirocysts are present only in the Anthozoa, whereas ptychocysts are found exclusively in cerianthids. The capsule wall of spirocysts is thin, and the encapsulated tubule is strongly coiled. The everted tubule lacks spines entirely but secretes a unique adhering, hygroscopic substance (K. W. England, unpublished manuscript). The everted tubules of ptychocysts are woven into the cerianthid's body tube (Mariscal *et al*., 1977).

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Nematocysts are conspicuously diverse. Weill (1934) divided them into 16 categories. Additional nematocyst types were subsequently identified, climaxing today in over thirty varieties and subvarieties (Carlgren, 1940, 1945; Cutress, 1955; Werner, 1965; Mariscal, 1974; Bouillon *et al*., 1986; Östman, 1983, 1997; Rifkin, 1996; Östman and Hyman, 1997). Various systems of nomenclature have been devised to cope with this diversity, including Stephenson's (1929), which was also used by den Hartog (1977, 1980), and Schmidt's (1969, 1972, 1974), although, generally, nematocyst classification is based on that of Weill (1934) with modifications made by Carlgren (1940), Cutress (1955), Mariscal (1974), Calder (1977), Rifkin (1996), Östman and Hyman (1997), and the convention on terminology proposed by Watson and Wood (1988).

Weill's nomenclature was based on observations made through the light microscope (LM) primarily of the discharged tubule and its spine pattern. The appearance of the inverted tubule, coiled inside the capsule also contributed distinct diagnostic characteristics (Carlgren, 1940, 1945; Cutress, 1955). The higher resolution achieved by modern LMs and the scanning electron microscope (SEM) have revealed some errors in the interpretation of the fine structures of the nematocysts, in estimates of the tubule diameter and the pattern of spines and thus effects the basic nomenclature of the different categories of nematocysts. For example atrichs are now known to be spined rather than smooth (Cutress, 1955; Calder, 1974; Westfall, 1966a; Heeger *et al*., 1992; Östman *et al*., 1995); basitrichs are spined throughout their whole tubules rather than merely spined basally (Cutress, 1955; Westfall, 1966b); tubules of isorhizas taper gradually toward their base rather than remaining isodiametric (Cutress, 1955; Östman and Hyman, 1997); everted microbasic b-mastigophores have no shaft rather than a small shaft (Cutress, 1955; Östman, 1983, 1988); amastigophores have a thin tubule beyond the shaft rather than no tubule (Cutress, 1955; England, 1991). Furthermore, isorhizas and anisorhizas, basitrichs and b-mastigophores represent overlapping categories rather than completely separate categories (cf. Cutress, 1955; England, 1991).

The evidence with improved visualisation techniques on structure also alters concepts of nematocyst function, in particular, their function as mini-hypodermic needles. Weill (1934) split the nematocysts into two main groups, the astomocnidae, possessing a tube closed at the tip, and the stomocnidae having an open tip. The stomocnidae generally have coarse spines of different sizes and shapes (Mariscal, 1974; Tardent, 1988; Östman and Hyman, 1997) and were thought to penetrate and inject toxin into prey, predators and competitors. Astomocnidae generally have spineless tubules or tubules armed with only slender, weak spines and were thought to entangle prey (Mariscal, 1974; Östman *et al*., 1995). Studies by Rifkin (1996) in *Chironex fleckeri* and by Heeger *et al*. (1992) in *Cyanea capillata,* however, have shown that the tips, of at least some fully discharged stomocnide nematocysts are closed, and Rifkin (1996) has shown that toxin can be released from discharged tubules with closed tips. Furthermore, pressure from droplets of capsular matrix flowing down the length of the hollow tubule may cause the end of a closed tubule to rupture. The value of cnidae for systematics, thus, depends on re-evaluating their diagnostic characteristics.

MATERIAL AND METHODS

Fresh cnidocysts from Hydrozoa, Scyphozoa and Anthozoa have been studied with LM and SEM techniques. Hydroids, family Campanularidae and Tubularidae (see Östman, 1979a,b, 1983, 1988; Östman *et al*., 1995), scyphopolyps and medusae of *Cyanea capillata* and *Cyanea lamarckii* (see Östman, 1997; Östman and Hyman, 1997), sea anemones *Gonactinia prolifera*, *Diadunema cincta, Metridium senile* and *Sagartiogeton viduatus,* and the solitary coral *Cariophyllia smithi* were from the Gullmar Fjord on the Swedish west coast. The hydroid *Halocordyle disticha* (see Östman *et al*., 1991) and the Scyphomedusa *Cotylorhiza tuberculata* were from the coast of Ischia in the Gulf of Naples, Italy. The siphonophore *Apolemia uvaria*(?) was collected off the coast of Catalina Island, California, near the Catalina Marine Science Centre. *Physalia* sp. and the scyphomedusa *Cassiopeia xamachana* were collected from the coast of Florida near the Whitney Laboratory of the University of Florida. Squash preparations for nematocyst studies were made by the method of Östman (1987) and Östman *et al*. (1991).

GENERAL CONSIDERATIONS AND RESULTS

Nematocyst nomenclature based on Weill (1934) distinguishes between nematocysts whose tubules are undifferentiated along their length (isorhizas,

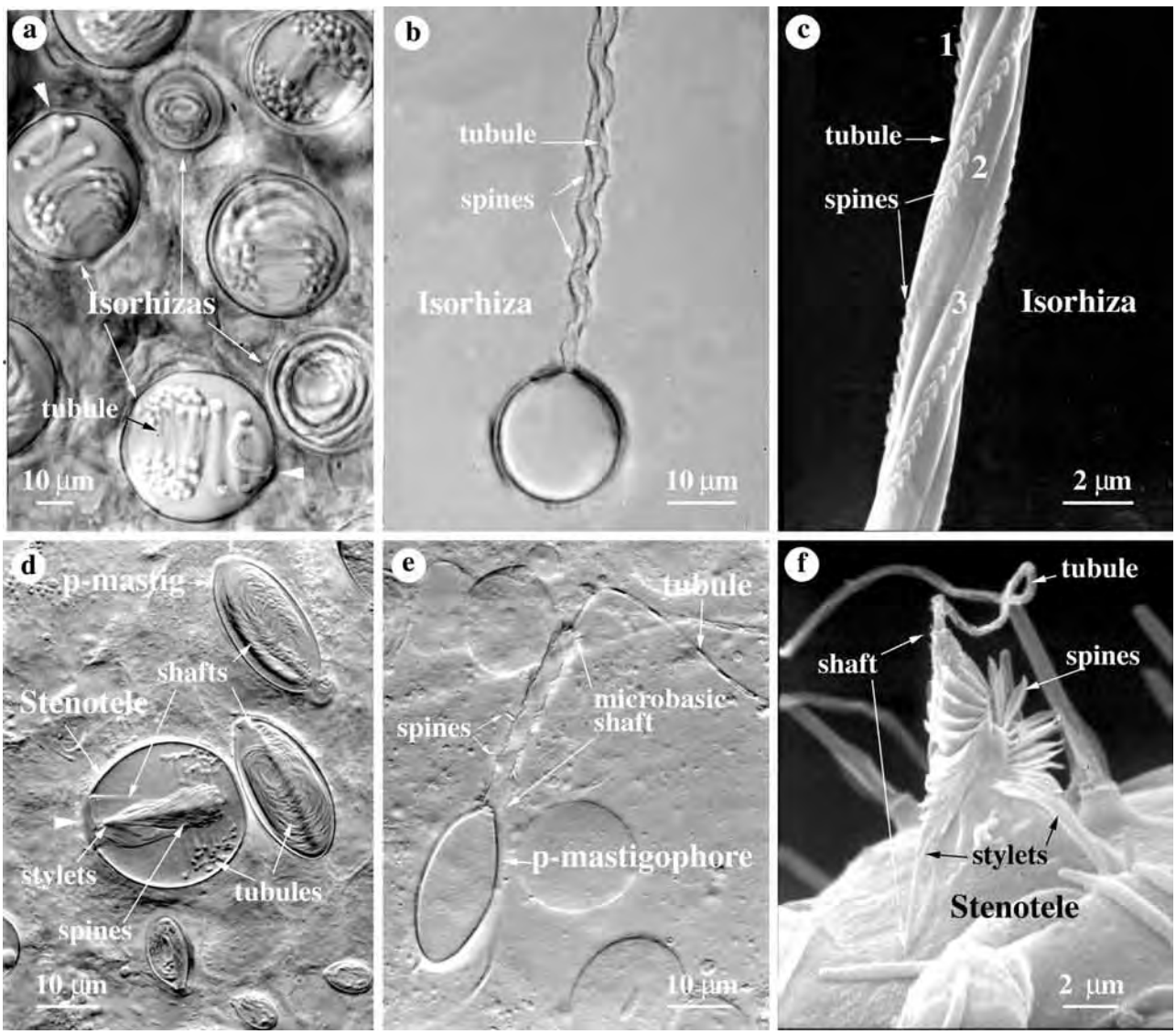

FIG. 1. – a) LM of undischarged O-isorhizas of two size classes of *Physalia* sp. The tubule is making loosely packed coils perpendicular to the main axis of the capsule. Note the pattern of the first loop of the tubule adhering to the apical tiny, but broad protruding tip (arrowheads). b,c) LM and SEM of isorhizas of *Apolemia* sp. Note the three rows (1,2,3) of broad-based arrow-shaped spines and the helical structure of the discharged tubule. d) LM of undischarged microbasic p-mastigophores of two size classes of *Apolemia* sp. and one stenotele. The stylets inside the inverted shaft of the stenotele are pointing towards the aperture of the capsule (arrowhead). Note the pattern of the spines and of the coils of the remaining tubule. The rod-shaped shafts of the p-mastigophores are slightly curved. Their narrow tubules are densely coiled along the main axis of the capsules. e) LM of a discharged microbasic p-mastigophore of *Apolemia* sp. Note the broad shaft in comparison to the narrow tubule. The spines on the shaft are larger and more prominent than those on the tubule. f) SEM of a discharged stenotele of *Halocordyle disticha*. Note the stylets and the broad-based spines or lamellae on the upper part of the broad based and apically tapering shaft. The helically twisted pattern is faintly shown on the narrow tubule. *1,2,3*, rows of spines; *p-amastig*, p-amastigophore.

Fig. 1a-c), those whose tubules are broader close to the capsule and taper gradually (anisorhizas), and those whose tubules are divided into a dilated portion or proximal shaft and a thread-like distal tubule (Fig. 1d-f). Tubules may be without spines or armed with three helically-coiled bands of spines (Fig. 1b,c), and these may extend throughout the length of the tubule or be restricted to areas. The spines may vary in shape and size from very long to short, from broad-based to slender, from pointed to hook-shaped, from strong to weak, and from prominent to tiny and virtually imperceptible in the LM (Fig. 1b,c,e,f). The initially large penetrating spines of the stenoteles are described by the term stylet (Fig. 1d,f) (see Tardent, 1988). The pattern of spines also varies from close-set to open. These differences in armature may appear along the length of a single nematocyst and between nematocysts.

Large, closely-set spines form a prominent feature of the encapsulated shaft as well as the everted

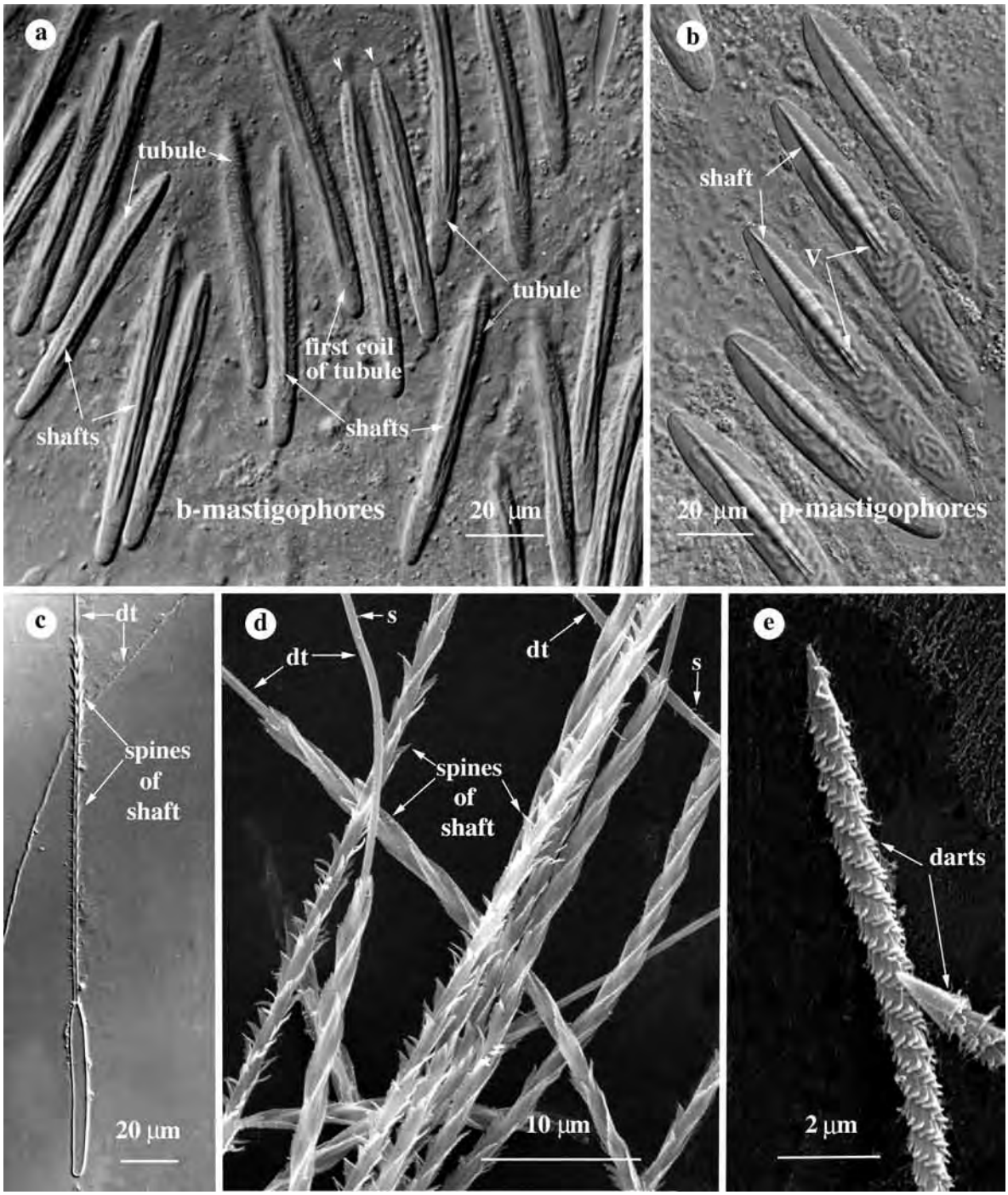

FIG. 2. – LMs and SEMs of b- and p-mastigophores. a) Undischarged mesobasic b-mastigophores of *Metridium senile*. Note the rod-shaped shafts and the first coil upwards of tubule at end of shaft (arrow). b) Undischarged p-mastigophores of *Cariophyllia smithi*. Note the V-shaped notch (V) at end of shaft. c) A discharged mesobasic heterotrichous b-mastigophore of *Metridium senile*. Note the different spinepattern on the shaft. d) Parts of shafts of homotrichous mesobasic b-mastigophores of *Metridium senile*. Note also the distal tubule (dt) armed with tiny spines (s). e) Parts of darts from *Metridium senile*. Arrowheads, point at anterior end of capsule; *dt*, distal tubule; *s,* spines on distal tubule; *V*, V-shaped notch at end of shaft

shaft or tubule (Figs 1d, 2a-d). Isorhizas, lacking closely-set, proximal spines (Fig. 1a,b) also lack prominent features in the unfired capsule. The tubule of many nematocysts is simply inverted within the capsule, whereas the broad, inverted shaft of a stenotele or p-mastigophore can be folded back within itself (Figs 1d, 2b; Cutress, 1955; Tardent, 1988).

Carlgren (1940) split nematocysts into two categories based upon the appearance of a prominent

straight shaft inside undischarged capsules: b-mastigophores (Fig. 2a) and the p-mastigophores with a V-shaped notch at base of unfired tubule (Fig. 2b). Cutress (1955) discovered that the shaft of some p-mastigophores had one or more darts and classified these nematocysts as q-mastigophores. Hand (1961), Werner (1965) and Mariscal (1974) did not accept Cutress's proposal. They regarded darts as artefacts produced by the cohesion of spines. Schmidt (1974), however, also identified darts, and some structures, interpreted as arrows or darts, were in the present work, identified in SEM preparations of *Metridium senile* (Fig. 2e). The term mesobasic was coined by England (1991) for microbasic b-mastigophores with a proximal, tightly folded shaft.

Nematocyst nomenclature

Traditionally, spines and spine patterns were described as follows:

atrichous	without spines
basitrichous	spines at base of tubule
heterotrichous	two or more kinds of spines
holotrichous	tubule spined throughout
homotrichous	all same kind of spines
spines	the armature decorating the surface of an everted tubule of a nematocyst
stylets	initially penetrating large spines

Tubules in the various types of nematocyst were described as follows:

anisorhizas	tubule tapers gradually toward distal end
astomocnidae	closed tubule (lacks opening at tip)
b-mastigophores	lacks V-shaped notch at base of unfired shaft (Fig. 2a)
birhopaloids (type I)	shaft with a distal and a proximal dilation (Fig. 3a-d)
euryteles	shaft dilated beyond a basally constricted region (distal dilation; Fig. 3f)
haplonemes	tubule lacking well-defined shaft (Fig. 1a,b)
heteronemes	tubule with well defined shaft (the shaft is an enlarged basal portion of the tubule, Figs. 1d-f, 2a,b,c)
isorhizas	isodiametric tubule (Fig. 1a,b)
macrobasic	shaft more than four times longer than the capsule's long axis
mastigophores	tubule extends beyond shaft (Fig. 1e)
p-mastigophores	V-shaped notch at base of unfired shaft (Fig. 2b)
pseudostenoteles	shaft with short unarmed base separated by a constriction from the armed part of shaft much longer and armed with rows of short spines. Two to four big spines are present at the level of constriction. Other big spines may be present along the row of short spines (Bouillon *et al*., 1986)
q-mastigophores	shaft bears one or more darts (Fig. 2e). The dart is considered to be an unattached structure neatly fitting over the end of the invaginated shaft (Cutress, 1955)
rhabdoids	cylindrical shaft, often isodiametric (Figs 1d,e, 2a)
rhopaloids	diameter of shaft changes (Figs 1d,f, 3a-f) shaft enlarged basal portion of tubule
trirhopaloids	discharged shaft dilated at more than 2 points along length; largest (=middle) swelling bearing spines (Rifkin, 1996)
shaft	enlarged basal portion of tubule
stenoteles	discharged shaft dilated at base, three spines (stylets) especially strongly developed at level of constriction between unarmed basal part and distal spinous portion (Fig. 1d,f)
stomocnidae	tubule with opening at tip

The following definitions are new or altered:

amastigophores	= p-amastigophores having a V-shaped notch at base of unfired shaft (Fig. 4c)
birhopaloids (new) (type II)	the two dilations on shaft close together (Fig. 3e, similar to the trirhopaloids of Rifkin (1996))
b-mastigophores	no V-shaped notch at base of unfired, narrow shaft; discharged shaft or proximal tubule approximately the same diameter as remaining tubule, proximal tubule with prominent armature (Fig. 2a,c,d)
isorhizas	tubule of uniform or nearly uniform thickness proximal to midpoint of tubule (Fig. 1b)

mesobasic	medium-sized shaft or prominent proximal armature, more than one and a half times but less than for times the capsule length (Fig. 4a,b)
microbasic	shaft or prominent proximal armature less than one and a half times the capsule length (Figs.1e, 4a,b)

Isorhizas, anisorhizas and basitrichs. No rod-shaped shaft visible inside unfired capsule

The tubules of the isorhizas and anisorhizas are simply inverted without portions folded back upon themselves; no shaft (enlargement of the proximal tubule) is visible inside undischarged capsules. Inverted as well as everted tubules of isorhizas are isodiametric or nearly so (Fig 1a,b) in contrast to the

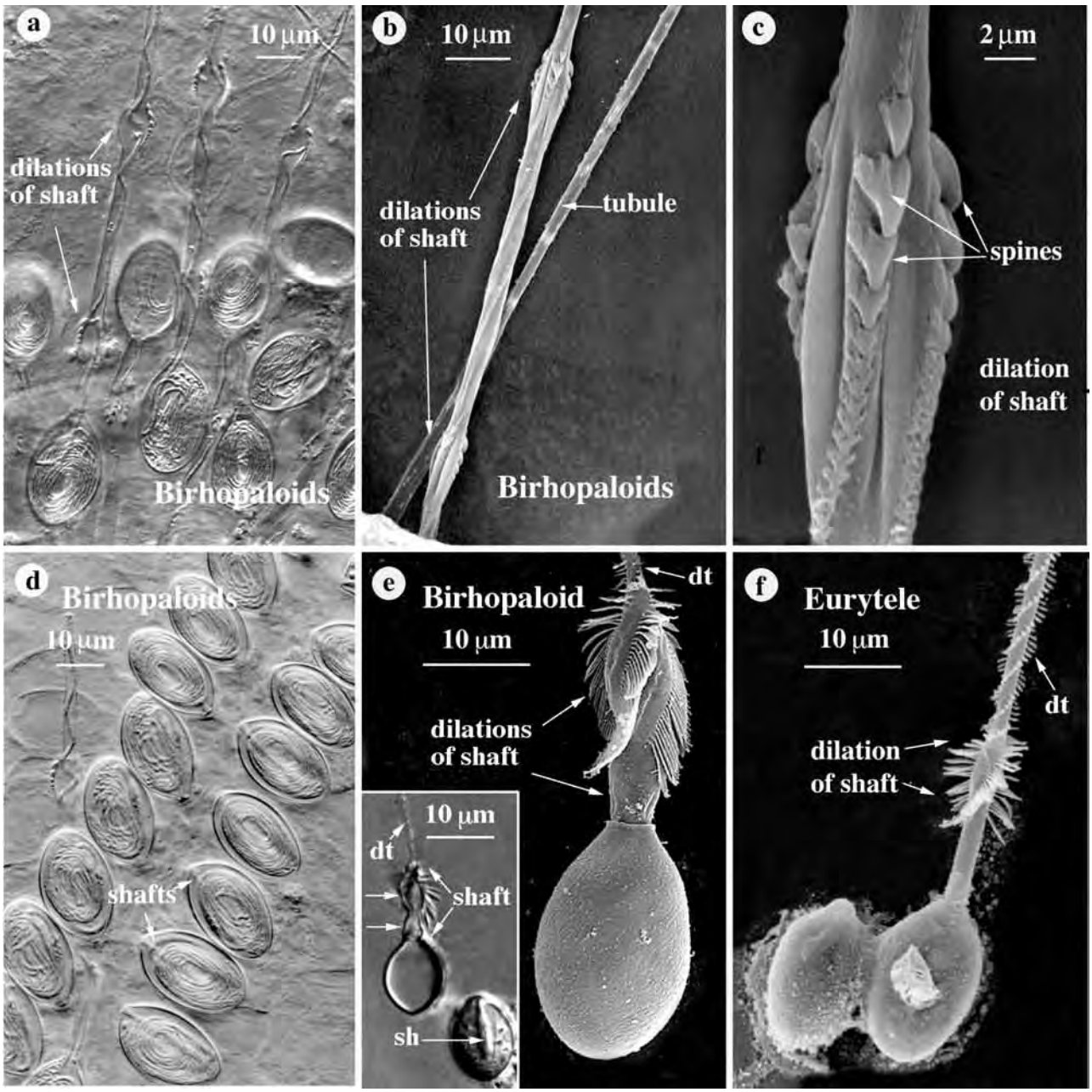

FIG. 3. – a-d) LMs and SEMs of birhopaloids type I of *Apolemia* sp. a,b) Note the distance between the two dilations or swellings on the shaft. Rows of spines are shown on the dilations and along the shaft. c) The distal dilation showing tree rows of broad-based arrow-shaped spines. d) Undischarged birhopaloid type I showing the inverted shaft and the densely coiled narrow tubule. e) SEM of discharged birhopaloid type II from *Cyanea capillata*. The two swellings of the shaft are close together. Only the large distal dilation is armed with spines. Insert: LM of discharged and undischarged birhopaloids type II from *Cassiopeia xamachana*. f) SEM of a eurytele from *Cyanea capillata* showing the distal dilation of shaft armed with long spines. Note the small spines on the distal tubule (dt). *dt,* distal tubule; *sh*, shaft.

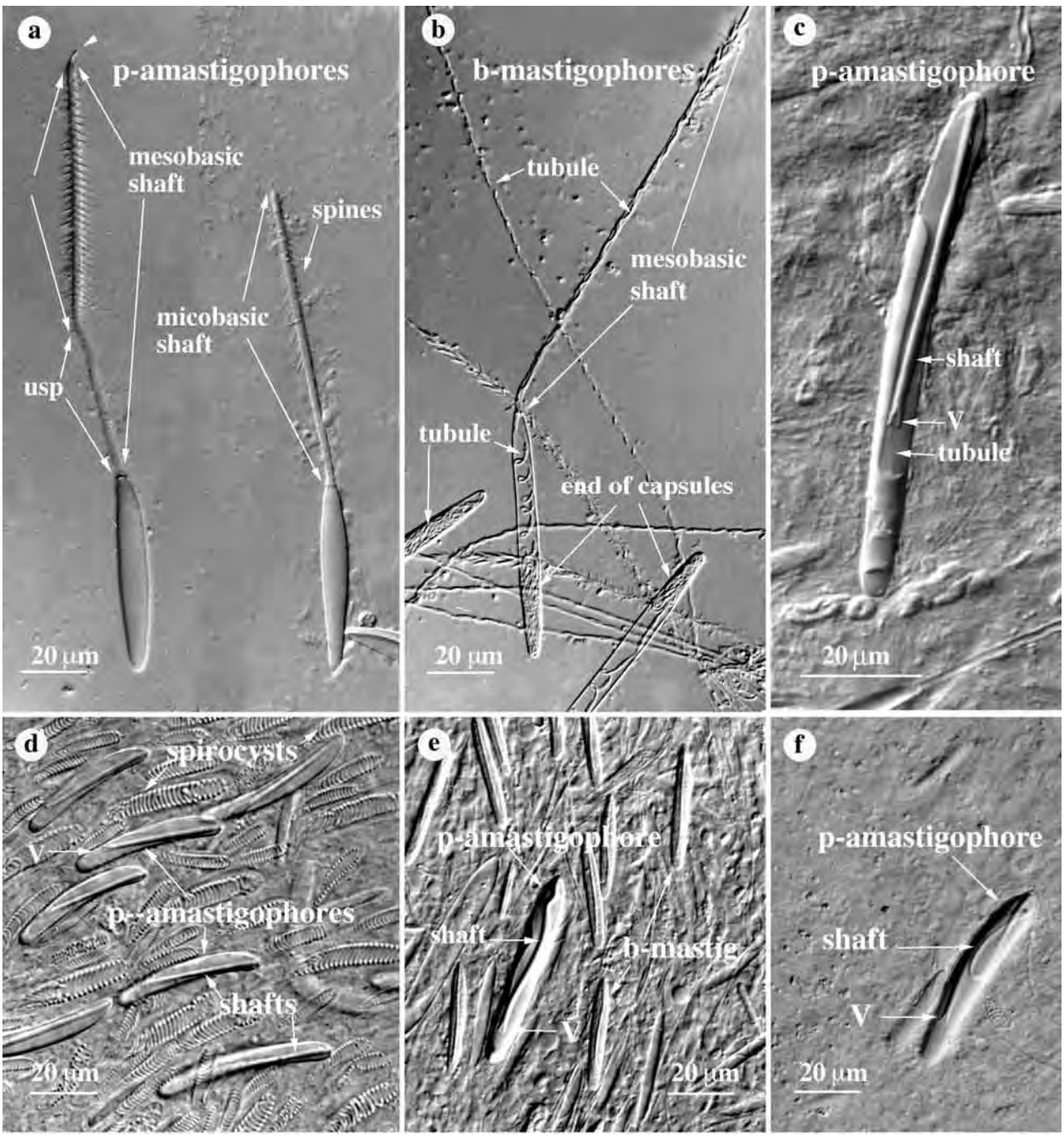

FIG. 4. – LMs of p-amastigophores, b-mastigophores and spirocysts. a) Discharged mesobasic- and microbasic p-amastigophores from *Sagartiogeton viduatus*. Note the tiny tubule (arrowhead) at end of the mesobasic shaft. b) partly discharged mesobasic b-mastigophores from *Metridium senile*. Note the coiled tubule inside shaft and capsules. c) An undischarged p-amastigophore from *Metridium senile*. Note the hardly visible narrow tubule emerging from the V-shaped notch at end of shaft. d) Spirocysts and p-amastigophores from *Gonactinia prolifera*. Note the position of the shaft. e) b-mastigophores and one p-amastigophore from *Sagartiogeton viduatus*. Note the undulating shaft of the p-amastigophore. f) An undischarged p-amastigophore from *Diadunema cincta*. Note the coiled shaft. Arrowhead, point at the tiny tubule at end of shaft; *V*, V-shaped notch at end of shaft.

tapering tubules of anisorhizas. In some hydrozoan isorhizas, the loops of the tubule are longitudinally coiled along the capsule axis (Östman, 1983, 1999). In most isorhizas, the inverted tubule begins to form coils close to the aperture, and coils loop back perpendicularly from wall to wall (Fig. 1a). The first coil to be discharged is the one closest to the aperture, and the last one is that at the end of the capsule. This pattern of discharge has been found in scyphozoan isorhizas (Östman and Hyman, 1997), siphonophoran (Fig. 1a), in other hydrozoan isorhizas (Östman *et al.*, 1991) and in actiniarian

isorhizas. The same pattern has been reported for actiniarian basitrichs (Cutress, 1955).

The inverted tubule of basitrichs with coarse armature close to the capsule can appear shaft-like but is coiled (Cutress, 1955) and not rod-like as in the b-mastigophores (Fig. 2a). Basitrichs thus resemble isorhizas (Cutress, 1955; Östman, 1982) or anisorhizas when their tubules are thick basally.

Nematocysts with shafts visible in the unfired capsule

The shaft is recognised by its large diameter compared with that of the remaining inverted tubule (Figs 1d, 2a,b, 3c-f). Large, closely-set spines are generally present on shafts, whereas spines are smaller and loosely-set on the distal tubule. The inverted shaft is twisted. Some shafts or parts of shafts are twisted more tightly proximally than distally (Fig. 5a). Other shafts are tightly twisted both proximally and distally (England, 1991). The lengths of shafts vary. Shafts shorter than their capsules generally form a straight axial rod in the centre of the capsule (Figs 2a,b, 4c), while some may follow the curvature of the capsule (Fig. 1d) and others run obliquely across the posterior of the capsule (Fig. 4d). Shafts longer than the capsule may be undulating or coiled (Fig. 4e,f). The diameter of the shaft may vary throughout its length.

Microbasic, mesobasic and macrobasic shafts

The terms microbasic and macrobasic refer to the length of the discharged shafts. Weill (1934) identified microbasic as nematocysts whose discharged shafts were less than three times the capsule length. Macrobasic nematocysts had discharged shafts longer than four times the capsule length. Rod-shaped shafts, similarly tightly twisted throughout and isodiametric, generally bear spines of the same size (i.e., they are homotrichous, Fig. 2d).

Shafts longer than the capsule are accommodated to the confined space by proximal undulations or coils, which are more tightly twisted than in the remaining part of the shaft (Fig. 4e,f, 5a). Schmidt (1969) interpreted the proximal part of the shaft as highly folded. The spines inside this portion are generally smaller and not as closely-set as those of the remaining part of the shaft (i.e., the nematocysts are heterotrichous, Fig. 5a,b,d). Since encapsulated shafts with small and loosely-set spines can be more tightly twisted compared to shafts with large closely-set spines (Fig. 5a-e), when discharged, these folded or twisted shafts are generally longer than less twisted and nonconvoluted shafts. England (1991) introduced the term 'mesobasic' for microbasic nematocysts whose shafts were more tightly folded proximally than on the remaining tubule. Thus only nematocysts with homogeneously twisted shafts were assigned to the category of microbasic by England (1991).

'Mesobasic' would seem more appropriately defined in terms of the shaft's length, rather than its degree of folding, since it is intermediate between micro- and macrobasic. All inverted rod-shaped shafts are more or less tightly twisted and when discharged they are slightly longer than the capsule (Fig. 5a). Encapsulated shafts with a distinct tightly twister, slightly undulating or coiled portion are often longer at discharge than one and a half times the capsule length. Thus, shafts longer than one and a half time the capsule length, but shorter than four times the capsule length, are here designated mesobasic (Fig. 4a).

Microbasic and mesobasic b-mastigophores. Simple inverted shafts in unfired capsules.

By definition (Carlgren, 1940; Cutress, 1955; Mariscal, 1974) microbasic b-mastigophores have a proximal, cylindrical enlargement of the tubule called the shaft. The shafts are less than three times the length of the capsule and gradually taper into the narrower distal tubule (or thread).

The b-mastigophores are recognised by the pattern of their inverted tubule. In unfired capsules, the tubule is simply inverted, forming a narrow, distinct rod-shaped shaft (Fig. 2a). In actiniarian b-mastigophores, the inverted tubule coils around the shaft against the capsule wall (Figs. 2a, 6a). The first coils to leave the discharging capsule are those closest to the aperture, and the last coils to evert are those at the posterior end of the capsule. Thus, immediately at the distal end of the unfired shaft the tubule coils back toward the capsule aperture. A similar situation may prevail in the elongated actinarian b-mastigophores (Fig. 2a). Studies of broken capsules of *Metridium senile* under SEM have revealed one loop of the tubule which may coil upward toward the aperture of the capsule (Fig. 6a,c). The remaining tubule forms regular coils from wall to wall around the shaft (Figs. 4b, 6a,c), although some irregular coils are found in the end of the capsule posterior to the base of the shaft (Fig. 6b).

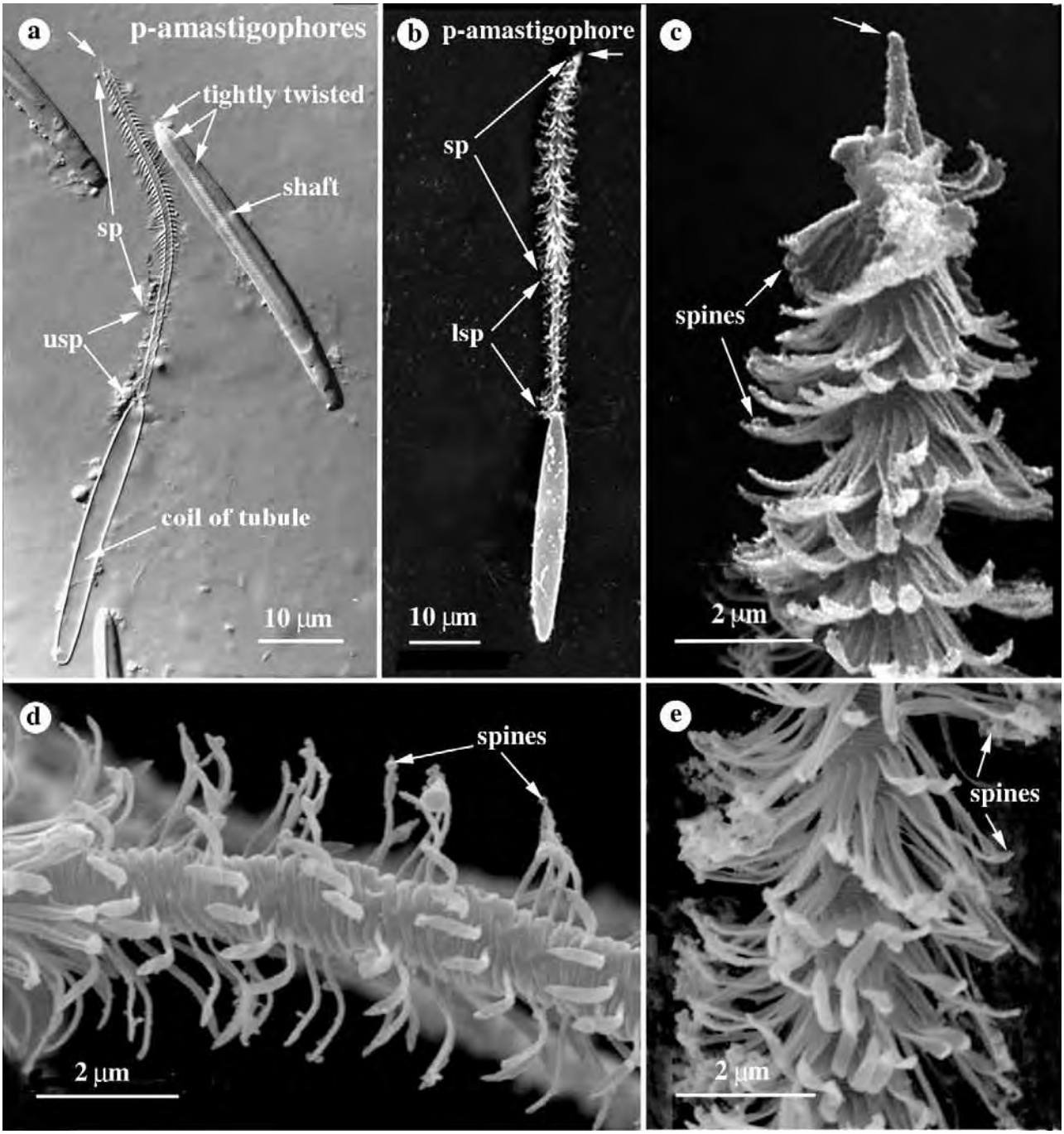

FIG. 5. – LM and SEMs of microbasic p-amastigophores. a) Discharged and undischarged p-amastigophores of *Metridium senile*. Note the undischarged twisted shaft. The more tightly twisted part of shaft (between arrows) corresponds to the unspined or loosely spined region (usp) of the discharged shaft. The remaining loosely twisted part of shaft corresponds to the heavily spined region (sp). Note coil of tubule inside dischargedcapsule. b) Discharged microbasic p-amastigophores of *Sagartiogeton viduatus*. Note the heavily spined part of shaft (sp) compared with the loosely spined part (lsp). c) Distal shaft with tip and large closely-set spines. No opening of tubule is seen at the tip. d) Part of shaft showing long loosely-set spines from the lsp-region in fig. b. Note the tight foldings on the shaft corresponding to the posterior more tightly folded part of the inverted shaft (between arrows in fig. a). e) Middle part of shaft showing large closely-set spines (from the sp-region in fig. b). Arrows point at tip of shaft; *sp,* spined region of shaft; *lsp,* region of shaft with loosely-set spines; *usp*, unspined or loosely-spined region of shaft.

The spines on the shaft are of considerably different sizes and configurations compared to the remainder spines of the tubule. In hydrozoan (Östman, 1983, 1988) and actiniarian microbasic b-mastigophores, the shaft armature consists of large, up to 3-4 μm long, closely-set spines generally of the same size (Fig. 2d). In the larger mesobasic b-mastigophores of *Metridium senile,* the spines of the shaft differ in size (Fig. 2c). The spines on the distal tubule are slender, loosely-set and seldom longer than 1 μm (Fig. 2d).

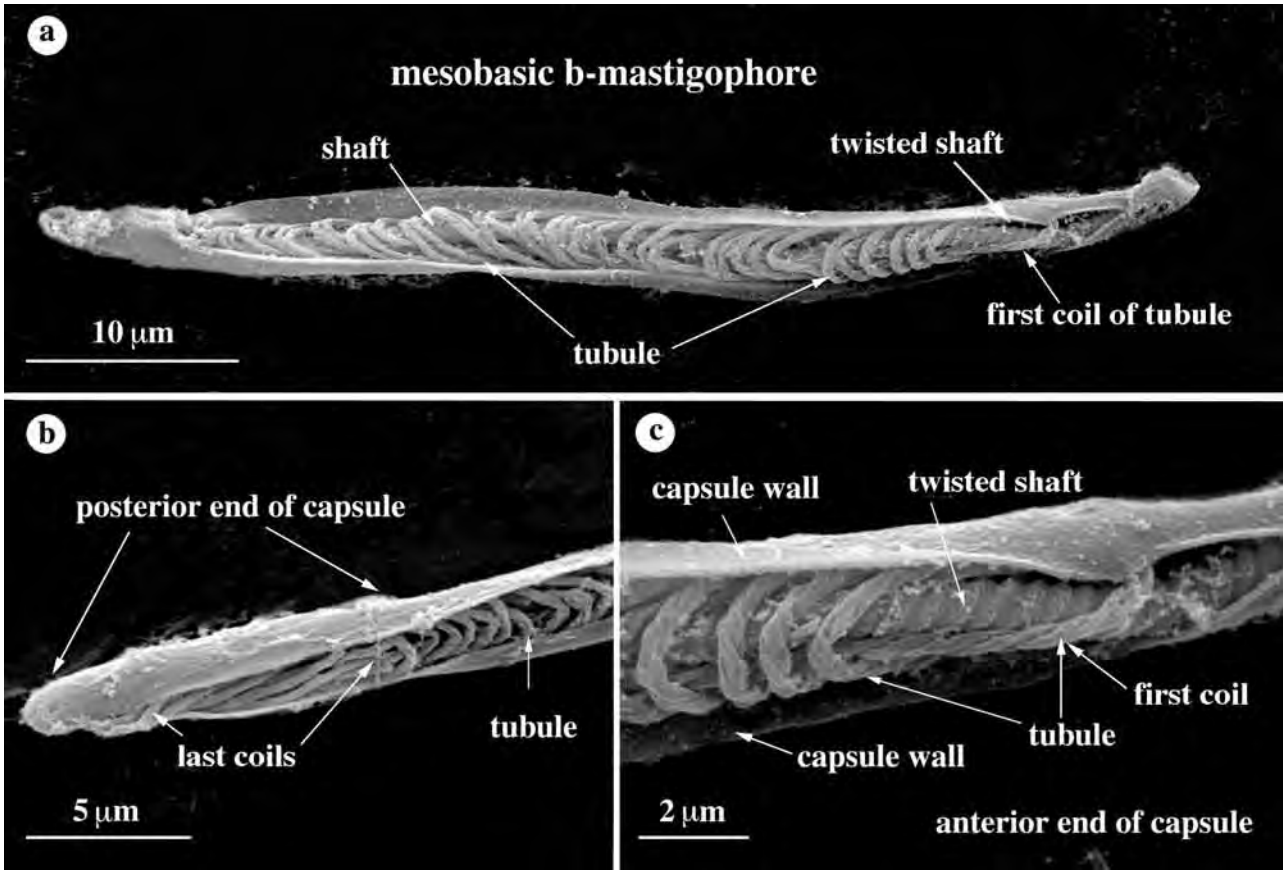

FIG. 6. – SEMs of broken capsules of mesobasic b-mastigophores of *Metridium senile* showing the twisted shaft and the coiled tubule. a) Note the regular coils of tubule around the shaft. b) Posterior end of a capsule showing the last longitudinal coils of tubule. c) Anterior part of a capsule. Note the twisted shaft and the first coil of the tubule coming from the posterior end of the shaft towards the anterior part of the capsule.

Microbasic and mesobasic amastigophores and microbasic p-amastigophores. Inverted shaft folded back within itself in unfired capsule

The p-mastigophores are by definition (Carlgren, 1940) identified by their inverted shaft, which at its end has a V-shaped notch. Nematocysts with shafts of considerably greater diameter than their distal tubules can be folded within themselves while yet encapsulated. The notch is formed where the broad, encapsulated shaft is folded back within itself (Cutress, 1955; Fig. 2b). First the shaft inverts inward into the capsule, and long shafts can then be folded back inside the shafts again. These shafts are generally broader than shafts without the V-shaped notch, which have no part of shaft folded back within themselves. Since a similar notch is present in amastigophores (Fig. 4c-f) these nematocysts can also be identified by the V-shaped notch at end of unfired shaft.

The remaining narrow, distal tubule of the p-mastigophores is attached at the end of the folded shaft nearest the capsule aperture (Cutress, 1955). Thus part of the inverted distal tubule is within the lumen of the folded shaft. Depending on its length, the remainder of the distal tubule is loosely coiled posteriorly outside the shaft (Fig. 2b).

The amastigophores have, by definition (Carlgren, 1940; Mariscal, 1974), no distal tubule and only a pointed shaft (Figs 4a, 5a,b,c). As in the p-mastigophores, inverted shafts of amastigophores are folded back within themselves forming the V-shaped notch at their base (Cutress, 1955). According to Cutress, these nematocysts have a distal tubule at least when undischarged. This short and very thin distal tubule is difficult to see and is easily overlooked (Fig. 4c). As in the p-mastigophores, the distal tubule of the amastigophores is attached inside the folded shaft at its end nearest the capsule aperture. The remainder of the distal tubule might be faintly visible. It can be straight or making few irregular coils or loops posterior to the shaft and ending attached to the posterior inner part of the capsule (Fig. 4c).

Since undischarged amastigophores are equipped with a tubule, Cutress (1955) suggested that the category amastigophores should be eliminated and amastigophores should be merged into the category of p-mastigophores.

Upon discharge, however, the distal tubule of amastigophores seems to break off at or close to the attachment to the shaft (Cutress, 1955), and coils of thin tubule are sometimes visible within the otherwise evacuated capsule (Fig. 5a). The everted cylindrical, broad shaft narrows abruptly to a pointed tip (Fig. 5a-c) or to a tiny, short tubule when parts of the distal tubule are everted along with the shaft (Fig. 4a, arrow).

Cutress (1955) also found that some p-mastigophores had a shaft divided into one or more darts and classified these nematocysts as q-mastigophores, although Hand (1961) and England (1991) regarded the darts as artefacts of spines, which were broken off when shaft everted. Schmidt (1974) noted darts and dart-like structures and regarded them valid. In the present investigation some dart-like structures were observed in SEM preparations of acontia from *Metridium senile* (Fig. 2e).

Stenoteles, euryteles and birhopaloids

The inverted shafts of hydrozoan stenoteles are broad, straight and folded back within themselves (Fig. 1d; cf. Tardent, 1988; Östman *et al.*, 1995). The large stylets pointing toward the apex of the capsule and the smaller lamellae or spines of the shaft are readily seen inside the inverted shaft (Fig. 1d). The remaining tubule is irregularly coiled posterior to the shaft (Tardent, 1988) or coiled from wall to wall perpendicularly to the capsule axis in siphonophoran stenoteles (Fig. 1d). When everted, the pointed stylets emerge from the capsule first followed by closely set lamellae or spines (Fig. 1f; Tardent, 1988).

Scyphozoan euryteles (Fig. 3f) and a new type of birhopaloid (type II, Fig. 3e), recently identified in the scyphomedusae *Cyanea* spp. (see Östman, 1997; Östman and Hyman, 1997), *Cotylorhiza tuberculata* and *Cassiopeia xamachana*, have broad and prominent shafts. It has not yet been investigated if the shafts are folded back within themselves. The distal tubules of the euryteles and the birhopaloids make the first loop towards the aperture of the capsule directly after the end of the shaft (Östman and Hyman, 1997).

Systematic value of nematocysts

Anthozoan species have different cnidomes compared to medusozoan (hydrozoan, schyphozoan and cubozoan) species. Only two categories of cnidocysts, the isorhizas and the microbasic b-mastigophores, are present throughout the cnidarian classes (Shostak and Kolluri, 1995). The common actiniarian nematocysts, the p-mastigophores and the amastigophores (Fig. 2), are not present in Scyphozoa. Hydrozoa shares only the p-mastigophores with Siphonophora (Fig. 1e). The large, narrow, elongated nematocyst capsule is also specific for the sea anemones (Fig. 2). These capsules have no lid and three flaps close their aperture. A thickened ridge or flange surrounds the aperture at the junction of the capsule wall with the tubule (Cutress, 1955).

Hydrozoa and Scyphozoa are regarded as more closely related to each other than to Anthozoa and may be merged in the subphylum (or superclass) Medusozoa along with Cubozoa, presumably derived from Scyphozoa. The nematocysts of Hydrozoa and Scyphozoa are also more similar to each other than to those of Anthozoa. The capsules of these medusozoans have a lid, are often broad and rounded to sub-spherical (Fig. 1a,b), and are seldom of the narrow, elongated shape of nematocyst capsules in sea anemones (Fig. 2).

Euryteles, birhopaloids and isorhizas are common nematocysts in hydrozoans as well as in scyphozoans. Microbasic b-mastigophores, which are common in Hydrozoa are, however, only present in cubozoan medusae and not in scyphozoan medusae. The cubozoan are also reported to have the common p-mastigophores of the anthozoans (Rifkin and Endean, 1983). Desmonemes and stenoteles are specific for Hydrozoa (Bouillon, 1985; Tardent, 1988; Östman *et al.*, 1991, 1995). No category of nematocyst is limited specific to Scyphozoa or Cubozoa.

Differences in the cnidomes have taxonomic value at the species level (Gravier-Bonnet, 1987; Östman, 1982, 1988). For more than a century, cnidarian systematists have recorded information on the size and distribution of nematocysts. Carlgren (1940) pointed out that the size of the cnidae is of systematic value and that no description of a species is complete unless it includes annotation on the size of nematocysts. The sizes of cnidocysts are especially valuable for distinguishing those species, which have the same cnidome (Östman, 1979a,b; Östman and Hyman 1997).

Nematocysts also differ in size within the animal (Carlgren 1940, 1945; Schmidt 1974; Östman and Hyman, 1997) and some are large in animals of larger size (Östman and Hyman, 1997). The sizes of nematocysts differ in specific organs and parts of organs. The same nematocyst type can appear in several different size classes present in the same organ or in different organs of the same animal. The b-mastigophores within *Metridium senile,* for example, can differ by up to 50 μm (Fig. 2a). Furthermore, young animals frequently possess categories of nematocysts that are absent in older animals (Kubota, 1978a,b; Östman *et al*., 1995) and animals in different stages of metagenesis (their life-cycle) have different cnidomes. This is the case, for example, in planulae, scyphistomae and medusae of Scyphozoa (Calder, 1971, 1972, 1974, 1977, 1983; Östman, 1997; Östman and Hyman, 1997), the actinulae and polyps of *Tubularia larynx* (see Östman *et al*., 1995), and for the colony and medusa of *Clytia hemispherica* (see Östman, 1979a,b). To be of value for taxonomic diagnosis, therefore, measurements must be taken from all the major types of nematocysts present in a species throughout its life cycle, and in different organs and parts of the same animal.

DISCUSSION

The criteria for identifying different nematocyst types proposed by Weill (1934) have been enormously influential on cnidarian scientists; although the nomenclature for basic nematocyst categories has altered, new categories have been added (Carlgren, 1940; Cutress, 1955, Bouillon *et al*., 1986), and other modifications have been made (Carlgren, 1940; Cutress, 1955; Mariscal, 1974; Calder, 1974; Östman, 1988; Östman and Hyman, 1997). Due to the improvement of the light microscope and the advent of SEM, some additional modifications ought to be made. This is most simply accomplished by retaining as far as possible the descriptive nomenclature of basic categories while adjusting definitions to suit new data.

Proposed changes in definitions of extant nematocyst nomenclature

Isorhizas and anisorhizas

Small spines, of only a few micrometers in length, were not observed by Weill (1934) and earlier nematocyst workers. Atrichs and basitrichs of Weill have later been found to be spined throughout the length of their tubules (Cutress, 1955; Westfall, 1966a,b; Calder, 1974; Heeger *et al*., 1992; Östman, 1983). Calder (1974) suggested changing Weill's terminology of the a-atrichs and A-atrichs to a-isorhizas and A-isorhizas. Some isorhizas, however, are spineless and can thus be regarded as atrichous isorhizas (Östman, 1982). Moreover, during part of their development, some isorhizas are spineless.

The definition of basitrich as 'nematocysts with a tube of uniform diameter, which bears coarse armature proximally for a distance less than three times the capsule length' is valid for the basal spines of some isorhizas and anisorhizas (see Cutress, 1955). Some isorhizas within the family Campanulariidae (Östman, 1982) and basitrichs of sea anemones (Cutress, 1955) can be referred to as basitrichous isorhizas.

The distinction between the anisorhizas and isorhizas is not clear, however (see Cutress, 1955; England, 1991; Östman *et al*., 1995; Östman and Hyman, 1997 for thorough discussion). Rather than being of uniform diameter throughout, the tubules of isorhizas may gradually become more slender towards the distal end. Isorhizas, therefore, are better defined as nematocysts whose tubule proximal to the mid-region is of constant or nearly constant diameter (Östman and Hyman, 1997).

Microbasic and mesobasic b-mastigophores

A distinct shaft is not present in all discharged b-mastigophores in species of hydrozoans (Östman, 1983, 1987) and in sea anemones (Fig. 2d), contrary to the assertions of Carlgren (1940) and Mariscal (1974). The difference in diameter of proximal and distal tubule of b-mastigophores is often very small or non-existent (Cutress, 1955; Östman, 1979a,b, 1987, 1988). Cutress (1955) remarked that the difference between the diameter of the everted 'shaft' and of the distal tubule of b-mastigophores could be as small as 0.1 μm. The proximal armature, however, is prominent and clearly visible in LM (Fig. 2c) compared to the unspined or loosely spined remaining tubule (Fig. 2d).

Differences in the relative diameters of the proximal and distal tubule cannot always be ascertained in the LM when a proximal tubule is armed with closely set spines. Thus, most discharged b-mastigophores can only be identified by their proximal armature, although in undischarged b-mastigophores, the proximal tubule is distinguished

from the remaining distal tubule by a straight, rod-shaped structure (Fig. 2a). The diagnostic criteria for the b-mastigophores should include, in addition, differences between the prominent proximal armature and smaller spines on the distal tubule or the spineless tubule (Fig. 2d).

The distinction between the basitrichs and the b-mastigophores made by England (1991) is also blurred following eversion. The basitrichs are more readily distinguished from the b-mastigophores when undischarged (cf. Cutress, 1955). The tubule of basitrichs is completely coiled proximally (Cutress, 1955), whereas that of b-mastigophores is uncoiled basally, forming the rod-shaped proximal tubule. The distinction between b-mastigophores and basitrichs is further obscured, because the first coil of some basitrichs is large enough to resemble the rod-shaped proximal tubules of b-mastigophores, whereas some thin, short-shafted b-mastigophores coil much like the tubules of basitrichs (cf. Cutress, 1955).

Microbasic p-mastigophores and micro- and mesobasic p-amastigophores

The shaft in undischarged amastigophores has a V-shaped notch resembling that of an unfired p-mastigophores (Carlgren, 1940) (Fig. 2b, 4c). The broad, invaginated shaft forms the V-shaped pattern when it is folded back within itself (Cutress, 1955). Contrary to Cutress (1955), who merged the amastigophores into the p-mastigophores, the amastigophores are recognised in the present work as a valid category, even while acknowledging that a tiny tubule is visible in the undischarged capsule (Fig. 4c). The prefix 'p' is thus added before 'amastigophore' to indicate the similarity in the structure of the shaft with the p-mastigophores. The category amastigophore is thus changed to p-amastigophore. Undischarged p-mastigophores and p-amastigophores are difficult to separate in sea anemones, since the tubules of some of the p-mastigophores are mostly invisible, but, following discharge, p-amastigophores are readily distinguished from the p-mastigophores due to the absence of a tubule beyond the shaft (Fig. 4a).

Microbasic and mesobasic

England (1991) proposed the term mesobasic for shafts that had a tightly twisted part corresponding to the folded portion described by Schmidt (1969, 1972, 1974). Following discharge, the mesobasic shaft was less than four times the capsule length.

Defining the degree of shaft folding in small and medium-sized nematocysts can be problematic, but 'mesobasic' is easily defined exclusively to terms of shaft length. Accordingly, mesobasics are redefined as nematocysts whose shafts are slightly longer than one and a half time but less than four times the capsule length. Shafts longer than one and a half time the capsule length when discharged are generally more tightly twisted proximally (corresponding to the folded portion of Schmidt, 1969) and slightly undulating or coiled when undischarged (Fig. 4e,f). Undischarged microbasic shafts can often be distinguished from mesobasic shafts by their rod-shaped straight, short shaft (Fig. 4c,d).

Nematocyst nomenclature and classification

Stephenson (1929) began the modern effort to systematise nematocyst nomenclature. He placed p-mastigophore and amastigophore nematocysts together and named them 'penicilli'. The basitrichs and some microbasic b-mastigophores were given the name 'spirule'.

Weill's (1934) nomenclature was, however, generally accepted by cnidarianists, and considerable effort was spent bringing cnidocysts described in the old literature into Weill's scheme (Shostak and Kolluri, 1995) even if it did not solve all the problems of classification. Weill (1934) had subdivided the nematocysts into two main groups: the stomocnidae (nematocyst with tubule open at tip) and astomocnidae (nematocysts with tubule closed at tip). The majority of penetrating nematocysts were assigned to the stomocnidae. Generally nematocysts whose tubules entangle prey were assigned to the astomocnidae (Weill, 1934; Mariscal, 1974).

Werner (1965) doubted the reliability of observations on tubule tips, and Rifkin (1996) stated that some penetrating nematocysts assigned to the stomocnidae had closed tips when fully discharged, while droplets of capsular matrix ruptured tubules. The tip of discharged p-amastigophores of sea anemones examined in the present study seemed also to be closed (Fig. 5a,c). Furthermore, the large A-isorhizas of *Cyanea* spp. entangle prey and were apparently not penetrators (Östman and Hyman, 1997), although isorhizas were assigned to the stomocnidae. Thus, if the categories of astomocnidae and stomocnidae were to be retained for identifying nematocysts, their definitions would require modification (see below).

Schmidt (1969, 1972, 1974) also introduced a rational nomenclature, but it caused confusion by using synonyms for nematocysts already well known in other nomenclatures. For example the b-rhabdoids of Schmidt are virtually the same as the microbasic b-mastigophores of Weill (Östman, 1987). The pseudo-microbasic b-mastigophores (Östman, 1989) can also be merged with microbasic b-mastigophores. The polyspiras of Spangenberg (1965) are certainly one type of isorhiza (Östman and Hyman, 1997).

Cnidocyst nomenclature would seem best served by modifying the definitions of widely used terms in accordance with descriptions from modern LM and SEM of the everted tubule with its pattern of spines as well as the form of tubule-coiling within the unfired capsules. The classification system of Mariscal (1974) based on the nomenclature of Weill (1934) is most easily changed to accommodate these modification.

The desmonemes are the only astomocnidae examined by the author (Östman *et al*. 1991, 1995). The main concern of this work is thus the nomenclature of nematocysts assigned to the stomocnidae, which have been examined by the author during previous and the present study.

Modified classification based on Mariscal (1974)

I. ASTOMOCNIDAE: Tubule closed at tip; generally entangles prey.
  Desmonemes: tubule forms a corkscrew-like coil. Few coils of tubule visible in undischarged capsule.
II. STOMOCNIDAE: Most tubules open at tip; mainly penetrants.
  A. HAPLONEMES: no prominent, rod-shaped shaft visible inside undischarged capsule.
    1. Isorhizas: tubule isodiametric or nearly isodiametric proximal to the mid-point and tapering thereafter.
      a. Atrichous or holotrichous: tubule unspined or armed with tiny spines throughout.
      b. Basitrichous: prominent large spines close to capsule. Remaining tubule unspined or armed with tiny spines.
    2. Anisorhizas: tubule slightly dilated towards base.
      a. Atrichous or holotrichous: tubule unspined or armed with tiny spines throughout.
      b. Basitrichous: prominent large spines close to capsule. Remaining tubule unspined or armed with tiny spines.
  B. HETERONEMES: prominent shaft visible inside undischarged capsule.
    B1. Microbasic: discharged shaft or proximal tubule with prominent armature short, less than one and a half times capsule length.
    B2. Mesobasic: discharged shaft or proximal tubule with prominent armature medium-sized, more than one and a half times longer but shorter than four times capsule length.
    B3. Macrobasic: discharged shaft or proximal tubule with proximal armature long, more than four times the capsule length
      1. Rhabdoids: inverted shaft rod-shaped, everted tubule with prominent spines generally of the same size.
        a. Mastigophores: tubule continues beyond shaft or proximal armature.
          (a) p-mastigophores: V-shaped notch at base of unfired, broad shaft; discharged shaft tapers abruptly into tubule.
          (b) b-mastigophores: No V-shaped notch at base of unfired, narrow shaft; discharged shaft or proximal tubule approximately the same diameter as remaining tubule.
        b. Amastigophores: no tubule beyond everted shaft;
          (a) p-amastigophores: V-shaped notch at base of unfired shaft (changed terminology.
      2. Rhopaloids: shaft of unequal diameter
        a. Euryteles: discharged shaft dilated distally.
          (a) Homotrichous: spines of shaft all of the same size.
          (b) Heterotrichous: spines of shaft of unequal size.
        b. Stenoteles: discharged shaft dilated at base, large spines at point of constriction between basal and distal part.
          (a) Stenoteles proper: three stylets or especially strong spines at constriction, distal portion armed by rows of lamellae or spines.
          (b) Pseudostenoteles: two to four large spines at constriction, distal portion of shaft long, armed with smaller spines; sometimes also with a few large ones (new category, Boullion *et al*., 1986).
        c. Birhopaloids: discharged shaft with one distal and one proximal dilatations.
          (a) Birhopaloides type I. The two dilations separated from each other.
          (b) Birophaloides type II. The two dilations

close together (new category or similar to trirophaloids of Rifkin (1996)).

III. Spirocysts: thin capsule wall, containing a long spirally coiled tubule of uniform diameter (Fig. 4d). No shaft or spines distinguishable.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Kinematic comparison of bell contraction by four species of hydromedusae*

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SUMMARY: Bell form strongly affects the hydrodynamic performance of swimming hydromedusae. Although the relationship between bell shape and hydrodynamic parameters has been documented for static models of different bell shapes, the dynamic differences in contraction characteristics of different bell shapes have not been described. This is an important issue in medusan motion because the way in which medusan bells contract may influence the effect of bell shape on swimming performance. We measured differences in bell morphologies and wake velocities during swimming by two prolate (streamlined) and two oblate (disc shaped) forms of hydromedusae. Our results indicate that propulsion by prolate medusae is fundamentally different than that of oblate forms. Prolate species were characterized by contraction over the entire length of the bell and produced a narrower, higher velocity jet. Oblate medusae contracted primarily near the bell margin, and produced a broader, lower velocity jet. Prolate medusae achieved higher velocities but lower Froude propulsion efficiencies than oblate medusae. The adaptive value of these patterns are interpreted in terms of foraging and life history patterns.

Key words: swiming, morphology, efficience, fluid flow, jet propulsion, foraging.

INTRODUCTION

The great variety of bell morphologies among the hydromedusae (Russell 1953, Kramp 1959) have clear hydrodynamic correlates (Daniel 1983, 1984, 1985; Colin and Costello 1996) which are related to foraging mode (Costello 1992). Medusae that sit in ambush for prey do not typically swim while capturing prey. For these species, swimming serves to change position of the medusa, either for escape or migration to a new feeding location. Rapid acceleration is crucial for escape swimming and prolate bell forms are optimally designed for rapid accelerations (Daniel 1983, Colin and Costello 1996). As a result, bell form among ambush-foraging medusae may be expected to fall towards the prolate end of the spectrum among hydromedusan bell shapes. In contrast, hydromedusae that entrain and capture prey by utilizing the currents created during swimming are more suited to oblate bell shapes. The reason for this is that an oblate form, although minimizing acceleration of the medusa's body, maximizes the acceleration of fluid surrounding the medusa's bell (Daniel 1983, 1985; Colin and Costello 1996). It is this fluid, known as the added-mass of the medusa's bell, that contains the prey that are sieved through the tentacles hanging in the wake created by the medusa. By maxi-

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mizing the volume of their wake, oblate medusae maximize the volume of fluid filtered for prey (Colin and Costello, 1996).

Although there is clear evidence that static models of different shapes affect hydrodynamic variables such as drag and the acceleration reaction (Daniel, 1985), the possibility that bell forms have different dynamic traits has remained unexplored. Specifically, we wanted to address the possibility that medusae of different bell forms contracted their bells differently. In order to resolve this question, we examined two representative prolate [*Aglantha digitale* (O.F. Muller, 1776), *Sarsia* sp. (probably *Sarsia apicula* (Murbach and Shearer, 1902), but also frequently referred to as *Sarsia tubulosa* in the local literature (A. Brinckmann-Voss, pers. comm.)] and oblate [*Aequorea victoria* (Murbach and Shearer, 1902), *Phialidium gregarium* (A. Agassiz, 1862)] species from a guild of co-occurring medusan predators which are seasonally abundant in the waters at Friday Harbor, WA, USA.

In order to evaluate the effect of differences in bell contraction patterns on swimming, we accompanied bell motion measurements with a measure of the energetic efficiency of swimming by calculating the Froude propulsion efficiency (Fr_p). A highly efficient propulsive mechanism yields an increase in the velocity of a swimmer equivalent to that of the water moved backwards in the wake by the act of swimming. Fr_p compares the velocity of an object with that of the objects wake (Vogel, 1994a) and provides an index swimming performance with which to compare medusae of different bell morphologies.

METHODS

Changes in bell morphology during swimming were measured for three individuals of each hydromedusan species. Swimming by the medusae was recorded using a backlit optical system (Costello and Colin, 1994). Medusae were observed while swimming freely in 0.22 um filtered seawater within rectangular vessels ranging in dimensions from 4.5 x 8.0 x 2.0 cm to 25.5 x 30.5 x 18.5 cm (width x height x depth) and volumes from 50-14,000 ml. Vessel choice depended upon medusa diameter. *Artemia salina* eggs were used as tracers of fluid movements within the vessels. Particle motions were used to determine the velocity of the jet produced by the contracting medusae.

Cross-sectional measurements were made at five different points along the oral-aboral axis of the bell to determine the differences in contraction patterns between the oblate and prolate forms. In order to accommodate the different sizes of the various medusae, five equidistant sampling points were determined for each individual medusa based on the maximum bell height of that medusa (Fig. 1). As a medusa's bell deformed during the pulsation cycle, the locations of the cross-sections maintained their relative spacing with reference to the bell margin. Changes in cross-sectional dimensions of each of the five bell locations were measured throughout one complete bell pulsation cycle for three individuals of each species of hydromedusae. Measurements of cross-sectional widths were made with image analysis software (Optimas Corporation). A

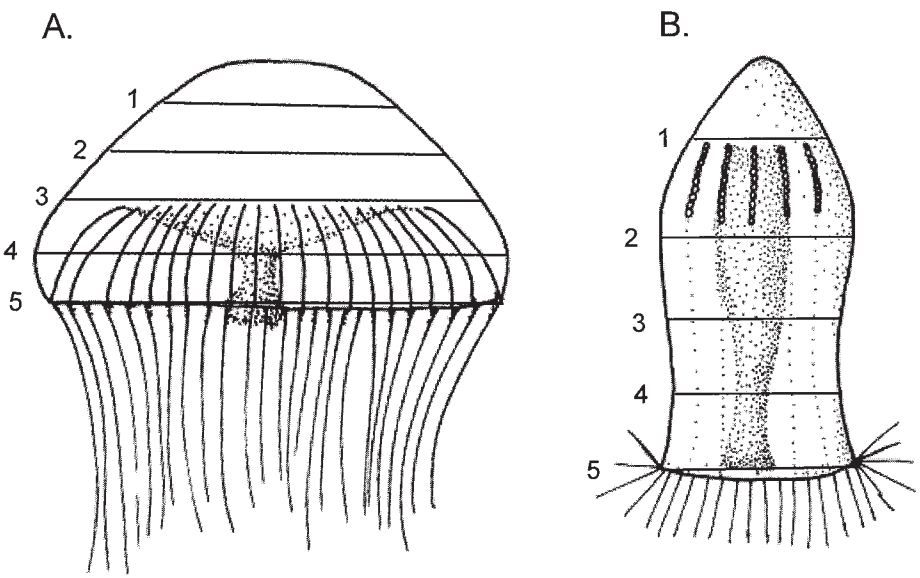

FIG. 1. – Representative cross-sectional sample locations along the bells of (A) *Aequorea victoria* and (B) *Aglantha digitale*.

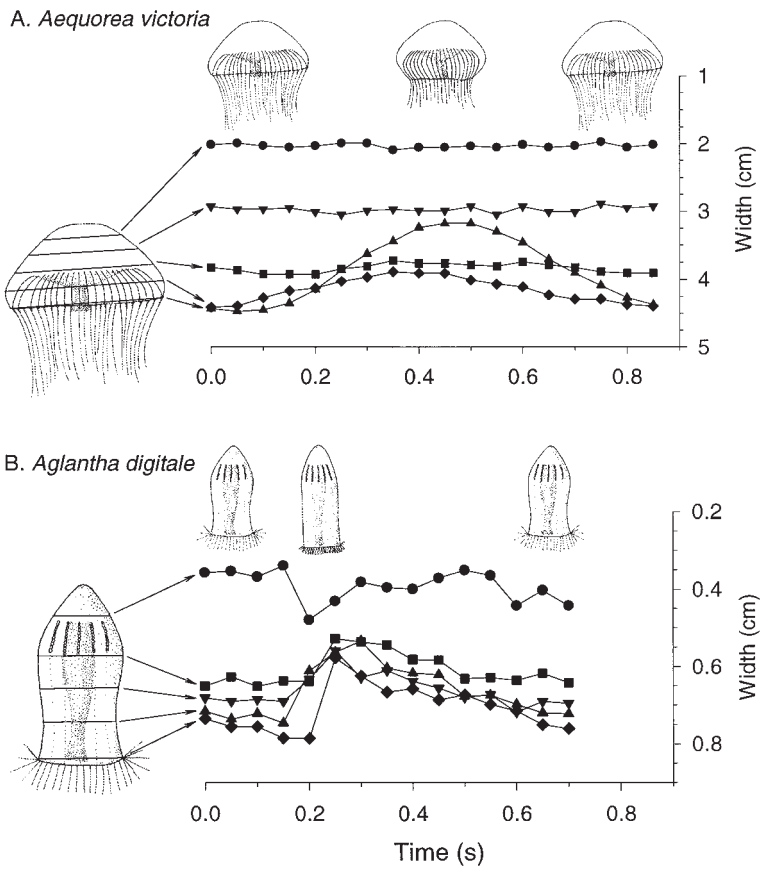

FIG. 2. – Typical cross-sectional widths (cm) during pulsation cycle for the hydromedusae (A) *Aequorea victoria* and (B) *Aglantha digitale*. Cross-sectional widths were measured every 0.33 sec during swimming at five evenly spaced locations along the height of the bell. Numbers along the medusa's bell indicate the position above the bell margin (cm) of each cross section.

metric scale was included within videotaped sequences in order to allow spatial calibration of the video sequences. Motion only within the two-dimensional viewing field was assured by using a sequence in which bell orientation was level and the medusa swam from bottom to top of the viewing field.

Froude propulsion efficiency (Fr_p; Vogel, 1994a) measures the energetic efficiency of thrust production by an organism or vehicle and was employed in order to compare swimming efficiencies of medusae possessing different bell forms. A medusa's velocity was measured as the rate of change in the position of the anterior point of the bell during the contraction phase of the pulsation cycle. The tip of *Aglantha*'s bell was sometimes difficult to locate precisely due to its transparency, so change in position of the bell margin was used to measure medusan velocity for this species. Jet velocity was determined by tracking particles (6-8 for each medusa) ejected through the velar aperture at the beginning of the contraction phase. Fr_p was calculated by comparing the medusa's body velocity (V_m, cm sec^{-1}) with the velocity of the jet expelled by the medusa (V_j, cm sec^{-1}), where:

$$Fr_p = \frac{2V_m}{V_j + V_m} \times 100$$

Fr_p was measured for 10-14 individuals of each species of hydromedusae.

Statistical analysis (Statistica, Statsoft Inc.) relied upon ANOVA of Fr_p and changes in cross-sectional dimensions during bell contraction. Differences between cross-sectional samples within a species were tested using Tukey's honest significant difference test (Tukey's HSD) and a value of a=0.05 was used as the critical value determining statistical significance.

RESULTS

Prolate medusae contracted their bells differently during swimming than did oblate medusae. Figure 2 illustrates representative examples of bell shape

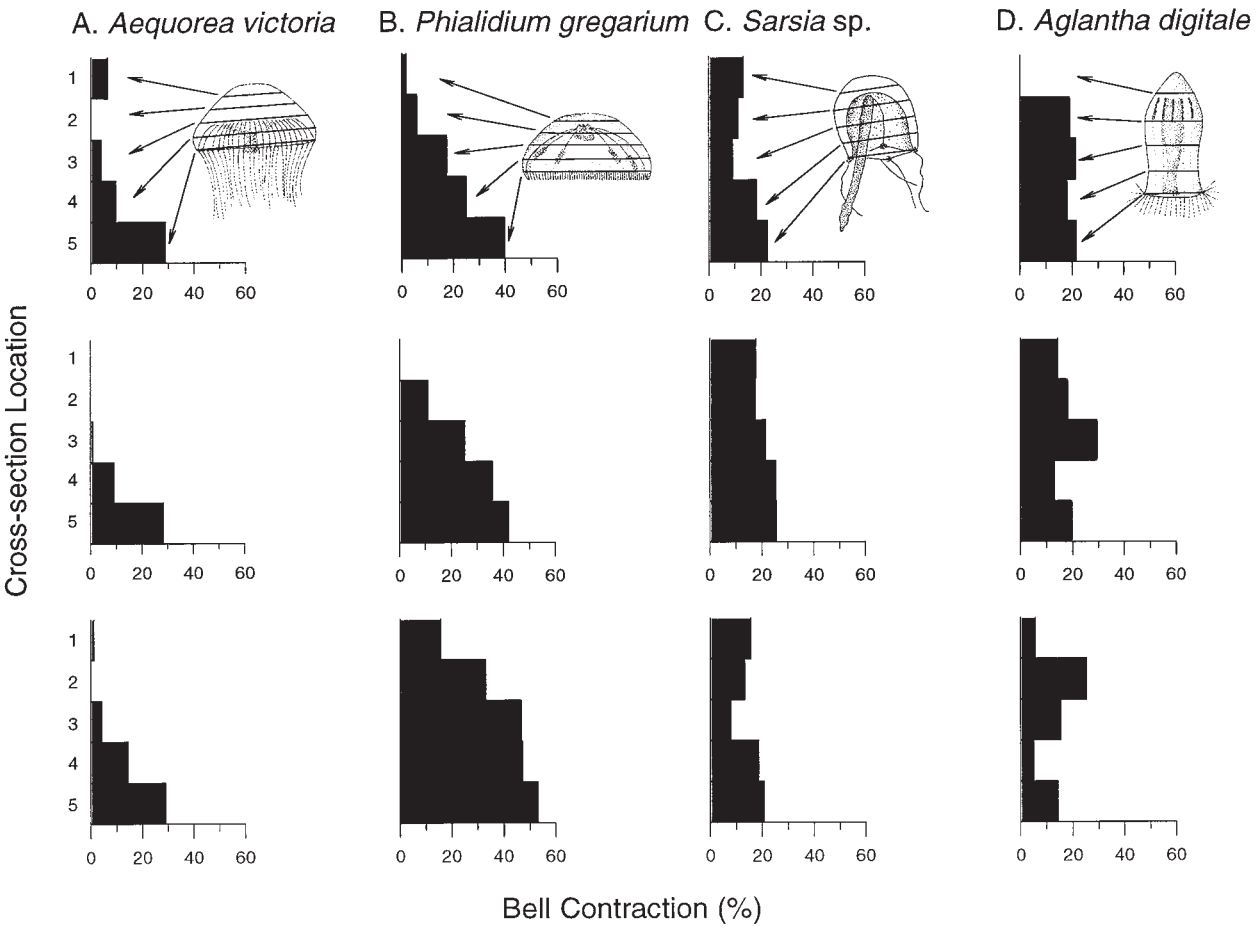

FIG. 3. – Percent contraction relative to resting bell width at different locations along the bells of (A) *Aequorea victoria,* (B) *Phialidium gregarium,* (C) *Sarsia* sp. and (D) *Aglantha digitale*. These values represent the total percent change in bell cross section that occurred over the course of bell contraction. Contraction lengths have been scaled to bell diameter for each individual hydromedusa.

alterations during swimming by *Aglantha digitale* and *Aequorea victoria*. Cross-sectional dimensions of *Aglantha*'s bell decreased essentially uniformly along the length of the bell during contraction. The anterior tip of the bell was an exception to this pattern because it appeared to bulge slightly as the rest of the bell contracted to its narrowest dimensions. In contrast, cross-sectional dimensions demonstrated that *Aequorea*'s bell contracted almost solely at the bell margin. Bell cross-sectional dimensions varied most nearest the bell margin but there was little or no change at the remaining three sampling locations that were successively more distant from the bell margin. Measurements of these individuals indicated that the jet expelled by *Aglantha* was created by a contraction that was evenly distributed along the full length of the bell while *Aequorea* used only the portion of the bell closest to its margin.

Further comparisons within and between medusan species required standardization of dimensional measurements for individuals of different sizes. For this reason, the change in width of each bell cross-section during bell contraction was expressed as a percentage of the relaxed (prior to contraction) bell width for that cross-section. This conversion from absolute lengths to a non-dimensional percentage allowed statistical comparisons of morphological changes by different size individuals of one species as well as between different species with divergent bell morphologies.

Comparisons of standardized cross-sectional data of medusae confirmed that bell contraction patterns differed significantly between genera and location along the bell (ANOVA, $p < 0.001$ for both variables). Although contraction patterns showed some intraspecific variation (Fig. 3), the average percent contraction (Fig. 4) was not significantly different for different locations along the bells of *Aglantha* (ANOVA, $p=0.088$) or *Sarsia* (ANOVA, $p= 0.066$). In contrast, average percent contraction varied significantly along the bells of *Phialidium* (ANOVA, $p = 0.014$) and *Aequorea* (ANOVA, $p < 0.001$). For

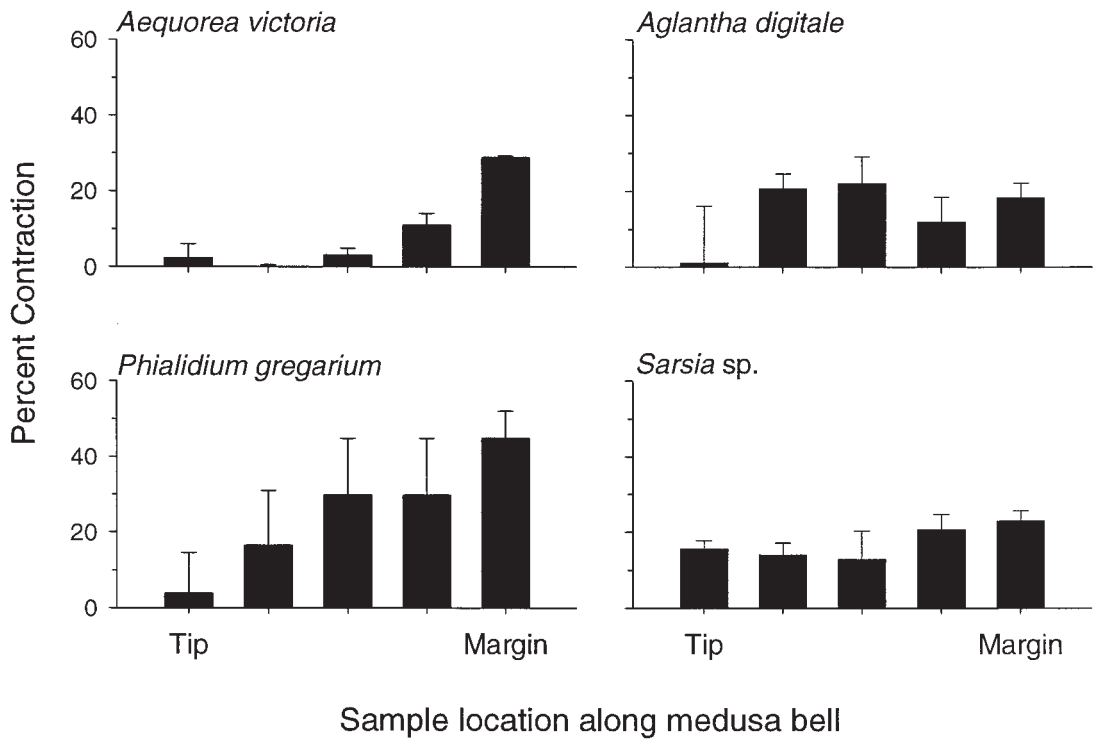

FIG. 4. – Average percent change in cross-sectional width of medusae during bell contraction. Values represent mean (±1 std dev) of three individual medusae sampled at each location spanning the bell tip to the bell margin.

both the latter species, contraction was greatest at the bell margin. However, the two oblate species differed significantly (ANOVA, $p = 0.002$) in the manner of bell contraction. The extent of contraction increased from the distal tip to the bell margin of *Phialidium*. In contrast, contraction by *Aequorea* was limited essentially to the lower portion of the bell and there were no significant differences between contraction at the bell tip and the nearest two bell sample locations. The overall bell contraction patterns indicated that contraction by the prolate genera was distributed essentially evenly along the bell length whereas oblate genera contracted primarily at the bell margin. Greater variation occurred within the oblate than the prolate species.

Froude propulsion efficiencies

The manner in which the medusae accelerate water to generate thrust during swimming, as measured by Froude propulsion efficiency (Fr_p), was distinctly different (ANOVA, $p < 0.001$) for prolate and oblate medusae. There were no significant differences between the two prolate genera (ANOVA, $p > 0.999$, Fig. 5) or the two oblate genera (ANOVA, $p > 0.999$, Fig. 5). However, the oblate (Fr_p avg. = 97%) medusae had significantly higher efficiencies

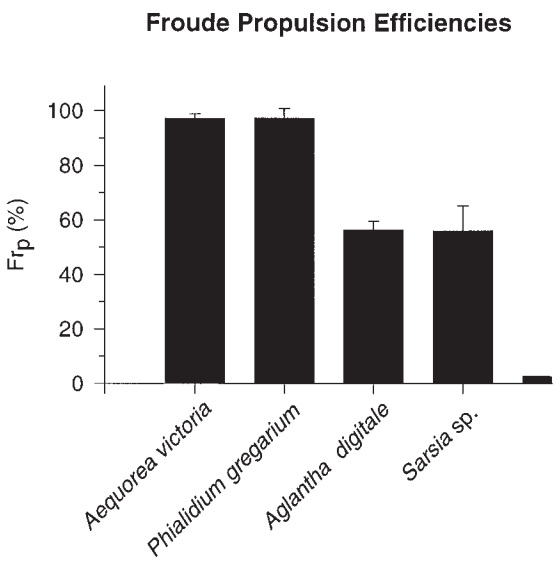

FIG. 5. – Froude propulsion efficiencies (Fr_p) of hydromedusae. Bars represent the mean of between 10-14 individuals of each species, error bars indicate ±1 standard deviation about the mean.

(ANOVA, $p < 0.001$) than did the prolate (Fr_p avg. = 56%) medusae.

DISCUSSION

Oblate and prolate shaped medusae function differently and these differences are expressed both in the mechanical nature of bell pulsation and in swim-

ming performance. Prolate medusae such as *Aglantha digitale* and *Sarsia* sp. contract the entire bell rapidly and essentially evenly along the full bell length, producing a high velocity, narrow stream of water jetting through the velar aperture and thrusting the medusa forward. The resulting accelerations of the medusa are relatively rapid (Daniel, 1983; Costello and Colin, 1996). However, from an energetic perspective, the process is inefficient by comparison to that of the oblate genera. The high velocity jet is energetically costly because the energy expended to accelerate a fluid increases as the square of the increase in velocity (Vogel, 1994a). Therefore, each incremental increase in the velocity of the jet costs an exponential increase in energy expenditure. The high cost per yield in forward motion of the medusa is expressed by the Froude propulsion efficiency, which is significantly lower for the prolate than for the oblate medusae. However, the low efficiency of jet propulsion of prolate medusae may be justified, in an evolutionary sense, by the advantages gained by rapid acceleration during escape from potential predators. Further, because they are ambush predators, swimming comprises a relatively low proportion of the time budget of these medusae (Costello and Klos, unpublished data) and, therefore, may represent a low expense in their composite energy budget.

The oblate medusae provide an informative contrast with the prolate forms. Bell contraction of *Aequorea victoria* and *Phialidium gregarium* occurs primarily near the bell margin. For *Aequorea*, the remainder of the bell moves little during contraction. The resulting stream of water ejected through the wide velar aperture is relatively wide and slow moving. The low velocities of the wake require less energy during acceleration of the fluid than high velocity wakes of prolate medusae. The wake velocities of the oblate medusae are similar in magnitude to the forward velocities of the medusan bells. The lower energy expenditure required for acceleration to these low velocities is expressed in the high Froude propulsion efficiencies of the oblate medusae. Essentially, the oblate medusae accelerate a much larger relative volume of water to a slower velocity than do the prolate forms. The lower acceleration minimizes the energy necessary to move a given volume of fluid; the larger fluid volume accelerated ensures adequate thrust to move the medusan body forward. The high efficiency and low energetic cost of this form of propulsion suits a foraging mode dependent upon continuous swimming. Further, because prey capture depends upon entrainment in the flow created during swimming, movement of larger volumes of fluid during bell pulsation contributes to the success of the cruising foraging strategy evolved by these oblate medusae. The tradeoff inherent in this swimming pattern is the loss of rapid acceleration for escape swimming. Apparently, this has not been the dominant selective force shaping evolution of oblate hydromedusae.

Rather than being simply isolated traits of variably shaped medusae, we suggest that the differences in bell contraction and propulsive efficiencies reflect the evolution of different propulsive systems by oblate and prolate medusae. Prolate medusae use their entire bell to contract a clearly bounded fluid-filled chamber and expel that fluid through a narrow orifice. This propulsive system is a classical example of the jet propulsion most commonly associated with medusae (Daniel, 1983; Denny, 1993; Vogel, 1994a, 1994b). Such propulsive systems are typified by low Froude propulsive efficiencies (Vogel, 1994a). By comparison, the subumbrellar cavities of oblate hydromedusae are less clearly bounded by their wide velar apertures (Costello and Colin, unpublished data). The relative importance of jet production for propulsion of these medusae has not been definitively measured. However, flapping of the bell margin during contraction clearly contributes significantly to wake production by large oblate medusae. Flows created by entraining fluid adjacent to the bell margin carry prey items into the tentacle mass trailing the swimming medusae. Propulsion by flapping a broad, flattened bell in this manner is more consistent with a drag-based propulsive system which creates thrust by pushing against a fluid (Denny, 1993). The resistance of the fluid to shear by the flapping body generates an opposite reactive force, thrust, which moves the medusa forward.

The advantages of this propulsive mode for oblate medusae are twofold. First, the flow generated during bell contraction entrains and transports prey to the medusa's tentacles. Swimming thus becomes an integral part of foraging. Second, oblate morphologies can result in substantial hydrodynamic advantages compared to prolate forms for medusae which are cruising predators. The most extreme examples of this coupling of oblate morphology with a cruising foraging mode occur in the scyphomedusae. Pelagic representatives of this cnidarian class have no velum, and thus no orifice for jet propulsion, and depend completely on flows produced during essentially continuous swimming

(Costello *et al*., 1998) to carry prey to capture surfaces (Costello and Colin, 1994, 1995).

Whereas all pelagic scyphozoan classes exhibit drag-based propulsion coupled with a cruising foraging mode, the proportion of hydromedusae utilizing this method of propulsion appears to be low. Most adult hydromedusae are relatively small (<3.0 cm bell diameter) and large, oblate forms appear to have arisen primarily within the Leptomedusae (Costello and Colin, unpublished, based on Mayer, 1910). Thus, as a group, the hydromedusae are probably dominated by jet propulsion and the drag-based propulsion of large, oblate genera is less common. The ecological consequences of these morphological and functional patterns are significant and affect patterns of prey selection (Mills pers comm.; Costello and Colin, unpublished data). These interrelationships will need to be elaborated in order to fully understand the role played by hydromedusae in their natural environment.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Morphology and distribution of a deep-water Narcomedusa (Solmarisidae) from the northeast Pacific*

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SUMMARY: Specimens of *Solmaris* sp. (Solmarisidae, Narcomedusae) with only four tentacles were collected in the northeast Pacific. The majority were collected in Canadian Pacific waters by Tucker trawl from stations outside the 500-m contour off the west coast of Vancouver Island, British Columbia, at depths of 300-700 m. Six were collected in vertical hauls at Station *P* (50ºN, 145ºW). The oral stomach wall contains three prominent rings of tissue. There is a peripheral ring of aggregates of gonadal tissue. Nematogenic areas with three types of nematocysts form an intermediate ring closer to the mouth than the gonads, and they also overlie the gonadal position where nematogenic tissue extends out to the base of each tentacle. Immediately surrounding the mouth, the epithelium of the oral stomach wall is greatly thickened, and this central ring contains large numbers of secretory cells.

Key words: nematocysts, *Solmaris,* Hydrozoa, distribution, anatomy.

INTRODUCTION

The inshore fauna of the Hydrozoa (medusa stage) from Puget Sound and off British Columbia is fairly well known (summarized in Arai and Brinckmann-Voss [1980] and Mills [1987]). However, there are few published data on the deep-water fauna from Canadian and adjacent waters of the northeast Pacific Ocean. A recent book by Wrobel and Mills (1998) has included some data on deep-water forms of the region in a guide to the west coast of North America from Baja California to Alaska. Since 1980, collections in Canadian Pacific waters (within the 200-mile limit) have provided specimens of a number of deep-water species of coelenterates (Fulton *et al.,* 1982; Arai *et al*., 1993), and a series of papers on this fauna has been started. A paper on the Anthoathecatae and Tiarannidae was recently published (Brinckmann-Voss and Arai, 1998). The present paper describes a previously-undescribed narcomedusa, and it is expected that other papers will follow.

In spring, the most abundant narcomedusa from outer Canadian Pacific waters is a small *Solmaris* with only four tentacles. In recent years, approximately 900 specimens have been collected. The

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species is similar to *Solmaris quadrata* Bouillon, Boero and Seghers, 1991, described from seven specimens collected off Papua New Guinea. The present paper describes the known distribution, anatomy, and histology of the Canadian form.

MATERIAL AND METHODS

Collection of specimens

Most specimens were collected off the west coast of Vancouver Island, British Columbia, Canada. Of these, the majority were collected off the southwest coast in January to May of 1987 and February to March of 1988 (Arai *et al*., 1993). The study area included the La Perouse Bank area and associated slope waters. Twenty stations were sampled along two transect lines, approximately 36 km apart, laid out approximately perpendicular to the bottom contours from off Barkley Sound and Pachena Point. Each line extended from 8 to 200 km from shore, with sampling stations 10-20 km apart (see maps and individual station locations in McFarlane and Beamish [1992] and Arai *et al*. [1993]).

Discrete samples were taken at 300, 500, and 700 m at each station using a 1-m^2 Tucker trawl that opened and closed at depth. Each trawl unit consisted of three nets. When deploying this gear, the bottom net was open with the middle and top nets closed. After reaching the desired discrete depth, the middle net was opened and the top and bottom nets were closed for a tow duration of approximately 15 min at that depth. During retrieval the top net was open. The three depths were chosen for sampling (together with surface neuston tows) in order to give maximum information about sablefish larvae and associated fauna during their ontogenetic migration to the surface from a depth of approximately 1,000 m (McFarlane and Beamish, 1992). Each net of 335-μm black nitex mesh was equipped with a rigid cod end with a 335-μm screen and a flowmeter. Samples were preserved in 4 or 5% buffered sea water-formaldehyde.

Earlier, in 1980 and 1986, samples off the Canadian west coast were obtained by oblique bongo tows which did not yield discrete depth data (see maps and station locations in Fulton *et al*. [1982] and Arai *et al*. [1993]).

Canadian weatherships at Ocean Station *P* ("Papa"; 50°N, 145°W, outside Canadian waters in the northeast Pacific) were used as oceanographic sampling stations from 1956 to 1980 (Waddell and McKinnell, 1995). In addition to daily vertical hauls to 150 m, occasional vertical hauls were made to 1,200 m using SCOR, Miller, or NORPAC nets.

Histology and morphometry

Several specimens were prepared for light-microscopic examination in glycol-methacrylate sections. For photography, two specimens (4.8 and 5.0 mm diameter) were soaked in 0.1 M phosphate buffer (pH 8) to remove excess fixative, and infiltrated and embedded in glycol methacrylate, using a Historesin kit (LKB, Heidelberg, Germany). Sections (3-5 μm in thickness) were cut with glass (Ralph) knives on a rotary microtome. Sections were transferred to puddles of demineralized water on microscope slides, and they were then stretched and affixed by heating. To augment staining, sections were treated with 1% periodic acid for 10-15 min. An alcoholic solution of methylene blue and basic fuchsin was applied to the sections at room temperature (Bennett *et al*., 1976). Background staining was eliminated by dipping sections in 95% ethanol, and the sections were then allowed to air-dry thoroughly. Cover glasses were mounted with Fisher Permount.

The semithin histological sections were viewed and photographed with a Nikon Optiphot compound microscope equipped with planachromatic objective lenses and an AFX-II photomicrographic attachment. The microscope was calibrated with a stage micrometer (100 lines/mm).

Three whole specimens (4.0-4.5 mm diameter) were squashed in distilled water. Nematocyst masses were transferred to microscope slides, and supported cover glasses were added. Nematocysts were viewed and photographed with Nomarski differential interference-contrast optics.

Three types of nematocysts, initially recognized morphologically in the glycol-methacrylate sections, were also evident in the squashes. Photomicrographs of the nematocysts were digitized, and for each type, the circumferences of 25 specimens were measured, using "Sigma Scan" 3.02 software (Jandel Scientific, San Rafael, California, U.S.A.). Since all three types of nematocyst are essentially spherical in shape, each circumference (c) was treated as the perimeter of a circle. By calculating the radius (r) of a circle ($r = c/2\pi$), it was then possible to insert the radius into the formula for the volume (v) of a sphere ($v = 4\pi r^3/3$). Calculations were made in

"Quatro Pro" 6.0 software (Corel Corporation, Ottawa, Ontario, Canada), and the volumetric data were plotted with "Sigma Plot for Windows" 4.0 software (SPSS, Chicago, Illinois, U.S.A.).

RESULTS

Distribution of medusae

Off the west coast of Vancouver Island, specimens of *Solmaris* sp. have so far been collected only from stations outside the 500-m contour at latitudes between 47°57'N and 50°30'N. In 1987 and 1988, Tucker samples were collected along two lines of stations southwest of Vancouver Island as detailed in the above. Although collections off Pachena Point and Barkley Sound were made at inshore stations and across the La Perouse Bank (as well as beyond to the 1,200-m contour line), no specimens were obtained from inshore or bank stations.

Weather permitting, samples were collected at each station at the three discretely-sampled depths (300, 500, and 700 m). Table 1 presents the data from the six stations outside the 500-m contour for which 1987 data are most complete. This year is presented because, in 1988, no sample exceeded 3.1 individuals per 1,000 m^3, whereas in 1987, populations of up to 20 medusae per 1,000 m^3 were recorded (Table 1). Of the three depths, the medusae were usually most abundant at 500 m during daylight (Table 1), whereas at night, they may be more abundant at 300 m. In 1987 *Solmaris* sp. constituted about 40% of the narcomedusae collected at these stations.

In addition to the specimens from Canadian Pacific waters, six specimens were obtained from Station *P* (50°N, 145°W). These medusae were collected in vertical hauls to 1,200 m with a SCOR net, so no depth data are available for this location.

TABLE 1. – Abundance of *Solmaris* sp. at six stations off southwest Vancouver Island in 1987. For each set of tows, abundance was assessed at two or three depths.

Depth (m)	Specimens per 1,000 m^3	Date
47°57'N 126°26'W		
300	1.1	26 January (night)
500	0.0	26 January (day)
300	0.0	21 February (night)
500	9.8	21 February (night)
700	0.3	21 February (night)
300	17.8	21 March (night)
500	2.9	21 March (night)
700	0.8	21 March (night)
48°09'N 126°00'W		
300	0.7	27 January (day)
500	6.6	27 January (day)
300	0.0	21 February (day)
500	8.9	21 February (day)
700	0.3	21 February (day)
300	2.5	16 March (night)
500	1.4	16 March (day)
700	0.0	16 March (day)
48°11'N 125°56'W		
300	2.1	03 February (day)
500	1.0	03 February (night)
300	0.0	18 February (day)
500	5.6	18 February (day)
700	1.9	18 February (night)
300	0.0	20 March (night)
500	5.9	20 March (day)
700	2.8	20 March (day)
48°15'N 126°40'W		
300	0.0	25 January (day)
500	0.8	25 January (day)
300	1.8	17 February (night)
500	0.6	17 February (night)
700	2.1	17 February (day)
300	4.1	16 March (night)
500	1.9	16 March (night)
700	1.0	15 March (night)
48°22'N 126°20'W		
300	0.0	24 January (day)
500	21.8	24 January (day)
300	0.0	16 February (day)
500	14.1	16 February (day)
700	0.0	16 February (day)
300	1.0	15 March (night)
500	2.3	15 March (day)
700	0.5	15 March (day)
48°26'N 126°14'W		
300	0.0	23 January (day)
500	7.5	24 January (day)
300	1.5	16 February (night)
500	6.9	16 February (day)
300	7.8	22 March (night)
500	2.8	22 March (night)

Anatomy

Like all narcomedusae, the medusae lack radial canals. The umbrella is a somewhat-flattened, slightly-quadratic dome with a diameter in the present collections from 4 to 11 mm. Four tentacles leave the umbrella at the level of the stomach (Fig. 1A), each above a peronium so that the margin is divided into four lobes (Fig. 1A). The tentacles are solid, each with a root curving upward into the mesoglea above the stomach (Figs. 1A, 1D) and with an external length of at least 2.5 times the diameter of the bell.

The stomach is broad and circular, without pouches. The oral stomach wall of mature specimens contains a peripheral ring of aggregates of gonadal tissue

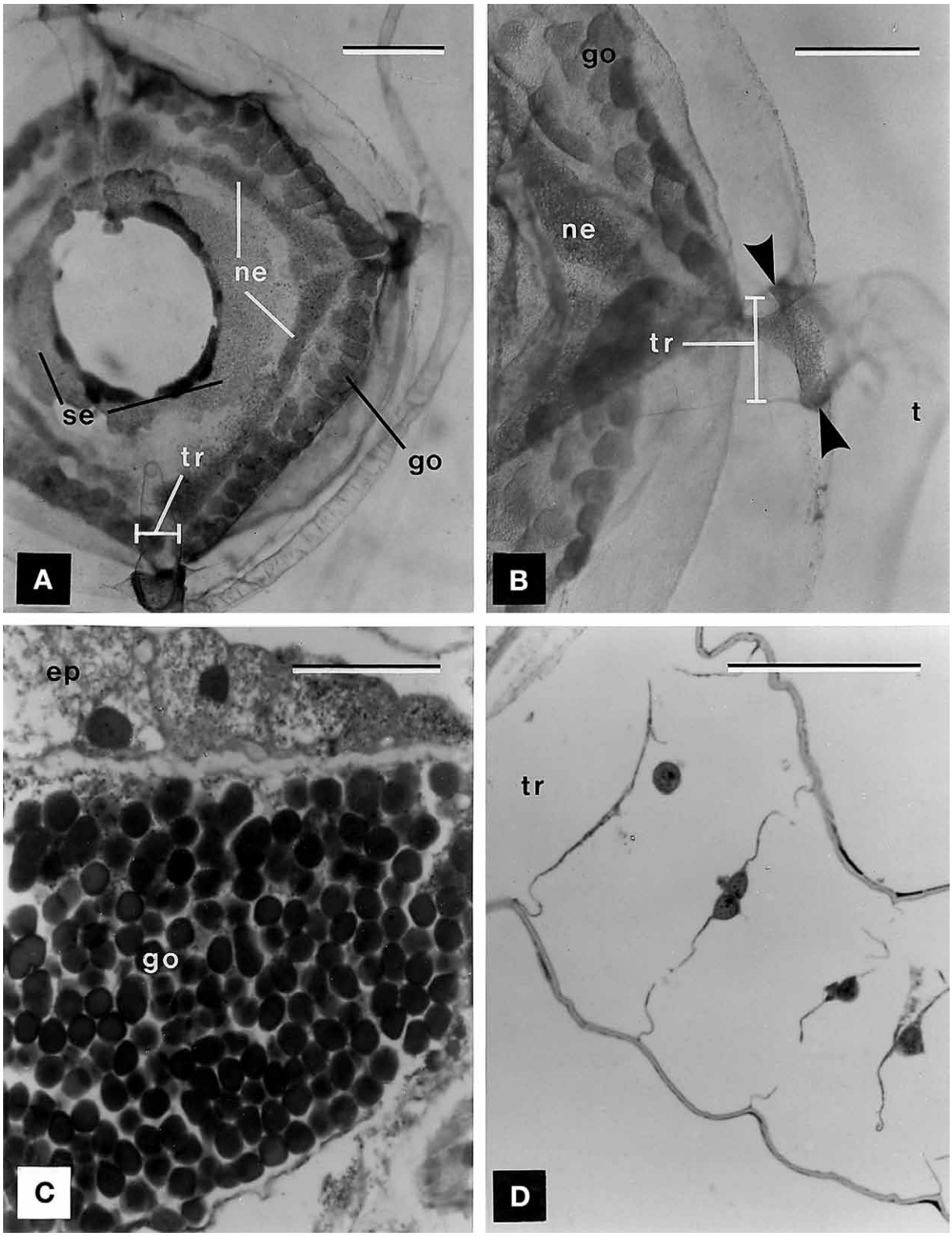

FIG. 1. – **A**. Aboral view of a 6.5-mm medusa revealing the three tissues (go, gonadal tissue; ne, nematogenic tissue; se, secretory tissue) in the oral wall of the stomach observed though the transparent umbrella. Note the ring of discrete aggregations of gonadal tissue. tr, root of a tentacle. Scale bar = 1 mm. **B**. Aboral view of a medusa illustrating the connection of nematogenic tissue (ne) of the oral stomach wall to a semicircular band (between arrowheads) at the base of a tentacle. go, gonad; t, tentacle; tr, root of a tentacle. Scale bar = 0.5 mm. **C**. Photomicrograph of a glycol-methacrylate section through the oral wall of the stomach, illustrating the structure of a single aggregate of gonadal tissue and the overlying epithelium (ep). go, gonad. Scale bar = 100 μm. **D**. Photomicrograph of a glycol-methacrylate section through the endodermal root of a tentacle (tr). Scale bar = 100 μm.

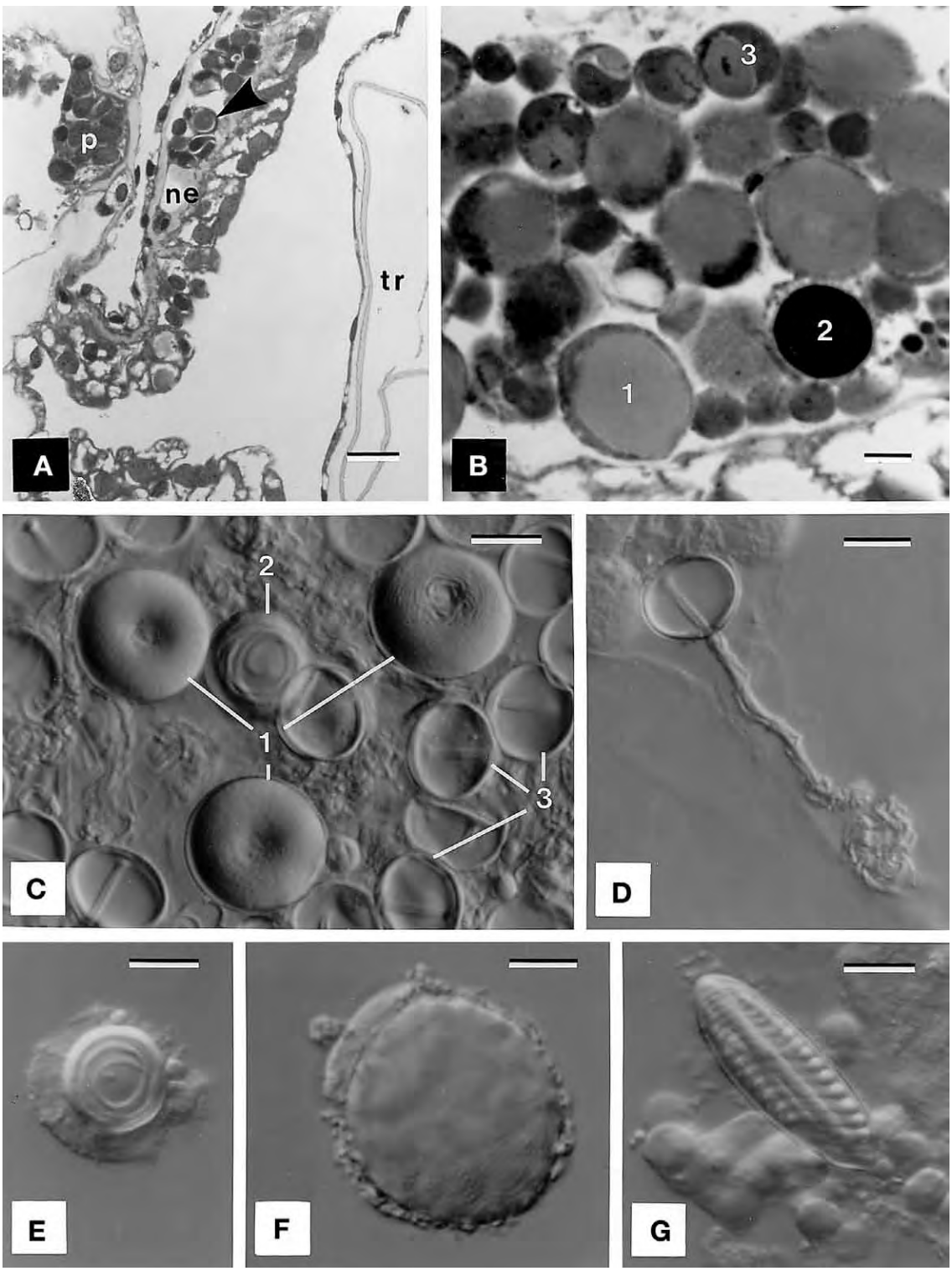

FIG. 2. – **A**. Photomicrograph of a glycol-methacrylate section of the margin of the umbrella, where nematogenic tissue (ne) of the oral stomach wall approaches the margin. Nematocysts (arrowhead) are apparent. p, peronium without canal; tr, root of a tentacle. Scale bar = 10 μm. **B**. Photomicrograph of a glycol-methacrylate section of the nematogenic tissue in the oral stomach wall. Three types of nematocysts can be identified on the basis of morphology and size (see text): Type-1 nematocysts (1) are large and without stained internal structure, Type-2 nematocysts (2) are intermediate in size and intensely stained, and Type-3 nematocysts (3) are small with a stained irregular bar. Scale bar = 10 μm. **C**. Squash of a whole medusa viewed with Nomarski optics. The three types of spherical nematocysts (1, Type-1 nematocyst; 2, Type-2 nematocyst; 3, Type-3 nematocyst) are easily identified. The Type-3 nematocysts are invariably everted in squashes. Scale bar = 10 μm. **D**. Everted Type-3 nematocyst. Squash viewed with Nomarski optics. Scale bar = 10 μm. **E**. Type-2 nematocyst in its nematocyte. Squash viewed with Nomarski optics. Scale bar = 10 μm. **F**. Type-1 nematocyst in its nematocyte. Squash viewed with Nomarski optics. Scale bar = 10 μm. **G**. Rare Type-4 ovoid nematocyst which has not been observed in sections of whole animals and which may belong to prey. Squash viewed with Nomarski optics. Scale bar = 10 μm.

(Figs. 1A and 1C). Nematogenic areas form an intermediate ring closer to the mouth than the gonads and also overlie the gonadal position where nematogenic tissue extends out to a semicircular band at the base of each tentacle (Figs. 1A and 1B). Immediately surrounding the simple mouth, the epithelium of the oral stomach wall is thickened with large numbers of secretory cells (Fig. 1A).

The margins of the medusae are very fragile. The umbrella with attached tentacles is easily distinguished from any other species in the tows. However, in spite of examination of almost 900 specimens, none have been found with exumbrellar lobes undamaged from the collecting stress, so the sense organs and any associated otoporpae have not been observed. The tissue in the peronia below the tentacle bases lacks canals (Fig. 2A), so a peripheral canal system is probably not present on the exumbrellar lobes.

Histology

The glycol-methacrylate sections were used to identify the gonodal tissue, nematogenic tissue, and secretory tissue of the oral stomach wall. Both sections and squashes of whole animals were used to examine the nematocysts present in the medusa within the limitations of bright-field and Nomarski differential interference-contrast microscopy.

In stained sections, three types of spherical nematocysts within nematocytes were identified by their morphology (Fig. 2B of nematogenic tissue). The largest nematocyst (Type 1) shows no stained internal structure when sectioned (Fig. 2B). The next- smaller size (Type 2) is often intensely stained in sections (Fig. 2B). The smallest spherical nematocyst (Type 3) has a stained irregular bar in sections (Fig. 2B).

The above three types of nematocysts and a rare fourth ovoid type were also present in squashes of whole medusae (Figs. 2C-2G). Types 1 and 2 did not evert, presumably due to prior fixation. Examined with Nomarski optics Type 2 is clearly seen to contain loosely-coiled structures, presumably tubules which are isodiametric or of evenly-reduced diameter (Figs. 2C and 2E). Some indication of finely-coiled structures may be present in Type 1 using Nomarski optics but details could not be observed (Figs. 2C and 2F). In squashes, Type 3 is everted with a tubule that is apotrichous (with spines on the distal portion of the tubule) at the level of light microscopy and with a remaining bar within the capsule (Figs. 2C and 2D). The ovoid nematocyst (Type 4) has a longitudinal shaft surrounded by a coiled tubule. It is extremely rare, observed only twice in squashes.

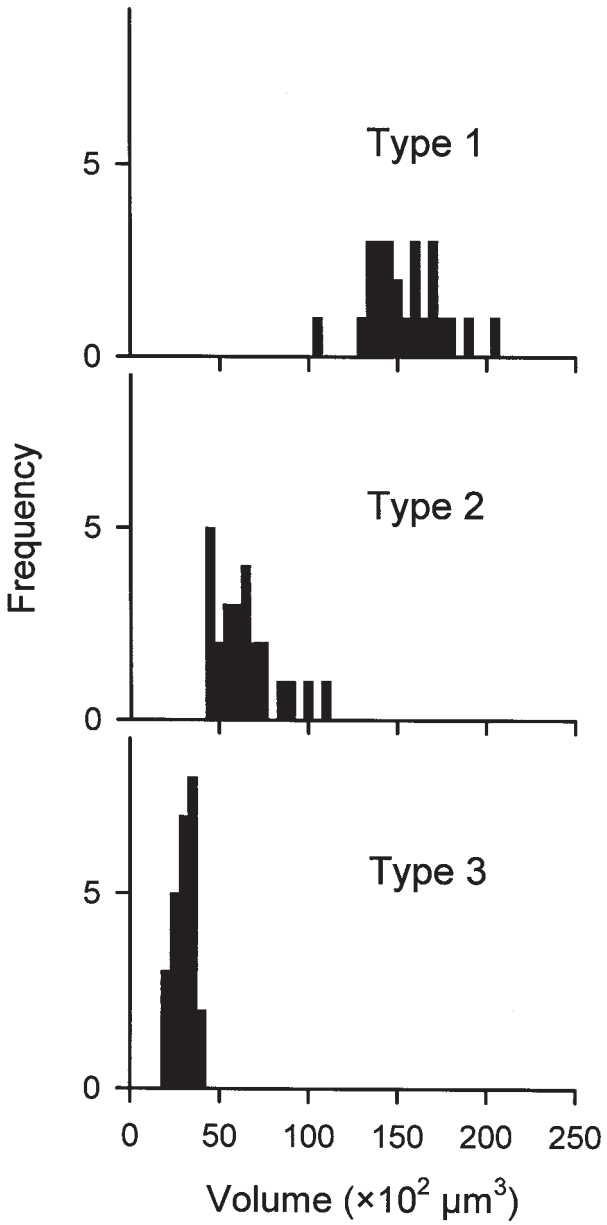

FIG. 3. – Volumetric comparison of the spherical (Type-1, Type-2, and Type-3) nematocysts. Volumes were calculated for 25 nematocysts of each type and plotted at increments of 500 μm^3. There is little overlap between the three types.

In squashes the diameters of 25 Type-1 nematocysts ranged from 27.0 to 33.8 μm (mean 30.7±1.4 μm), of 25 Type-2 nematocysts ranged from 19.8 to 27.4 μm (mean 22.6±2.0 μm), and of Type-3 nematocysts ranged from 15.4 to 19.8 μm (mean 17.5±1.2 μm). The volumes of the three types of spherical nematocysts were calculated, and the frequencies were plotted at increments of 500 μm^3 (Fig. 3). Due

to eversion of the Type 3 and fixation of all three types of nematocysts, caution must be used in comparing these measurements of diameters with absolute values from fresh squashes of other narcomedusae. The measurements do, however, clearly demonstrate that the three morphological types present in whole animals fall into three distinct size classes as shown in Figure 3. Similar size classes are found in other narcomedusae.

DISCUSSION

Taxonomy

The medusae are clearly narcomedusae of the family Solmarisidae, because they lack stomach pouches. Since there is a simple ring of gonadal tissue without diverticula and since no canals have been seen in the peronial tissue near the bases of the tentacles (Fig. 2A), the medusae are referred to the genus *Solmaris* rather than *Pegantha* (Kramp, 1961; Bouillon, 1985). In the northeast Pacific, species of *Solmaris* with more than four tentacles (including *S. coronata* and *S. rhodoloma*) have been reported off central and southern California and Mexico (Alvarino, 1980, 1985; Segura-Puertas, 1984; Wrobel and Mills, 1998). The present specimens, however, most resemble *S. quadrata* Bouillon, Boero and Seghers, 1991, the only species previously described with only four tentacles.

Based on seven specimens from Papua New Guinea, Bouillon *et al*. (1991) diagnosed *S. quadrata* as: Solmaridae of quadratic form, flattened, of small size, up to 1.2 mm diameter; exumbrellar lobes little developed, slightly convex; four tentacles; two statocysts per exumbrellar lobe, gonads differentiated on almost the whole subumbrellar wall of the stomach. They also noted relatively-short tentacles. In contrast to the diagnosis, the drawing shows a specimen of over 2 mm diameter with tentacles each approximately 1 mm in length. The authors state that the types are deposited in the Institut Royal des Sciences Naturelles de Belgique, however such specimens are not listed in the 1995 collection list of that institute (Bouillon *et al*., 1995) so their location is unknown.

Until specimens of the Canadian Pacific form are collected with intact lobes and sense organs, it is not possible to clearly identify the form as, or distinguish it from, *Solmaris quadrata*. It differs from the previous description of *S. quadrata* in larger maximum size to 11 mm diameter, longer tentacles to at least 2.5 times the diameter of the umbrella, and less extensive disposition of the gonads on the subumbrellar wall. These latter characters may not prove to be of specific value when specimens of the Canadian form of less than 4 mm diameter are available for examination.

Although the present data show that this species is abundant offshore, at least in spring, it has not been recorded previously from Canadian or other North Pacific waters. The small and delicate specimens are usually damaged in nets. Offshore collections from submarines have not been made in this area, and individuals are in any case smaller than is normally visible to observers in manned submarines (C.E. Mills, pers. comm.). These factors will make it difficult to collect the even smaller juvenile specimens necessary to resolve the taxonomic questions. Until those questions are resolved, it is premature to speculate on the range of the species beyond the northeast Pacific.

Nematocysts

This is the first paper to examine the nematocysts of a solmarisid narcomedusa. In other species of narcomedusae, spherical nematocysts of two sizes have been observed on the tentacles (Bouillon *et al.,* 1988; Purcell and Mills, 1988; Carré *et al.,* 1989). The tubule is of the same diameter throughout (isorhiza), or tapers very gradually, so that it is evenly coiled in the undischarged capsule. Depending partly on the degree of magnification used in the observation, these nematocysts have been described as atrichous (without spines) or apotrichous (with spines on the distal portion of the tubule) when examined by light microscopy. However Carré *et al*. (1989) showed that small spines may be present throughout the length of the tubule which are visible only with the transmission electron microscope. These nematocysts correspond to the Type-1 and Type-3 nematocysts of the present *Solmaris* sp. The appearance of the unexploded Type-1 nematocysts is similar to that of the larger nematocysts of *Aegina citrea* tentacles (see Fig. 2 in Mills and Miller [1984]).

A third type of spherical nematocyst has been observed in the subumbrella and exumbrella of *Aegina citrea* and *Solmissus* spp. (Mackie and Mackie, 1963; Mills and Goy, 1988). Three types are also present in the aberrant hydrozoan *Polypodium hydriforme* which has been placed in the nar-

comedusae by some authors (Raikova, 1990). This third type corresponds to the Type-2 nematocyst of the present *Solmaris* sp. Figure 2E of the present paper may be compared with Figure 4b of Mills and Goy (1988).

In addition, a fourth type of nematocyst was rarely present in squashes of whole animals of *Solmaris* sp. This nematocyst (Type 4) is ovoid with a tubule possessing a well-developed shaft (*i.e*. it is a heteroneme) (Fig. 2G). This would be very unusual for the narcomedusae. It was not found in the histological sections, so it is not documented as belonging to the tissue of *Solmaris*. Narcomedusae usually have a diet of other gelatinous animals, including other hydromedusae (Mills and Goy, 1988; Purcell and Mills, 1988; Larson *et al*., 1989). It is probable that this nematocyst belongs to the remnants of a prey species in the stomach.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Approaches to the ethology of hydroids and medusae (Cnidaria, Hydrozoa)*

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SUMMARY: The behavioural patterns of 26 species of Antho- and Leptomedusae (with or without medusa stage) were investigated by video recordings. The analysed activities were: answers to mechanical stimuli, prey capture and ingestion, digestion, egestion, and swimming. The quantity of behavioural patterns identified in the small number of hydrozoan diversity studied so far is sufficient to demonstrate that these supposedly "simple" animals have evolved a complex array of responses to both external and internal stimuli.

Key words: Cnidaria, Hydrozoa, Anthomedusae, Leptomedusae, ethology, feeding.

INTRODUCTION

Cnidarians are the most primitive metazoans with complex behaviour. The other phyla with which they are placed at the root of the animal kingdom (i. e., Porifera, Placozoa and Mesozoa) have simple reactions to external stimuli only. Cnidarians, furthermore, have a well-developed nervous system and some have complex sense organs. They are, thus, the ideal group to investigate the origins of animal behaviour.

The nervous system of hydromedusae is more complex than the one of hydropolyps, being formed by a network of cells and fibres forming two rings connected by sparse fibres, one in the exumbrellar ectoderm and the other in the subumbrella. In some medusae, like *Sarsia*, the outer nervous ring passes at the base of tentacles, forming a tentacular ganglion that receives inputs from the ocelli. This probably serves as a centre for the directioning of nervous impulses between the tentacles and other body regions. Its role, thus, is that of a primitive and rudimentary brain (Mackie, 1971). Such medusan structures have no counterpart in hydroids, whose nervous system is a simple network. Both hydroids and medusae, however, answer a vast array of stimuli.

Hydroid feeding behaviour has not been studied much, although the first observations date back to the beginning of the 1900. Jennings (1906) was the first to describe as "feeding reaction" a behavioural pattern consisting in the movement of a hydranth after contact with a prey (tentacle retraction, mouth opening, etc.). Jennings (1906) stated that the feeding reaction is not a constant reflex, but depends on

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general physiological conditions, and reported on the importance of both mechanical and chemical stimuli. Many authors described the behaviour of single species, but without attempting a general classification of behavioural patterns. For instance, Vannucci (1956) observed that starved *Dipurena* hydranths move randomly in all directions while searching for a prey. Passano (1957) reported on the search movements of the hydranth of *Corymorpha*. Brinckmann-Voss (1970), followed by Edwards and Harvey (1983), studied the behaviour of the polymorphic colony of *Thecocodium brieni*. Orlov (1997) described the feeding behaviour of *Sarsia producta*.

Marfenin (1981) was the first to recognise the ecological importance of hydroid behaviour. He stated that every species, if examined in sufficient detail, has a unique feeding behaviour, this being determined, for instance, by the ability in prey capture, and by ingestion, digestion, and egestion times. Differences in these processes are related to environmental features, colony architecture, form and size of the hydranth and, if present, of the hydrotheca.

Medusae, being able to move in the water column, have more recognisable behavioural patterns than hydroids. Numerous authors, thus, studied medusan swimming and feeding behaviour. A common belief of past researchers (e. g., Hyman, 1940) was that medusae impact casually with their prey, so that both hydro- and scyphomedusae feed passively while swimming with their tentacles outstretched. If this were true, medusan-feeding efficiency would be simply related to tentacle number and length. Many recent observations on single species, however, led to a refined view of medusan behaviours. Mills (1981) and Costello (1988, 1992) distinguished ambush and cruising predators, relating foraging strategies to both tentacle morphology and arrangement and umbrella shape. Zamponi (1985) described an "alimentary space" and measured a "predation cylinder" from both tentacle number and length, and umbrellar diameter: a discrete volume of water in which a medusa can find a prey. Prey quality and quantity, furthermore, can induce varied foraging behaviours; physiological adaptations to low food availability are important aspects in hydromedusan ecology and account for the predatory efficiency of medusae (Costello, 1992). A particular case is that of the benthic and crawling medusae of *Eleutheria*, whose behaviour has been described in detail by Hadrys *et al*. (1990).

Valuable information on hydrozoan behaviour is scattered in literature on other topics. Behavioural data are present in many life cycle studies but are often mentioned neither in the abstract nor in the key words (e. g., Boero *et al*., 1991).

Most behavioural studies were centred on either polyps or medusae and the present paper is an attempt to identify general behavioural patterns for both stages from both Antho- and Leptomedusae, with or without a medusa stage.

MATERIALS AND METHODS

The behaviour of 18 species of Anthomedusae and 8 species of Leptomedusae was investigated (Table 1). Material was invariably sampled by collecting hydroids by SCUBA diving. Hydroids, fed with *Artemia* nauplii, were kept into hemispherical bowls, either attached to portions of their natural substrata or transplanted on microscopic slides. Hydroids were monitored seasonally in the field to identify fertile periods, and were collected and brought to the laboratory only when medusae were near to be liberated. Medusae were also kept into hemispherical bowls and fed with *Artemia* nauplii. Food was provided daily and water was changed after every feeding session. Both hydroids and medusae were kept in natural seawater filtered at 0.45 μm. The bowls were kept into thermostatic rooms having both temperature and photoperiod matching natural conditions. The behavioural patterns were studied under a Leica MZ12 stereomicroscope equipped with a videocamera and every pattern was recorded on videotape. Reaction to mechanical stimuli, capture of prey, ingestion, digestion, and egestion were investigated for both hydroids and medusae; swimming patterns were studied for medusae.

RESULTS

The observed behavioural patterns differed in the examined species, but it was possible to identify types of behaviour that are usually present in more than one species (Table 1). Although polyps and medusae constitute a single biological unit, they will be considered separately since their behaviours fall into different categories even within the same species.

TABLE 1. – Behavioural patterns of polyps and medusae (see text for further explanation and references).

Species	Polyp			Medusa	
	Mechanical stimuli	Prey capture	Digestion	Swimming	Prey capture
Anthomedusae					
Filifera					
Bougainvillia sp.	Escape	Passive	Decrease of feeding space	Move	Mouth to tentacle
Turritopsis nutricula	Escape	Passive	Decrease of feeding space	Move	Mouth to tentacle
Eudendrium spp.	Escape	Passive	Inhibition of capture ability	Absent	Absent
Hydractinia sp.	Escape	Passive	Decrease of feeding space	Absent	Absent
Thecocodium brieni	Approach	Passive	Decrease of feeding space	Absent	Absent
Amphinema dinema	Unidirectional bending	Passive	Decrease of feeding space	Move	Mouth to tentacle
Codonorchis octaedrus	Escape	Passive	Decrease of feeding space	Move	Mouth
Octotiara russelli	Unidirectional bending	Passive	Not seen	Not seen	Mouth
Stomotoca atra	Unidirectional bending	Passive	Not seen	Not seen	Mouth
Hydrichthys mirus	Not seen	Not seen	Not seen	Move	Mouth
Capitata					
Cladonema radiatum	Approach	Active	Decrease of feeding space	Move and catch	Tentacle to mouth
Coryne producta	Approach	Active	Decrease of feeding space	Absent	Absent
Dipurena halterata	Approach	Random	Decrease of feeding space	Not seen	Not seen
Eleutheria dichotoma	Approach	Passive	Decrease of feeding space	Crawling	Tentacle to mouth
Ectopleura wrighti	Approach	Passive	Inhibition of ingestion ability	Not seen	Mouth to tentacle
Cladocoryne floccosa	Approach	Passive	Decrease of feeding space	Absent	Absent
Zanclea sessilis	Approach	Passive	Decrease of feeding space	Move	Mouth to tentacle
Zanclea giancarloi	Approach	Passive	Decrease of feeding space	Move	Mouth to tentacle
Leptomedusae					
Aequorea forskalea	Escape	Passive	Decrease of feeding space	Catch	Not seen
Mitrocoma annae	Escape	Passive	Decrease of feeding space	Not seen	Not seen
Campalecium medusiferum	Unidirectional bending	Passive	Decrease of feeding space	Move (young)	Mouth to tentacle
Halecium pusillum	Escape	Passive	Not seen	Absent	Absent
Aglaophenia octodonta	Escape	Passive/Active	Inhibition of capture ability	Absent	Absent
Plumularia setacea	Escape	Passive	Inhibition of capture ability	Absent	Absent
Clytia linearis	Escape	Passive	Decsease of feeding space	Catch	Mouth to tentacle
Obelia dichotoma	Escape	Passive	Inhibition of capture ability	Catch	Mouth to tentacle

Hydroids

Mechanical stimuli

Single hydranths were stimulated mechanically with forceps, so to simulate harsh contact with a source of "disturbance". The behavioural responses fell within three categories: escape, approach, unidirectional bending.

Escape reactions (Fig. 1). Escape reactions were typical of the hydranths of filiferans and most thecates. They were classifiable in three patterns according to both morphological constraints and the intensity of the stimulus:

1 - tentacle contraction. Light stimuli usually caused tentacle contraction accompanied by tentacle folding towards the mouth. *Halecium pusillum* was the only species that folded the tentacles towards the column.

2 - hydranth contraction. Strong stimuli, or reiterated light stimuli, caused tentacle contraction and, simultaneously, hydranth contraction. In the species with a theca, this behaviour resulted in retraction into it. Well-fed hydranths of *Aequorea forskalea* and *Mitrocoma annae*, however, were not able to contract into their thecae and remained outside them even when contracted. The haleciids, having a reduced theca, resembled filiferans in this behavioural pattern.

3 - tentacle folding. *Eudendrium* spp. were not able to contract neither tentacles nor hydranth; disturbed hydranths folded their uncontracted tentacles around the peduncled hypostome.

Approach reactions (Fig. 1). All Capitata, and the dactylozooids of the filiferan *Thecocodium brieni*, bent their polyps towards the source of the mechanical stimulus, whatever its intensity. These species have short, capitate tentacles that are only slightly contractile. This active movement was related to the way these species catch their prey (see below).

Unidirectional bending (Fig. 1). The hydranths of some pandeids and of the thecate *Campalecium medusiferum*, when touched, bent in a constant

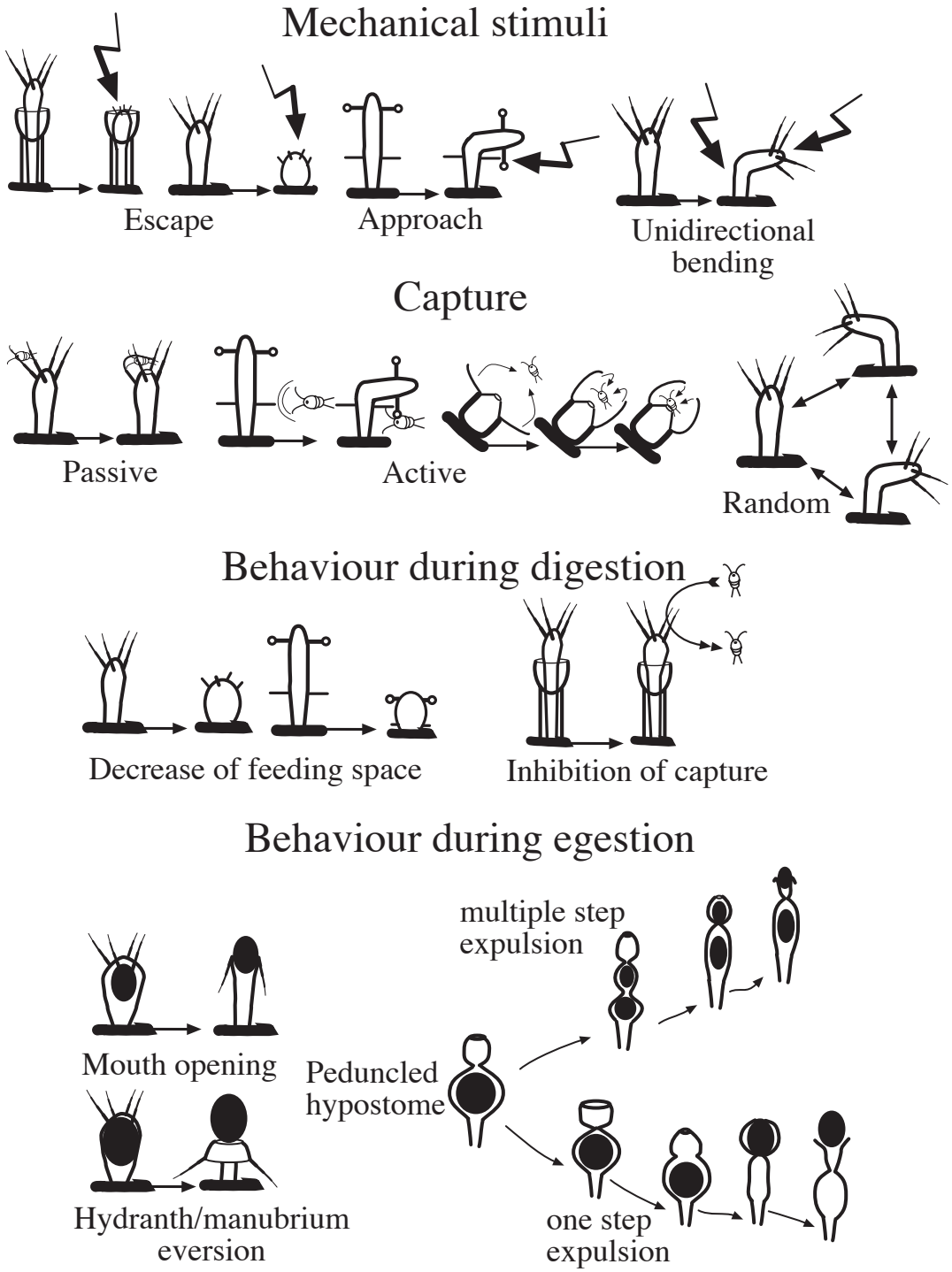

FIG. 1. – Answer to mechanichal stimuli, capture and behavior during digestion in hydroids; behavior during egestion in hydroids and medusae.

direction, irrespective of the direction of the stimulus. The hydranth, furthermore, bent at a well-defined place in its column; this allows to hypothesise the presence of a muscular "articulation" functioning as a knee or elbow. The histology of this region is not known in detail.

Feeding patterns

All feeding patterns were observed while using *Artemia* nauplii as food source. None of the investigated species will ever encounter such prey in the wild and it is known that hydroids do not feed on

crustaceans only (Barangé, 1988; Barangé and Gili, 1988; Coma *et al*., 1995; Gili *et al*., 1996). It is presumed, however, that the feeding behavioural patterns remain rather constant whatever the offered food is. The observed feeding patterns fell into three categories:

Passive (Fig. 1). This was the typical capture strategy of hydranth with long and usually filiform tentacles extended in the water, defining a wide feeding space. They wait for the prey to collide with tentacles after having entered their feeding space. This feeding strategy was observed in most of the investigated species.

Thecocodium brieni had a peculiar passive-active behaviour. The prey was passively captured by the dactylozooids. The gastrozooids, then, took it actively from the dactylozooids (see Brinckmann-Voss, 1970 and Edwards and Harvey, 1983 for detailed descriptions).

Active (Fig. 1). The active strategy occurred typically in hydranths with mechanoreceptors (Tardent and Schmid, 1972); these were able to feel an approaching prey, bent actively towards it and caught it with their tentacles. The hydranths performing this behaviour usually had short capitate tentacles, but their ability to "move" around the stalk led to the control of a hemispherical feeding space.

Another active feeding pattern was that of the hydranth of *Aglaophenia octodonta* whose relatively long and filiform tentacles beat the surrounding water creating a flow that conveyed little prey or organic matter towards the hypostome.

Random (Fig. 1). This behaviour was typical of *Dipurena halterata,* whose hydranths bent randomly in all directions exploring a hemispherical space, from which they captured every passing prey. The hydranth of *D. halterata* can also feel water vibrations and bend towards the source. Unfed polyps bent continuously to increase the probability to contact a prey, but the swinging frequency decreased in fed hydranths. In the latter case, hydranths bent only when excited by a swimming prey.

Behaviour during digestion

The main function of hydranths was to capture prey with their tentacles and nematocysts and to ingest it. Once the coelenteron was full, the captured food that would have remained unused would have been a waste of both energy and nematoblasts. Two main behavioural patterns were observed during prey digestion:

Decrease of feeding space. Full hydranths without mechanoreceptors tend to contract their body and tentacles, so to minimise the feeding space. This structural modification was particularly evident in small hydranths like *Codonorchis octaedrus* which, during digestion, became so small to nearly disappear against the substrate (Boero *et al*., 1997).

Mechanoreceptors, when present, shrunk to little buttons. This lowered the sensitivity to water movement and hydranths did not bend toward approaching prey.

Inhibition of capture (Fig. 1) *or of ingestion*. Some hydranths did not capture any prey during digestion, even if their tentacles remained extended in the water. This suggests that nematocyst discharge is under some kind of control (Burnett *et al*., 1960; Clark and Cook, 1986; Grosvenor and Kass-Simon, 1987), being prevented when the enteron is full. This inertia, however, could also be the answer to a temporary lack of nematocysts after many captures.

Well-fed hydranths of *Ectopleura wrighti* did not ingest prey and did not move. The long aboral tentacles, however, continued to catch prey and kept it until the coelenteron was at least partly empty. Prey items, then, were picked up, one by one, by the oral tentacles.

Medusae

The number of observations for medusae was lower than for hydroids since many of the investigated species had no medusae. For some species with medusae, furthermore, it was impossible to observe behavioural patterns due to rearing difficulties. Hydranth growth is rapid and there are no distinct stages, although tentacle number increases in the growing athecate hydranths, possibly influencing their possibilities of prey capture. Medusae, instead, usually change much more than hydranths during growth. The medusae of both *Clytia* and *Sarsia*, for instance, are almost round at release, whereas they become respectively more flat or more elongate at later growth stages. The velar opening of newly released medusae is usually small and jet propulsion is intense. Larger medusae often have much wider velar openings and their propulsion is

possibly due to flapping (Costello and Ford, in press). These changes surely influence behavioural patterns. In the present paper, we report mainly on fully-grown medusae.

Swimming behaviour

Swimming to move (Fig. 2). This behavioural pattern corresponds to the ambush predation described by Mills (1981). Medusae swam only to change the foraging site and prey. They remained still in either a horizontal (*Codonorchis*, *Amphinema*) or vertical position (*Bougainvillia*, *Zanclea*, *Turritopsis* and *Campalecium*), or while resting on the bottom (*Cladonema*).

Swimming to catch (Fig. 2). This behaviour corresponds to that of cruising predators (Mills, 1981). Medusae move actively in the water column searching for prey. Capture occurs when the prey contacts the tentacles. Capture rates depend on swimming velocity, prey type and predator morphological characteristics (Mills, 1981; Costello, 1988; 1992). *Cladonema* medusae can catch prey also while swimming as described by Rees (1979) but also can remain anchored to the bottom for a long time, so falling in the preceding category.

Crawling (Fig. 2). The medusae are anchored to the bottom with adhesive tentacular pads, and prey is captured with the tentacles. The change of foraging site occurs by walking on the bottom with the tentacles. This behaviour was observed by Hadrys *et al*. (1990) in *Eleutheria dichotoma*.

Prey capture and ingestion

Prey capture occurs with two main patterns:

With tentacles (Fig. 2). Two patterns of prey ingestion were recorded:

1 – Mouth to tentacle. Tentacles captured the prey and brought it to the subumbrellar margin by a fast retraction. After this, the manubrium moved out of the subumbrellar cavity to picked up the prey. Crumpling was a particular case of mouth-to-tentacle reactions: the umbrella contracted, pushed the manubrium out the velar opening, and the mouth ingested the prey. In *Obelia dichotoma* preys were killed by mouth nematocysts but were the continuous tentacle movement that brought them towards the mouth. This active filter feeding was similar to the one reported for the hydranths of *Aglaophenia octodonta*. Boero and Sarà (1987) described the same behaviour for the medusae of *Obelia longissima*.

2 – Tentacle to mouth. Captured preys are brought actively to the mouth by the tentacles. In *Cladonema radiatum*, prey items were forced into the subumbrellar cavity by the motile and branched marginal tentacles that passed them to the oral tentacles which passed them to the mouth. In *Eleutheria dichotoma* the casual contact of tentacles with a prey during crawling induced active tentacle movement towards the mouth, allowing prey ingestion.

With mouth (Fig. 2). Some species captured preys with their lips and not with their tentacles. *Stomotoca atra* and *Octotiara russelli* belong to this category (Boero and Bouillon, 1989). *Hydrichthis mirus* anchored one or two tentacles to the bottom, then it swam away while remaining attached, so stretching the attached tentacles for a rather long distance. Subsequent contraction of the attached tentacles caused a rapid backward movement of the umbrella, during which preys entered the subumbrellar cavity and contacted with the oral lips (Boero *et al*., 1991).

Codonorchis octaedrus used its lips to capture prey that entered by chance in its subumbrellar cavity (Boero *et al.,* 1997).

Egestion in hydroids and medusae (Fig. 1)

Undigested food remains were eliminated during egestion, allowing further uptake of food. Hydroids and medusae had similar egestion behaviours (performed by hydranths and manubria respectively), and this section covers both stages. The elimination of small amounts of waste occurred by contraction of manubrial/hypostomial walls, resulting in pushing undigested material towards the mouth, which was then opened to allow egestion. The oral tentacles, when present, were turned downwards in hydranths and upwards in medusae. When waste was more abundant, this behaviour was often followed by a turning inside-out of the distal part of the hydranth/manubrium, so that the digestive wall was at least partly exposed. This behaviour was commonly observed in medusae, during crumpling, but was not observed in the hydranths of the observed species of *Turritopsis*, *Halecium*, and *Plumularia*.

The hydranths of the Campanulariidae had particular egestion patterns, linked to the presence of a separation between the hypostomic and the gastric

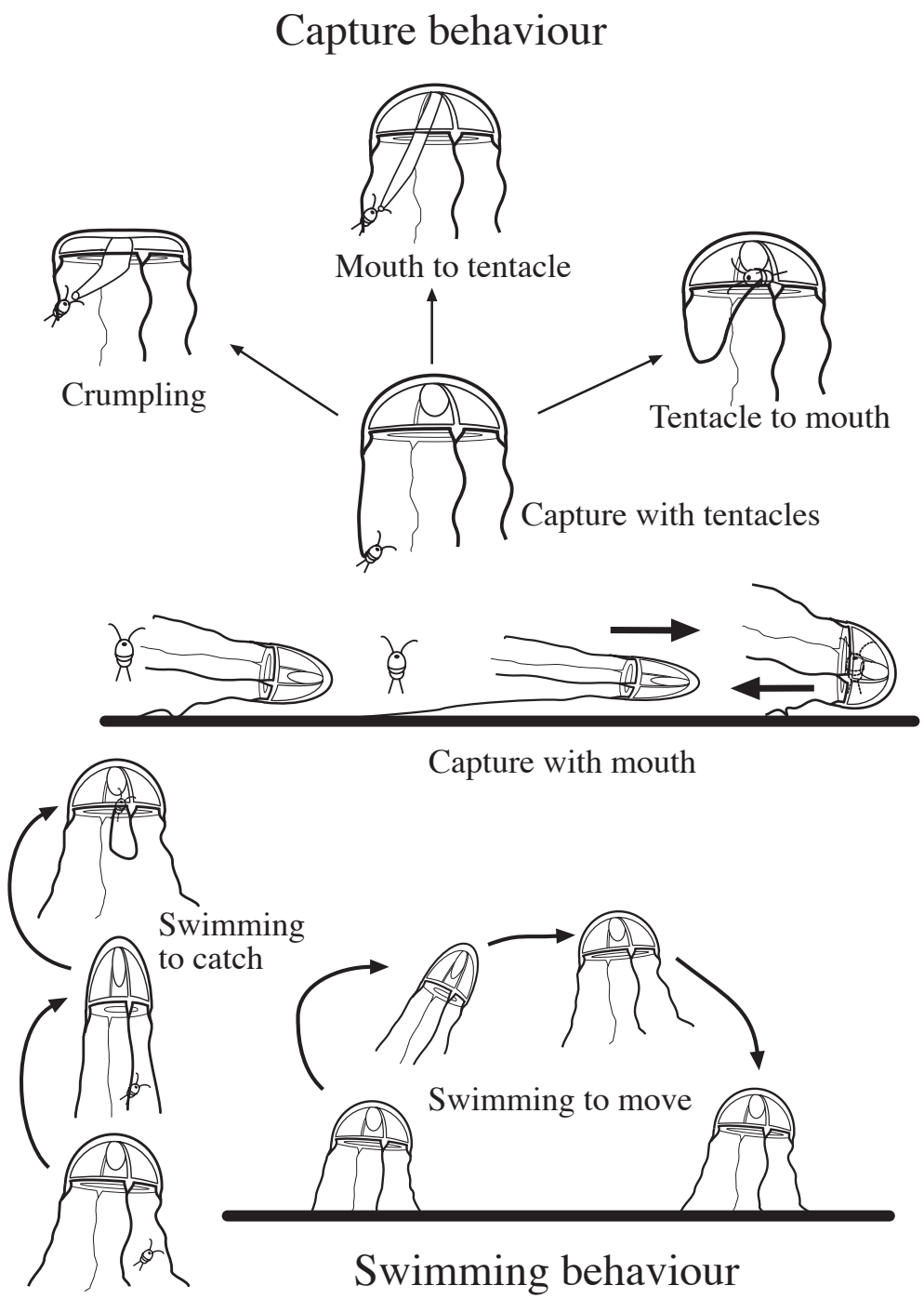

FIG. 2. – Capture and feeding behavior in medusae.

cavity, resulting in the so-called peduncled or trumpet-shaped hypostome. This constriction acted as a sphincter that played a paramount role in the functioning of the hydranth cavity. Three main ways of functioning of peduncled hypostomes were recognised:

1 - Multiple steps expulsion of egesta. Part of the undigested material was passed towards the hypostome while the sphincter was open. The sphincter contracted, causing fragmentation of the egesta, part of the egesta remained in the hydranth coelenteron, part in the hypostome. The mouth was opened and the constriction of the hypostome walls caused egestion. The expulsion of the rest of the egesta occurred in further steps, identical to the first one. This behaviour was observed in *Clytia linearis*.

2 - One step expulsion of egesta. The mouth was opened while the sphincter was closed, allowing the entrance of water into the hypostome. The mouth was closed and the sphincter was opened, the hypostome walls were contracted, pushing water into the hydranth coelenteron. The incoming water pushed up the egesta while the hydranth walls contracted; the egesta were passed into the hypostome. The sphincter contracted, separating the hypostome from the hydranth coelenteron; the mouth was opened, allowing waste elimination. This behaviour was observed in *Obelia*.

3 - Hypostome eversion. In *Eudendrium* waste was pushed up in the hypostome through the sphincter, but then it was egested by the turning inside out of the distal part of the hydranth.

DISCUSSION

The feeding patterns of capitate hydroids depend on tentacle number and on presence/absence of mechanoreceptors. Passive predators have a high number of tentacles controlling a wide feeding space in order not to fail the capture of a prey passing in their vicinity (e. g., *Zanclea*). Active predators (e. g., *Cladonema*, *Sarsia*, *Coryne*) have mechanoreceptors sensitive to prey movement. Their feeding space is wide due to the possibility of hydranth bending towards approaching prey, so that even a low number of tentacles are sufficient to kill an already located food item.

The feeding behaviour of *Dipurena halterata* is intermediate between passive (without mechanoreceptors and many tentacles) and active (with mechanoreceptors and few tentacles) patterns, with random bending movements, increasing the feeding space. *Dipurena halterata* has fewer tentacles than *Zanclea* (passive predator) but more than *Sarsia* or *Coryne* (active predators).

Most thecates are passive predators. *Aglaophenia octodonta* is exceptional since its active feeding pattern is not performed by using mechanoreceptors, and might be labelled as "random active". The water flow caused by beating tentacles suggests that some species of hydroids, always considered as passive filter feeders, can behave like active (but not ciliary) filter feeders such as barnacles.

Mechanoreceptors are known in some Capitata only and they possibly evolved only once. Unidirectional bending as a response to mechanical stimuli, but irrespective of their direction, on the other hand, was observed in both athecates (many Pandeidae species) and thecates (*Campalecium medusiferum*). The presence of the same behaviour in such different groups suggests convergent evolution that led to a still uninvestigated flexible zone.

The behaviour of medusae ranges from "classical" patterns of prey capture, to others such as the active filter feeding of *Obelia* (described by Boero and Sarà, 1987) or the yo-yo tentacle contraction of *Hydrichthys* (described by Boero *et al*., 1991). A world apart is the behaviour of symbiotic hydroids such as *Halocoryne* (described by Piraino *et al*., 1991) and *Eugymnanthea inquilina* (described by Piraino *et al*., 1994), reaching extremely high levels of complexity and specialisation.

Scattered unpublished observation, furthermore, suggest that the behavioural patterns of both hydroids and medusae are even more diverse than presented here and that the available observations cover just a minor part of hydrozoan ethology. Both ethology and behavioural ecology focused much on "higher" invertebrates and vertebrates but the available evidence suggests that even "lower" groups can perform behaviours of high complexity.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Non-indigenous hydromedusae in California's upper San Francisco Estuary: life cycles, distribution, and potential environmental impacts*

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SUMMARY: Two species of hydromedusae, assumed to be native to the Black and Caspian Seas, were routinely collected in Suisun Slough, California, at the Suisun City Marina, during late summer and fall of 1997. Suisun Slough connects directly with Suisun Bay, part of the biologically complex and commercially important upper San Francisco Estuary, and with San Francisco Bay via San Pablo Bay. *Maeotias marginata* (Modeer 1791), has been previously reported (as *M. inexspectata*) from the Petaluma River, another tributary entering San Pablo Bay, while an as-yet undetermined species of *Moerisia* represents a new distributional record for this genus in the eastern Pacific. Morphologies of the adults and immature growth stages of medusae of both species are described. The polyp stages of both species were reared in the laboratory following spawning of adult medusae collected from the field. Both species are apparently representative of the robust and aggressive Black and Caspian Seas brackish water fauna, many species of which have been introduced into estuarine habitats worldwide. The potential of these planktonic predators to alter zooplankton communities and feed directly on larval and juvenile stages of threatened native and commercially valuable estuary fish species are all possible, but remain uninvestigated.

Key words: *Maeotias marginata, Maeotias inexspectata, Moerisia* sp., *Moerisia lyonsi*, Olindiidae, Limnomedusae, San Francisco Bay, Cnidaria

INTRODUCTION

Maeotias marginata (Modeer, 1791) (see Mills and Rees, 2000) and *Moerisia* sp. are the first hydromedusae to be reported from Suisun Bay, an oligohaline, usually very low salinity portion of the San Francisco Estuary situated between San Pablo Bay and the Sacramento–San Juaquin River delta. Both species were collected in Suisun Slough, a sinuous arm of Suisun Bay, during September and October of 1997 and again in 1998. These two species are exotic introductions, and both are presumed members of the relict brackish-water Sarmatic fauna native to the Black and Caspian Seas. Both of these hydrozoans have complex life cycles consisting of asexual sessile polyps and sexual pelagic medusae. Polyps of both species were obtained in the laboratory following spawning of adult field-collected medusae. On the west coast of North America, *Maeotias marginata* (as *M. inexspectata)* has been previously reported in the San Francisco Estuary from the Petaluma River, which empties

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into San Pablo Bay (Mills and Sommer, 1995), while *Moerisia* sp. represents the first record of this genus from the Eastern Pacific.

The introduction of these medusae into Suisun Bay is significant, as no hydromedusae are known to be native to this bay. Jellyfish are important, though little-appreciated and understudied, secondary consumers on the zooplankton and zoobenthos in many other habitats. Under certain environmental conditions, hydromedusae can attain very high local abundances (blooms) and under such circumstances are capable of severely depleting and altering the structure of local zooplankton communities. Some hydromedusae are also known to feed directly on commercially valuable larval and juvenile fish. Here we document the life history stages of two introduced species and report initial results of field work which aims to investigate the capabilities of these hydrozoans to invade and impact sensitive estuarine environments. Knowledge of the basic biology of these cnidarians, as well as other aggressive invaders of estuarine habitats, is needed if appropriate preventative measures are to be instituted towards limiting negative impacts of alien introductions in vulnerable and economically important estuarine habitats around the world.

MATERIALS AND METHODS

On information that unidentified jellyfish had been observed in Suisun Bay, one of us (L.G.) undertook a field excursion to the marina adjacent to downtown Suisun City, located at the end of Suisun Slough, in early September 1997. At that time adult jellyfish, subsequently identified as *Maeotias marginata*, were seen in abundance at the water surface. Through routine plankton tows taken alongside the marina dock, a second hydromedusan species, later identified as *Moerisia* sp., was found to be sporadically abundant at the marina. Growth stages of both medusae were collected, photographed, and preserved.

Field Collections

Medusae of *M. marginata* and *Moerisia* sp. were collected weekly at the Suisun City Marina from mid-September to October 29, 1997, by taking horizontal plankton tows with a 0.25 m diameter, 500 μm-mesh net at about 0.5 m depth over a 1-2 meter bottom. A secchi disc was used to determine water clarity.

Large *M. marginata* were only occasionally collected in this manner, although immature stages of the medusa were routinely dip-netted from the surface where they were visible. A complete series of growth stages of *M. marginata* and *Moerisia* sp. were collected during the study period. Living medusae were further observed and photographed in the laboratory. Adult *Moerisia* sp. medusae were fed *Artemia* nauplii; *M. marginata* medusae ate bits of minced, cooked shrimp.

A cursory search in the field for the polyps of both species was made on local docks and other substrates, including scrapings from pilings and sides and bottoms of floating docks. None were found, although the polyps of both species were subsequently reared from mature medusae spawned in the laboratory. Initially, all *M. marginata* medusae collected were males, as previously reported by Mills and Sommer (1995) from the Petaluma River. In early September 1998, a few female *M. marginata* medusae were collected in the Napa River (which empties into San Pablo Bay), and primary polyps were obtained by spawning adult male and female medusae in the laboratory.

Laboratory Culture

Adult medusae were taken to the laboratory, sorted by sex using a dissecting microscope, and placed in 10 cm diameter glass rearing bowls filled with water collected from Suisun Slough. Laboratory culture techniques followed those described by J.T. Rees (1979) for *Eutonina indicans*. Release of eggs was observed, and development and subsequent settlement of planulae took place directly on the bottoms of the bowls. Primary and adult polyps thrived on newly hatched *Artemia* nauplii. Newly-released medusae obtained from polyps were transferred to small (5 cm diameter) plastic culture containers and reared to maturity. All cultures were fed once daily. Culture water was changed every second day prior to feeding. Culture temperature ranged from 18–21° C; salinity was maintained at about 10 psu (practical salinity units). No aeration was used in polyp or medusa culture containers. Salinities were measured in both the field and laboratory with a hand-held refractometer or a specific gravity meter calibrated with a refractometer. Nematocysts of both species were examined using a compound microscope at 500 and 1000X in squash preparations; measurements given are for unexploded nematocysts.

RESULTS

Photographic documentation of the life history stages of *Maeotias marginata* and *Moerisia* sp. from Suisun Slough is presented in Figures 1–6 and nematocysts are shown in Figure 7. Salinites and temperatures at the field site ranged from 7–10 psu, and 18–19° C, respectively, throughout the study period. Turbidity at the site was high and secchi disc readings were always 25 cm or less.

Maeotias marginata (Modeer, 1791)

M. marginata medusae were collected in the field in 1997 and 1998 between late August and early November. Despite numerous plankton tows, almost all medusae captured were adults 25 mm in diameter or greater, indicating that the peak period of release of the medusa from the hydroid had been earlier in the year. A few very small *M. marginata* medusae were collected in October, indicating that

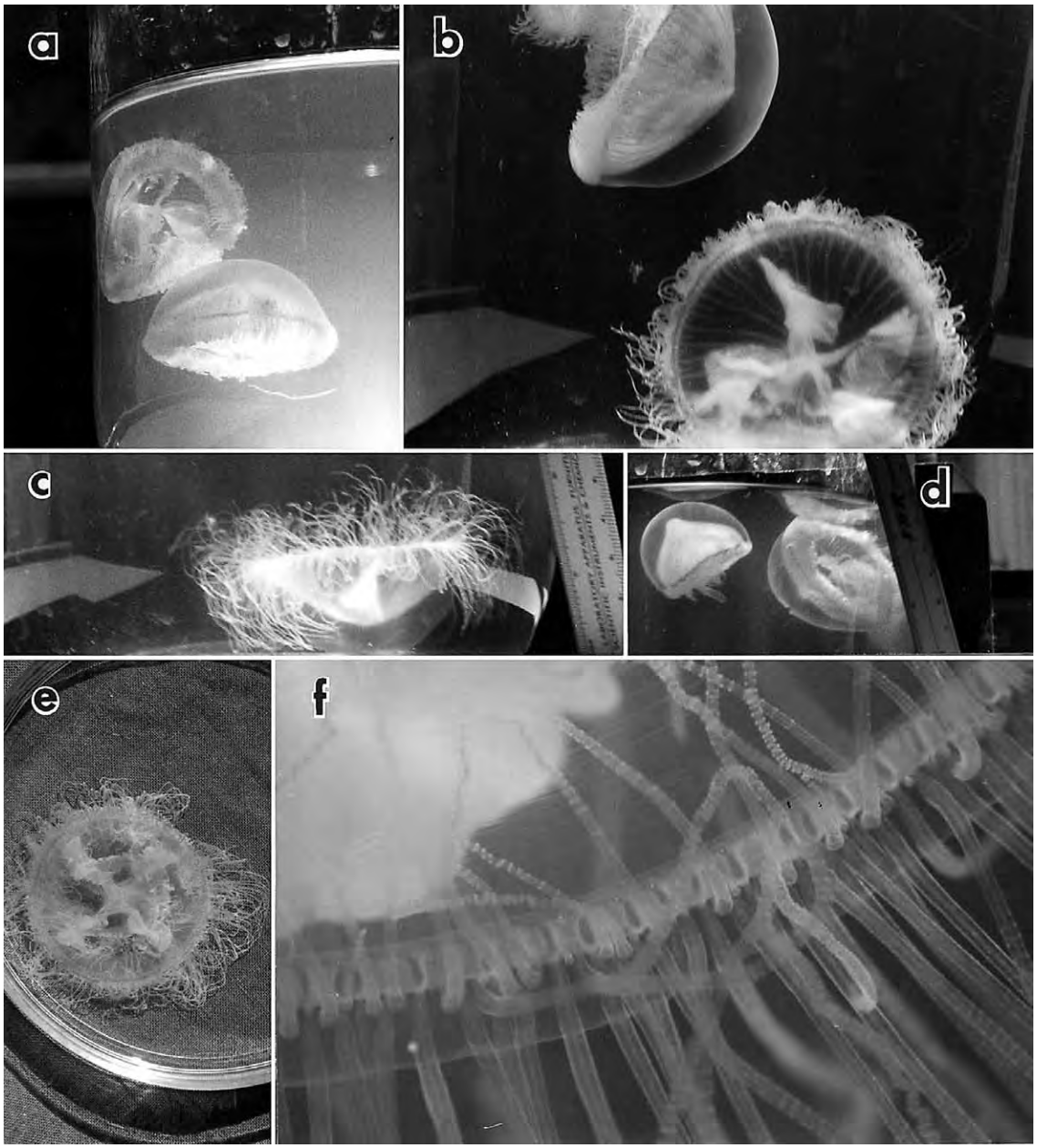

FIG. 1. – *Maeotias marginata*, adult medusae, 3.0–5.0 cm in bell diameter. (a,b) Lateral and oral views: note thickness of the mesoglea, conical subumbrellar profile, curtain-like gonads, centripetal canals, and numerous tentacles. (c) Lateral view of typical resting posture of medusa on the bottom. (d) Medusa at left shows maximum contraction of the bell while swimming. (e) Oral view of typical resting position on bottom. (f) Magnification of bell rim showing tentacles with rings of nematocysts, oval-shaped pigmented areas at bases of tentacles, and statocysts between the tentacle bases.

occasional medusae were still being released from the hydroid at this time. The biggest adults were 50 mm in bell diameter, among the largest reported for this species, including those collected and described from native habitat in the Sea of Azov and other areas in the Black Sea (Kramp, 1961; Mills and Sommer, 1995). Size class measurements taken in late September and October showed that the medusae were still increasing in mean size in October. According to local marina boat owners, smaller "dime-sized" (~15 mm bell diameter) medusae had been present and visible in great numbers at the surface in late July. These medusae were apparently juvenile *M. marginata*, with mature adults having 4–5 cm bell diameters still present and abundant in mid-September.

The time of introduction of *M. marginata* into Suisun Slough is unknown, but a preliminary review of field notes from California Fish and Game and U.S. Fish and Wildlife Service collections in the nearby Sacramento–San Juaquin River delta (upper San Francisco Bay Estuary) indicates that this species may have been present (and intermittently collected) there since 1959. Location of preserved specimens would be necessary to verify the species identification of these early records.

Morphology and Growth Stages of the Medusa (Figs. 1–3)

The morphology of the adult medusa is shown in Figure 1. Adult medusae were observed in various behavioral postures, including resting in a presumed feeding posture on the bottom, exumbrella downward, with tentacles outstretched (Fig. 1c, e). Live adult medusae at rest were wider than high, and in the field were generally an all-over light-brownish, or occasionally reddish, color. The mesoglea of the bell was clear in younger specimens, becoming more opaque as the medusae mature.

Gonads hung down from each of the four radial canals in folded sheets (Fig. 1b,e). The rim of the bell appeared as a characteristically brownish or reddish ring due to discrete areas of pigmentation at the bases of the tentacles. Alternating cycles of centripetal canals, each beginning at the ring canal and extending towards, but not actually reaching, the stomach, were visible between the four radial canals (Fig. 1b).

Mature medusae (~30–50 mm bell diameter) had about 450–600 tentacles; the tentacles had prominent and characteristic rings of nematocysts. Statocysts, each with a single inclusion, were located alongside the ring canal. No sensory clubs were present among the tentacles, confirming the observations of Mills and Sommer (1995). The large size, with bell wider than high, large number of tentacles, presence of very long centripetal canals, and a brownish or reddish bell rim render adult medusae of *M. marginata* distinctive.

Selected growth stages of the medusa are shown in Figures 2 and 3. The smallest medusa collected from the plankton (Fig. 3c,d,e) was 0.7 mm in bell height and 0.9 mm in bell diameter, and, since most new hydrozoan medusae are about 1.0 mm or less in bell height and grow rapidly, had probably just been released from the hydroid. The stomach had a characteristic quadrate base (Fig. 3d) and about 24 tentacles, each terminating in a club-shaped battery of nematocysts (Fig. 3e), all microbasic euryteles, surrounded the rim of the bell. Each tentacle appeared to have a solid endodermal core. There were four interradial marginal vesicles around the bell margin. Even at this very young stage, pigment granules were discernible around the bell margin (Fig. 3e); no centripetal canals were present.

In somewhat larger specimens (~1.5 mm bell height, 2.5 mm bell diameter) the marginal vesicles (now 3 per quadrant, each with a single statocyst), and broad radial and ring canals, all typical of larger medusae, were clearly visible (Fig. 2b). The manubrium hung down about 1/3 of the subumbrellar cavity length and there were about 50 tentacles. Four lips, precursors of the four elaborately-frilly manubrial lobes of adults, had begun to develop. The centripetal canals characteristic of adults were not yet present.

Medusae of 4–5 mm in bell height had about 200–250 tentacles, with the first signs of centripetal canals emanating from the ring canal. Gonads were beginning to develop on the radial canals near the manubrium.

In medusae 10 mm in bell diameter (Fig. 2c,d), developing gonads were clearly evident along the proximal half of the radial canals, and the four lips of the manubrium had begun to exhibit their characteristic frills. Centripetal canals were present in medusae of this size, although the animals were not yet sexually mature. The tentacle number was approaching 400. The broad ring canal, marginal vesicles with single statocysts, and numerous tentacles with prominent nematocyst rings enable this growth stage to be easily identifiable as *M. marginata*.

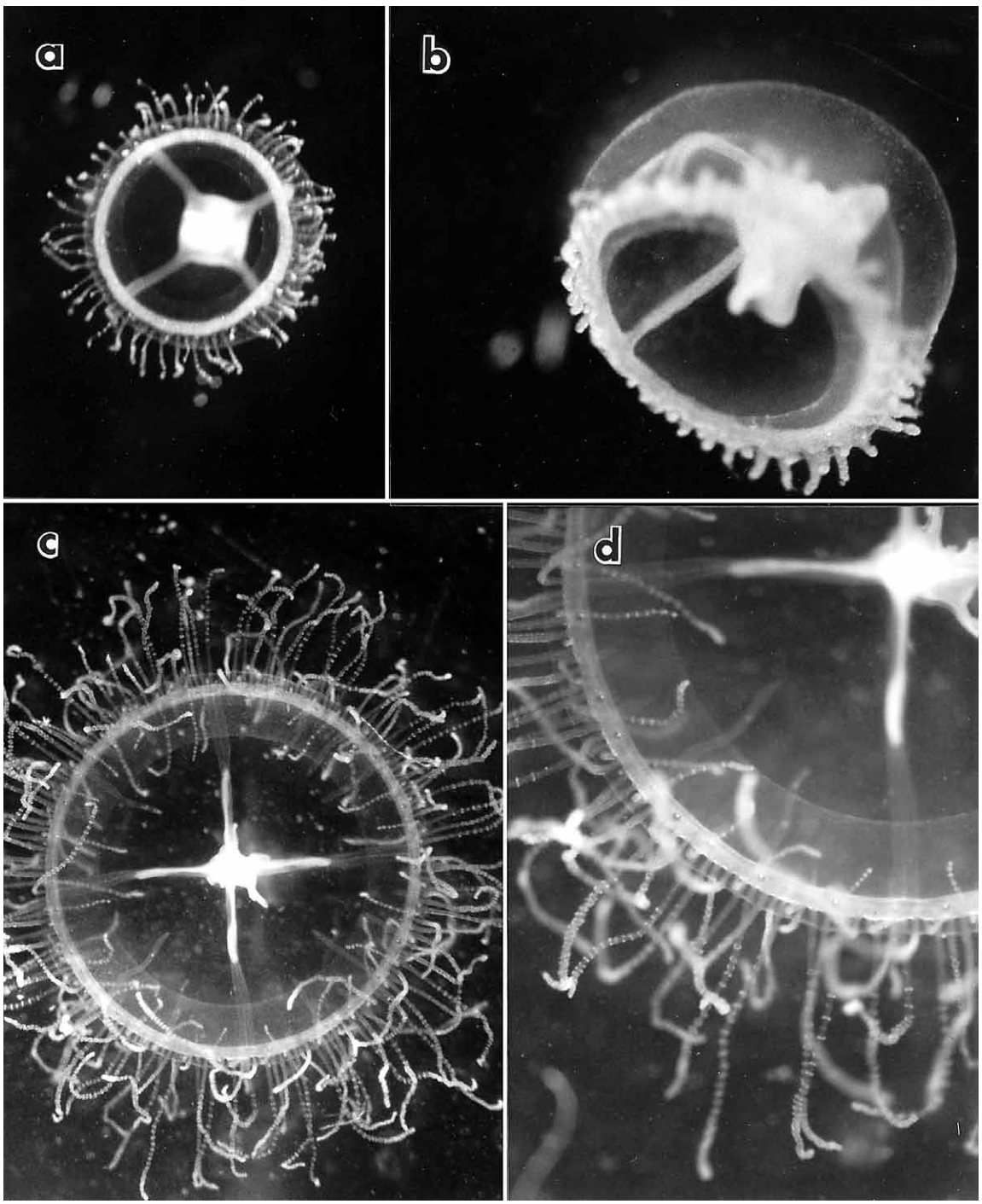

FIG. 2. – *Maeotias marginata*, growth stages of the medusa. (a) Very young medusa 1.0 mm in bell height and 1.5 mm bell diameter, showing numerous tentacles, broad radial and ring canals, and quadrate stomach. (b) Oblique view of young medusa about 1.5 mm bell height and 2.5 mm bell diameter: note beginning of formation of lips on the manubrium. (c,d) Two views of the same medusa, about 10 mm in bell diameter: note broad ring canal and marginal statocysts, numerous tentacles with prominent nematocyst rings, manubrium with frilled lips, and beginnings of gonad development along the radial canals.

The polyp (Fig. 3a,b)

The polyp described and figured as *M. marginata (=inexspectata)* by Mills and Sommer (1995) is undoubtedly that of *Moerisia* sp. (see below). In September 1998, one of us (J.T.R.) obtained primary polyps of *M. marginata* (Fig. 3a,b) by laboratory-spawning field-collected adult medusae. Eggs were small (~50 μm diameter) and settled immediately to the bottom of culture bowls after release. Swimming

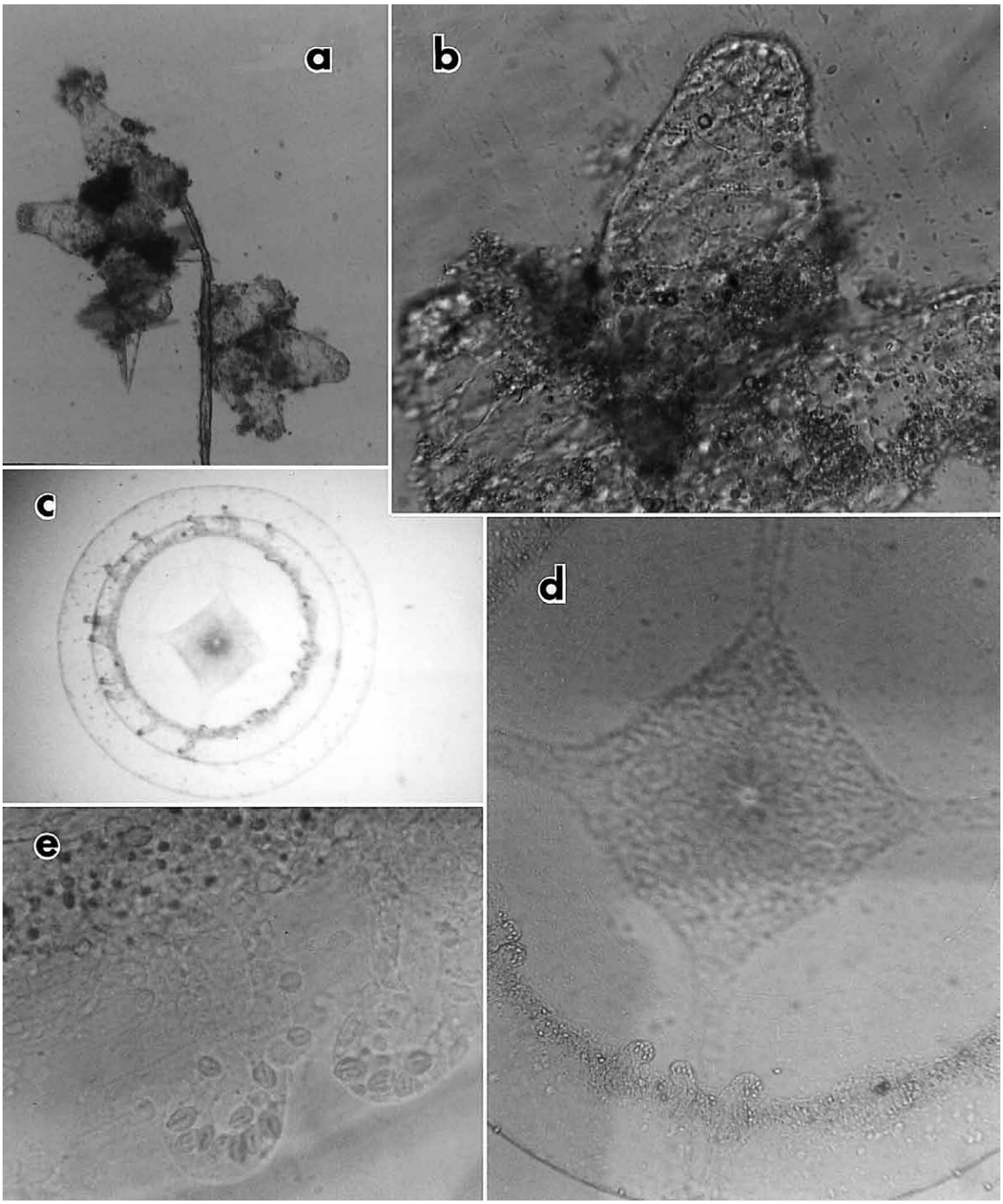

FIG. 3. – *Maeotias marginata*, primary polyp and very young medusa. (a) Group of primary polyps in the laboratory, each developed from a separate fertilized egg: each polyp is about 0.1 mm in height. (b) Enlargement of group of primary polyps: note nematocysts at the apex of the center polyp and large, clear cells of the polyp body. (c) Very young medusa, about 0.7 mm bell ht, 0.9 mm bell diameter with 24 tiny tentacles. (d) Enlargement of (c): note quadrate stomach and broad radial and ring canals. (e) Enlargement of tentacles: note microbasic eurytele nematocysts in the terminal tentacle knobs, solid endodermal core of tentacles, and pigment granules in the ring canal.

planulae did not develop, but polyps developed directly from eggs, on bits of loose detritus, which appeared to stick to the eggs and the bases of developing polyps, rather than on the glass of the finger bowls. Primary polyps were minute (~ 0.1 mm in height), inconspicuous, atentaculate, and similar in gross morphology to those few olindiid primary and adult polyps which are known, such as *Aglauropsis aeora* (Mills *et al*., 1976) and *Craspedacusta sowerbii* (see Russell, 1953). The body of the hydranth was composed of large, transparent cells. Nematocysts of primary polyps, which appeared to be all

microbasic euryteles, were oriented around what was presumed to be a mouth (Fig. 3b), although despite repeated attempts, polyps were never seen to feed, and no mouth or polyp gastric cavity was discernible. The entire behavioral repertoire of these tiny polyps consisted of slow and modest extensions and contractions of the polyp body. Efforts to rear the polyps were unsuccessful and whether further morphological change occurs is unknown.

Cnidom

All nematocysts in the medusa, on both the tentacles and the lips of the manubrium, were of one kind only, microbasic euryteles (Fig. 7a,b). In adult medusae of 40 mm bell diameter, these microbasic euryteles were of 2 sizes, with the larger size, 11–16 x 8–10 μm located on the tentacles, and the smaller size, 9–11 x 6–7 μm on the manubrium lips. Primary polyps also had a monocnidom of microbasic euryteles, but none were measured. Similarly-shaped euryteles have been reported in other olindiid limnomedusae such as *Aglauropsis aeora, A. conanti,* and *Eperetmus typus* (Mills *et al*., 1976).

Behavior

The adult *Maeotias marginata* medusa is a powerful swimmer, able to propel itself through the water several centimeters with each pulsation of the bell. In the field, medusae were routinely observed pulsing vertically to the surface, then flipping over and slowly sinking without pulsing, exumbrella-downward and tentacles extended. It is thought that *M. marginata* medusae spend the majority of their time on the bottom, so that medusae observed on the surface represent only a small portion of the total population present (Mills and Sommer, 1995). The medusae could not be observed on the bottom due to the turbidity of the water at the study site. When at rest on the bottom, the subumbrella and outstretched tentacles (Fig. 1c,e) are presumably used as a capture surface to snare food falling from the overlying water column. Prey are undoubtedly also captured as the medusae sink through the water column on their way to the bottom. Such a behavioral repertoire may be unique to some members of the family Olindiidae; it is also characteristic of *Gonionemus vertens,* whose species name recognizes the medusa's flipping over upon hitting the surface (Agassiz, 1862; C.E. Mills, personal communication).

In the laboratory, adult medusae spent most of their time resting on the bottom, with the oral opening oriented towards the surface (Fig. 1c,e). Resting medusae in the laboratory were fed by placing small bits of cooked shrimp or fish directly on the exposed subumbrella or tentacles. Adult medusae were maintained for 2–3 weeks in the laboratory, but did not thrive. Feeding habits of the smallest medusae are unknown. Although they presumably feed by capturing plankton with their tentacles and transferring the prey to the mouth in typical hydromedusan fashion, we were unable to keep the smallest medusae alive in the laboratory.

Systematics

Due to the presence of a monocnidom of microbasic euryteles and the presence of statocysts in immature and adult medusae, *M. marginata* should be retained in the order Limnomedusae, family Olindiidae. Confirmation of the ultimate taxonomic status of *M. marginata* must await detailed laboratory culture work on the entire life cycle, including the structure of the adult polyp and newly-released medusa. Molecular analyses and comparisons with other Anthomedusae and Limnomedusae should also aid in the systematic placement of this species as well as the entire family Olindiidae.

***Moerisia* sp.**

We found *Moerisia* sp. medusae within the same time frame and in the same location as *M. marginata*, although the presence of this species was more sporadic (and it was rarely seen by naked eye). *Moerisia* sp. medusae of all ages were occasionally collected in 5-minute plankton tows, but then not collected in replicate tows taken immediately afterwards. Presence of medusae did not seem to correlate with time of day. To determine if medusae were exhibiting a diurnal migration, a 24-hour series of surface plankton tows was taken at selected locations at the Suisun City Marina. Few *Moerisia* sp. were collected in total, and no correlation with time of day or night was evident. We now believe that these medusae are transported into the marina area on rising tides, a hypothesis which will require further sampling for verification.

Due to the apparent necessity for taxonomic revision of the family Moerisiidae, we were unable to assign a species name to the *Moerisia* from Suisun Slough. While a medusa very similar to ours has

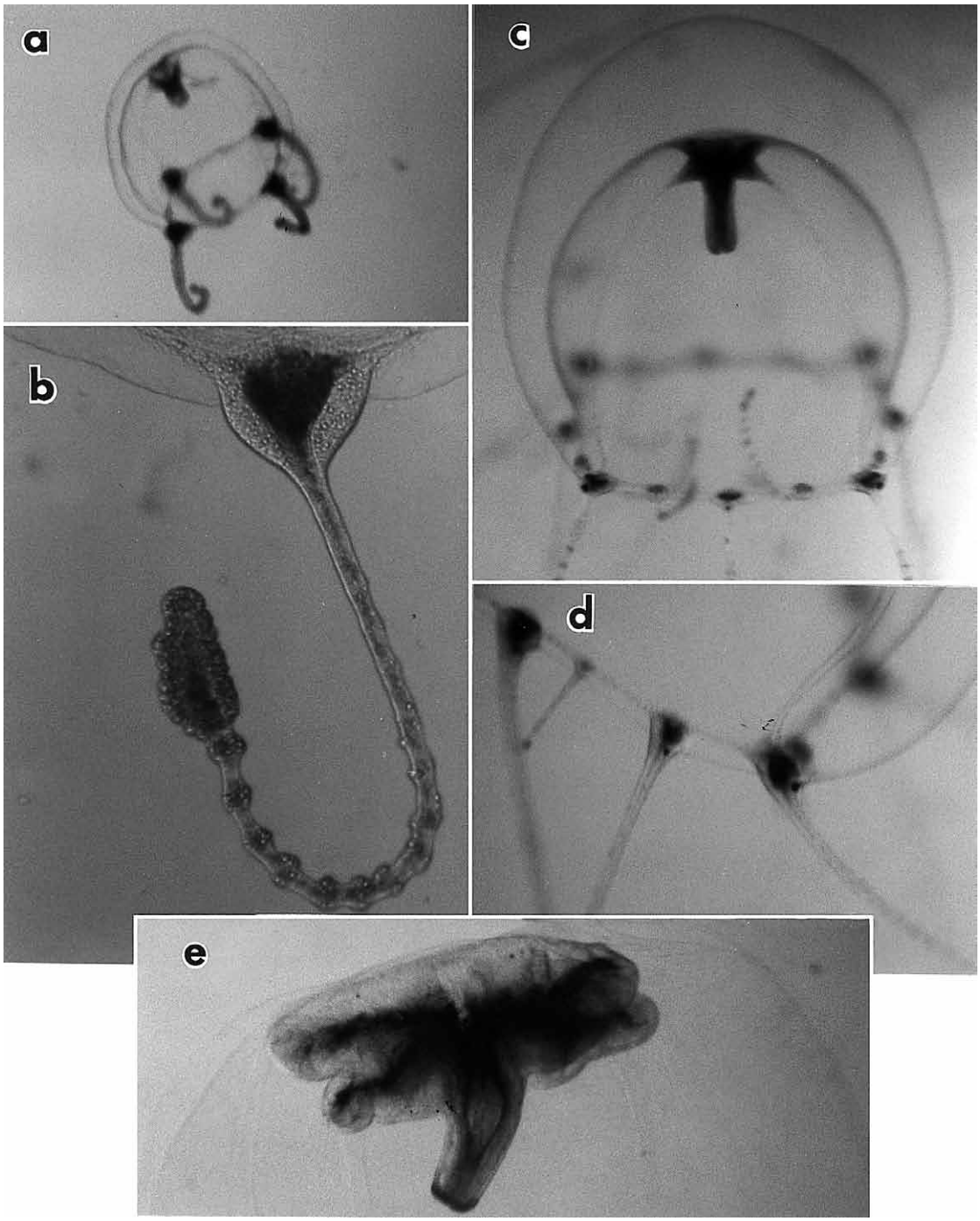

FIG. 4. – *Moerisia* sp., growth stages of the medusa. (a) Newly-released medusa, 1.0 mm bell height. (b) Close-up of one tentacle of newly-released medusa: note elongate terminal knob and nematocysts arranged in rings. (c) Ten-day old laboratory-reared medusa, 16-tentacle stage; bell height 2.0 mm. (d) Margin and tentacle bulbs on 24-day old laboratory-reared medusa, 4.0 mm in bell height: note the prominent ocelli. (e) Gonad of (d) appears to continue from radial canals onto the stomach wall.

been found in Chesapeake Bay in the eastern USA and designated *M. lyonsi* Boulenger, 1908 by Calder and Burrell (1967), morphological differences in both the medusa and polyp phases of the life cycle (discussed below) prevent us from assigning the Suisun Slough *Moerisia* to that species.

Morphology and Growth Stages of the Medusa (Figs. 4, 5)

Various size classes, including newly-released medusae, were collected from the plankton. Newly-released medusae (Fig. 4a) were about 0.8 mm in

bell height, as wide as wide, with four tentacles, each with a prominent tentacle bulb with red ocellus at its base, and with a terminal nematocyst knob. The nematocysts on the tentacles were arranged in rings (Fig. 4b). The mesoglea of the bell was uniformly thin, and the exumbrella was sprinkled with nematocysts. There were four radial canals. The simple tube-shaped manubrium extended about 1/4 into the subumbrellar cavity. There were no traces of gonads. In some newly-released medusae the beginnings of the tentacle bulbs in the next cycle of four tentacles were apparent. Statocysts were absent.

In those medusae raised in the laboratory from cultured polyps, age could be correlated with the size, tentacle number, and extent of gonad development in medusae from the plankton. Laboratory-reared medusae about 10 days old ranged in size from 1.5–2.5 mm bell height and diameter (Fig. 4c). The mesoglea in medusae of this size had gradually thickened, especially at the apex of the bell. Eight to sixteen tentacles and tentacle bulbs were evenly spaced around the bell margin, each bulb possessing a red ocellus. Tentacles arose from oval tentacle bulbs, and the upper margins of the bulbs were adnate to the exumbrella. Gonad development was just beginning on that part of the radial canals adjacent to the stomach. Laboratory-reared medusae of 24 days old (Fig. 4d,e) were about the same size as most of the largest found in the field, about 3.5–4.2 mm bell height, with 19–31 tentacles. Gonads extended only about 1/3 of the way down the radial canals in laboratory-reared medusae (Fig. 5a), but had the same general cruciform shape as those from the plankton (Fig. 5c and below).

The largest field-collected medusa was 8.1 mm in bell diameter and sexually mature, but most mature medusae ranged from 4.0–4.5 mm in bell height and diameter. The bell was dome-shaped and the mesoglea thick, especially at the bell apex (Fig. 5c). Gonads were cruciform in general appearance, and gonadal tissue extended from the base of the stomach along each of the four radial canals from 1/2 to 2/3 of the way to the ring canal (Fig. 5b,c). In some individuals, the distal ends of the gonads were somewhat pendant. The manubrium was simple and tubular, and extended only about 1/4 of the length of the subumbrellar space. The gonads appeared to extend continuously onto the upper part of the manubrial wall. The number of tentacles in mature medusae varied from 25 to a maximum of 32. There was a prominent red ocellus at the base of each tentacle bulb. The arrangement of nematocysts on the

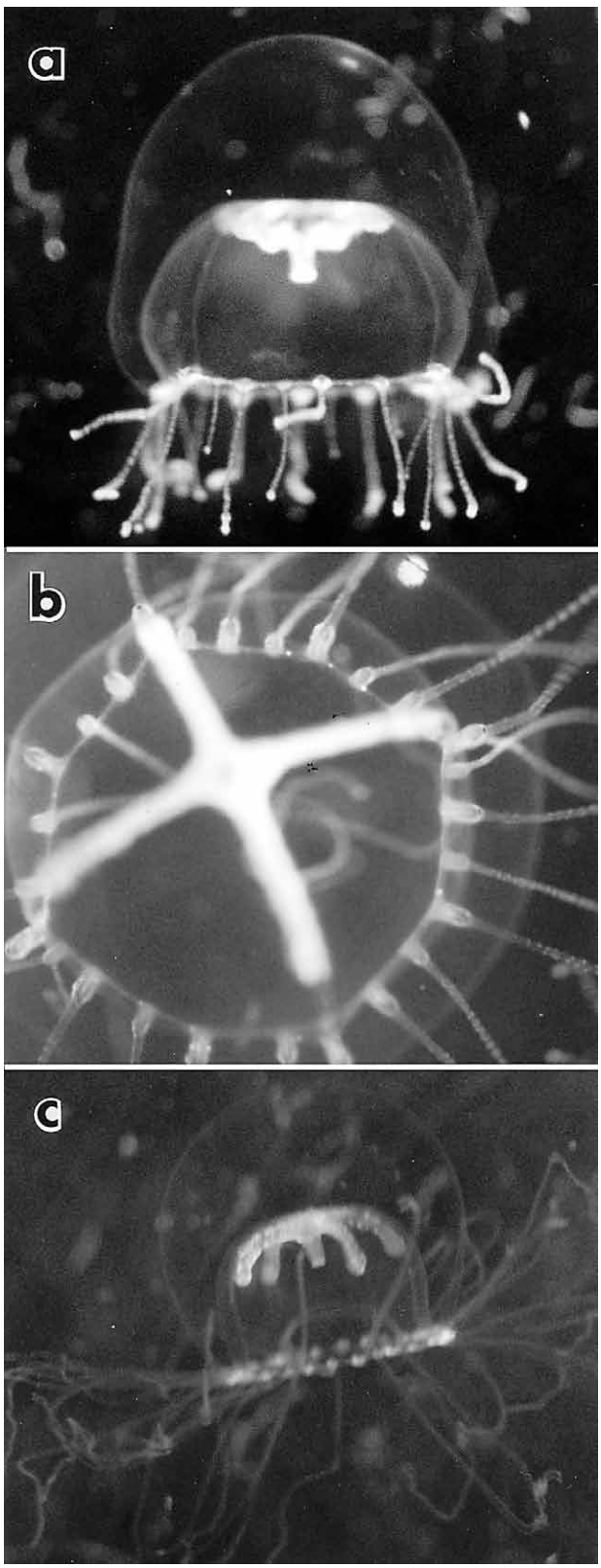

FIG. 5. – *Moerisia* sp., mature medusa. (a) Laboratory-reared medusa, lateral view, 4.0 mm bell height, 24 days old. (b) Field-collected medusa, aboral view, 4.5 mm bell height: note cruciform-shaped gonads. (c) Field-collected medusa, lateral view, 5.0 mm bell height.

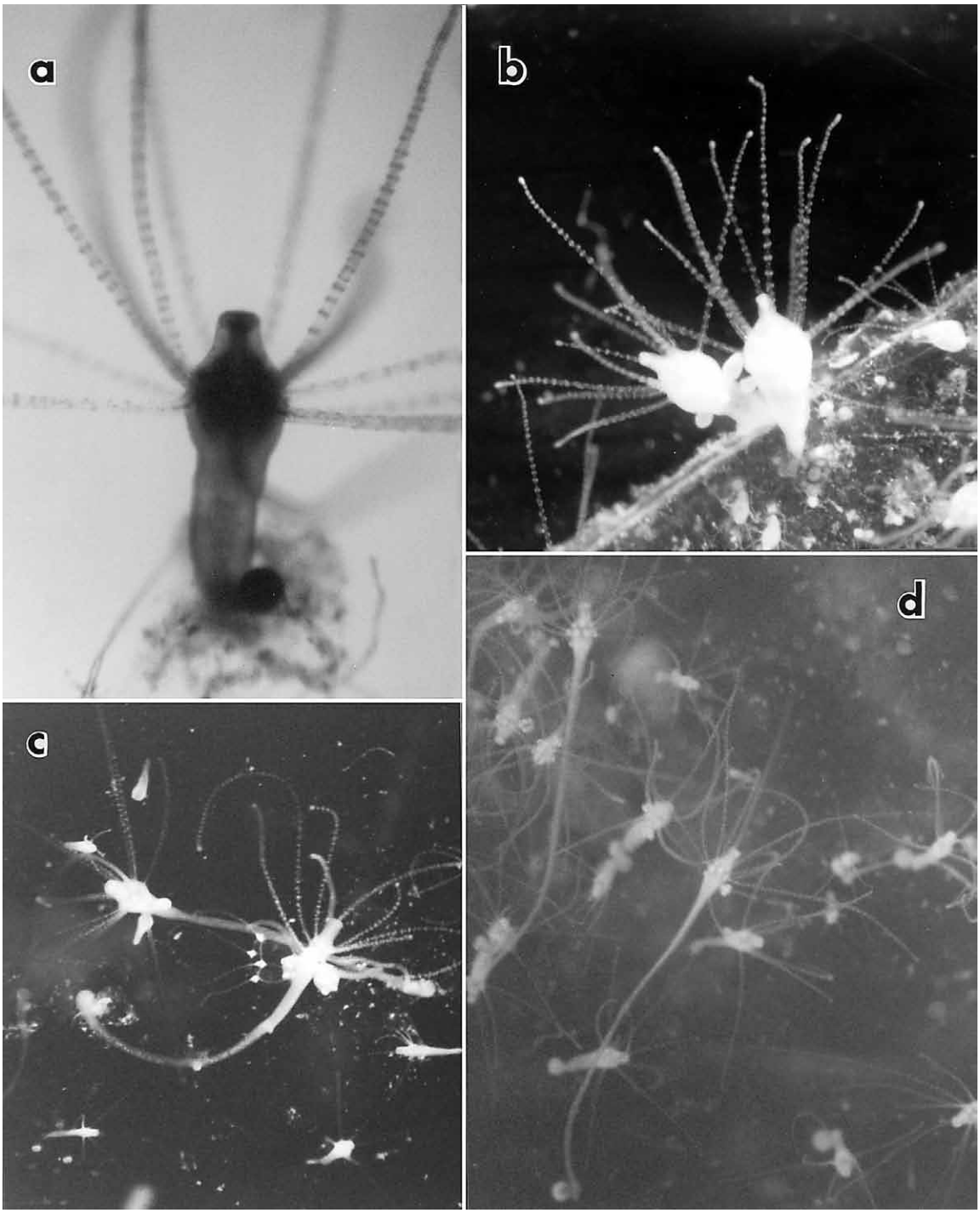

FIG. 6. – *Moerisia* sp. polyps, all reared in the laboratory. (a) Young polyp, 1 mm high: note nematocyst rings on tentacles and pedal disc at the base. (b) Two contracted polyps, 2 mm in height, both with planular buds: note slightly capitate nature of the tentacles. (c) Two expanded polyps, the larger hydranth bears a medusa, about 1 mm in diameter, ready for release; the hydranth at the left bears a planular body ready for release. (d) Group of polyps in various states of expansion; the longest polyps are more than 10 mm in length.

tentacles was usually in evenly-spaced rings, although occasionally nematocyst clasps (incomplete rings) were present. Translucent eggs were visible in the gonads of mature females, whereas mature male gonads appeared an opaque white color. The gonads and tentacle bulbs were opaque, and the mesoglea was transparent, rendering the medusae virtually invisible in turbid water. The cruciform opaque gonad, transparent mesoglea, and oval tentacle bulbs equipped with ocelli, coupled with a low salinity environment, allow adult *Moerisia* medusae to be easily recognized.

Behavior

Both laboratory-reared and field-collected *Moerisia* sp. medusae were relatively weak swimmers; medusae of all sizes were phototactic and pulsed towards a fixed light source in the laboratory. The medusae were occasionally observed positioned in mid-water in their culture containers, tentacles extended, presumably in a feeding posture, although in unaerated cultures *Moerisia* sp. medusae spent most of the time on the container bottom with tentacles extended. The medusae were very efficient predators, and any excess *Artemia* nauplii fed to them were quickly killed, but not necessarily consumed. Dead, uneaten nauplii fell to the bottom of the rearing containers and were removed immediately to prevent fouling of the cultures.

The polyp (Fig. 6)

Mature *Moerisia* sp. medusae from the field spawned readily in finger bowls. Eggs were opaque, about 100 μm in diameter, and sank to the bottom. Development into planulae occurred within 24-48 hours. Primary polyps were noted attached to the bottoms and sides of the bowls within 3-4 days after spawning. The primary polyps were minute, with a body diameter of about 0.1 mm. Three or four evenly-spaced tentacles surrounded the mouths of the polyps. The tentacles were very thin and highly extensile, and could stretch to a maximum of about 1 mm in length. The ends of each tentacle were slightly capitate. The primary polyps could capture and consume *Artemia* nauplii much larger than themselves. Newly hatched nauplii were occasionally eaten whole, but more often the highly expandable mouth of the polyp would engulf and digest a single naupliar appendage. As the polyps grew, they quickly began to consume entire nauplii of any size.

Polyps grew quickly, and within 2-3 weeks had attained heights of about 1mm (Fig. 6a). Nematocysts on the polyp tentacles were arranged in rings. The mouth, rimmed with nematocysts, was mounted on a conical hypostome. A pedal disc, or disc-shaped piece of tissue covered with perisarc, developed at the base of the hydranth at this early stage. The formation and release of planular bodies or buds, described by others who have observed *Moerisia*-type polyps (e.g. Uchida and Nagao, 1959), also began at this stage; the buds developed beneath the tentacles on the body of the hydranth (Fig. 6b). Within 1–2 weeks after release and settlement of the buds, they in turn developed into new polyps and produced their own buds. In this manner, dense mats of individual polyps developed in culture over 1–2 months.

In mature polyps, the medusa buds were formed among, or slightly below, the tentacles (Fig. 6c). While several (2–4) medusa buds were usually present on a mature polyp, development into liberated medusae occurred serially — only one medusa per polyp was released at any given time. Among the thousands of polyps observed in culture, virtually all were solitary, unlike the colonies of *M. lyonsi* polyps described by Purcell *et al.* (1999). Branching of polyps, or formation of double-headed polyps, was noted once, in an older, neglected culture (3–6 months old). Perisarc was never noted on any part of the hydranth, although older polyps developed several pedal discs covered by perisarc. The polyps could readily change shape, and the largest mature polyps were capable of great extension of the lower part of the hydranth, such that the polyp body appeared mounted on a thin thread. Such polyps were capable of extension up to 4 cm or more in length. The number of tentacles varied depending on the age of the polyp, with the youngest polyps having 3-4 tentacles and mature polyps with medusa buds having about 10 tentacles (Fig. 6d).

Cnidom

Nematocysts of *Moerisia* sp. polyps and medusae (Fig. 7c–f) was similar in complement and dimensions to those reported by Calder (1971) for *Moerisia lyonsi* from the Chesapeake Bay and by Uchida and Nagao (1959) for *Moerisia (=Ostroumovia) horii* from Japan. The polyp had both stenoteles (9–11 x 11–14 μm) and desmonemes (3–4 x 7–8 μm) on the tentacles, and stenoteles (7–12 x 9–16 μm) only on the lip of the mouth. The mature medusa had both stenoteles (7–10 x 8–12 μm) and desmonemes (3–4 x 7 μm) on the tentacles. The exumbrella of the newly-released medusa had small unidentified nematocysts (2–3 x 6–7 μm) that were never seen exploded, but were similar to the basitrich capsules figured by Uchida and Nagao (1959, p. 277) for *Moerisia horii*.

Systematics

The taxonomic status of the *Moerisia* found at Suisun City remains unresolved. It seems most similar to *M. lyonsi*, which however usually has only 4

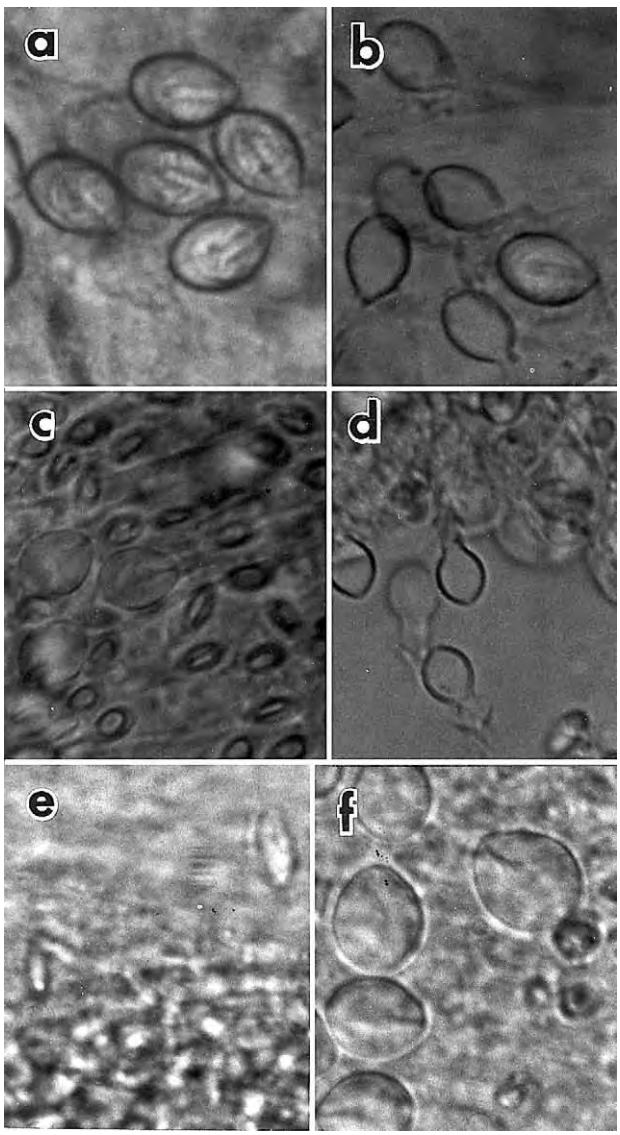

FIG. 7. – Nematocysts of *Maeotias marginata* and *Moerisia* sp.; see text for sizes. (a) Microbasic euryteles from tentacles of *M. marginata* adult medusa, unexploded. (b) Microbasic euryteles from tentacles of *M. marginata* adult medusa, exploded. (c) Larger stenoteles and more numerous smaller desmonemes from *Moerisia* sp. medusa, 1.3 mm bell height. (d) Exploded stenoteles from *Moerisia* sp. medusa, 1.3 mm bell height. (e). Basitrichs (?) from exumbrella of newly released medusa of *Moerisia* sp., unexploded. (f) Stenoteles and desmonemes from *Moerisia* sp. polyp tentacle, unexploded.

tentacles as an adult (Boulenger, 1908), with occasionally up to 22 (Kramp, 1961). We suggest, but have little evidence for, a scenario which would synonymize the genera *Odessia* and *Ostroumovia* with *Moerisia*, as proposed by Naumov (1960). Naumov lumped all three genera into two *Moerisia* species: *M. maeotica* and *M. pallasi*, found respectively in the Black and Caspian Seas (his monograph did not include *M. lyonsi*, which had not been collected in the USSR). Peterson (1990) retains both *Moerisia* and *Odessia* as separate genera. We suspect that the first *Moerisia* described, *M. lyonsi* from Lake Moeris in Egypt, is not unique and different from all other *Moerisia*s, because it seems very likely to be an introduction into the Nile basin. All *M. lyonsi* medusae described by Boulenger (1908) were males, indicating (as described for *Maeotias marginata* in the Petaluma River by Mills and Sommer, 1995) an entire population that could have descended from a single introduced male polyp. We suspect that other *Moerisia* medusae described from various parts of the globe, including *M. gangenica* in India and *M. lacustris* from fresh water in Trinidad, might also have been introduced, and that ultimately the family Moerisiidae may prove to contain only one or two species in the genus *Moerisia* with considerable morphological plasticity. Resolution of the taxonomic status of this genus will require collections in the Black and Caspian Seas, laboratory rearing studies, and ultimately genetic taxonomic work from specimens collected world-wide.

DISCUSSION

The San Francisco Estuary has the greatest number of recorded marine and aquatic introductions in North America, if not the world (Cohen and Carlton, 1995). There were probably no coelenterates (with the possible exception of *Hydra*) native to the low-salinity upper San Francisco Estuary prior to the Gold Rush in the mid-nineteenth century. The introduction of these two non-native hydrozoans probably represents the accommodation of a previously empty ecological niche by these secondary plankton consumers. Their potential and real effects on other native plankton and fish species, and the entire planktonic community structure of the upper Estuary, remains to be investigated.

There are few data on food preferences for either of these species, but both appear to specialize on crustaceans. Mills and Sommer (1995) reported that 70 field-collected adult *M. marginata* medusae had fed primarily on barnacle and copepod nauplii, planktonic and benthic copepods, and crab zoea larvae. Purcell *et al.* (1999) reported that Chesapeake Bay-resident *Moerisia lyonsi* in the laboratory demonstrated a clear preference for copepods and their nauplii.

Neither of these medusae is known to have predators, and both have the potential to invade all low-salinity San Francisco Estuary environments,

the sessile polyps of both species being transportable on the bottoms of boats of all sizes. The source and vehicle for the introduction of both of these species into the Estuary is unknown, but could have been ballast water from Black Sea or Chesapeake Bay ports being discharged into the Bay as described by Carlton (1985), or as part of a ship's fouling fauna. The San Francisco Estuary sees heavy commercial ship traffic from ports throughout East Asia, and a more likely introduction scenario might provide these hydrozoans arriving from an East Asian port into which they had been previously introduced, but not yet reported. Both species have the potential to invade any estuary world-wide with conducive environments, and additional reports and sightings elsewhere are to be expected.

Our lack of knowledge of the biology of both of these species precludes detailed predictions based on habitat preference and dispersal. Calder and Burrell (1967, 1969) reported *Moerisia lyonsi* in the Chesapeake Bay in salinities of 2.3 psu and *M. marginata* (as *M. inexpectata*) in salinities as low as 1.2 psu. Purcell *et al.* (1999) found *Moerisia lyonsi* in salinities from 0–5 psu in tributaries to the Chesapeake Bay. Laboratory studies on salinity and temperature preferences for all stages of the life cycles of both species are needed to fully assess their invasive capabilities.

The potential for severe ecological disturbance similar to that which befell commercial fish populations in the Black Sea following the introduction of the ctenophore *Mnemopsis leidyi* A. Agassiz, 1865 (Harbison and Volovik, 1994), could also result from hydrozoan introductions into estuarine habitats with depauperate faunas and vulnerable populations of native species. These nonindigenous hydrozoan species, including also *Blackfordia virginica* (see Mills and Rees, 2000), should be monitored as part of a Bay-wide effort to track both native and introduced coelenterate species. Monitoring results can be used to assess effects of introduced species on valuable fisheries resources, and to assess future efforts to limit further aquatic introductions.

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SCI. MAR., 64 (Supl. 1): 87-95 SCIENTIA MARINA 2000

TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Deep-water Hydromedusae from the Lacaze-Duthiers submarine canyon (Banyuls, northwestern Mediterranean) and description of two new genera, *Guillea* and *Parateclaia**

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SUMMARY: Several species of hydromedusae are reported from material collected by sediment traps placed in the Lacaze-Duthiers submarine canyon, off Banyuls (north-western Mediterranean). Two new taxa *Guillea canyonincolae* gen. nov. et sp. nov. and *Parateclaia euromarge* fam. nov., gen. nov. et sp. nov., are described. The existence of highly-specific hydromedusa populations in other Mediterranean canyons which appear to be related via geological history, topography, hydrographic and ecological features peculiar to each canyon, is discussed in relation to these new records.

Key words: Hydromedusae, submarine canyons, western Mediterranean, swimmers, deep-sea fauna, biodiversity, *Guillea canyonincolae*, *Parateclaia euromarge*.

INTRODUCTION

Interactions between the fluctuating continental runoff flows over the shelf and slope areas with abrupt topographies, together with local circulation and mass balance, give nearshore submarine canyons a key role in some coastal ecosystems, enhancing species richness and biological productivity (Hickey, 1995). The channeling of organic matter from the shelf to deep water through submarine canyons gives rise to high biomass levels and production rates in the plankton and benthos in such canyons (Greene *et al*., 1992; Vetter, 1994, 1995; Vetter and Dayton, 1998) resulting in biological communities in submarine canyons that are more productive and diverse than was thought only a short time ago (Gage and Tyler, 1992; Gage *et al*., 1995).

In the north-western Mediterranean, submarine canyons occupy nearly 50 % of the total continental slope. Recent investigations carried out in some of them have revealed a deep-water fauna composed mainly of meroplanktonic hydromedusae (see Gili *et al*. 1998, 1999, 2000). Spatio-temporal trends in the organic (carbon and biogenic silica) content of vertical fluxes of particulate matter have yielded a good match with the number of individuals and

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species of hydromedusae collected in these canyons (Gili *et al*., 2000). These observations led us to postulate the presence of unique plankton populations in these canyons, which are probably supported by the flux and storage of organic material coming from the continental shelf.

Previous studies carried out in three canyons (Fig. 1), Foix (near Barcelona), Lacaze-Duthiers (near Banyuls) and Planier (near Marseille) have shown that the specific composition and abundance of the medusa populations appear to be different between canyons (Gili *et al*. 1998, 1999, 2000). These studies covered an entire year in the Foix canyon while in other canyons, temporal trends had to be inferred from less sampling. The present study represents results derived from a second survey in the Lacaze-Duthiers canyon, carried out from December 1995 to January 1997. During that period, sediment traps collected several species of hydromedusae including the two new ones described here. Their occurrence is analysed, taking into account the previously proposed hypotheses (Gili *et al*., 1998, 1999, 2000), suggesting the existence of specific hydromedusa populations in each canyon investigated; such specificity seems closely related to the geological history, topography, and both hydrographic and ecological features of each of the studied canyons.

MATERIAL AND METHODS

A mooring line equipped with sequential sediment traps was deployed at a single station in the Lacaze-Duthiers canyon. One sediment trap was located at 1000 m depth, 30 mab (metres above the bottom) and another in intermediate waters 500 mab during a full-year sampling period (December 1995-January 1997). The sediment traps were Technicap model PPS3, which incorporates a carousel with 12 rotary collectors (Heussner *et al*., 1990). The sample collecting interval was set at 15 or 16 days, depending on the month. Before trap deployments, the sample tubes were rinsed and filled with a neutralized

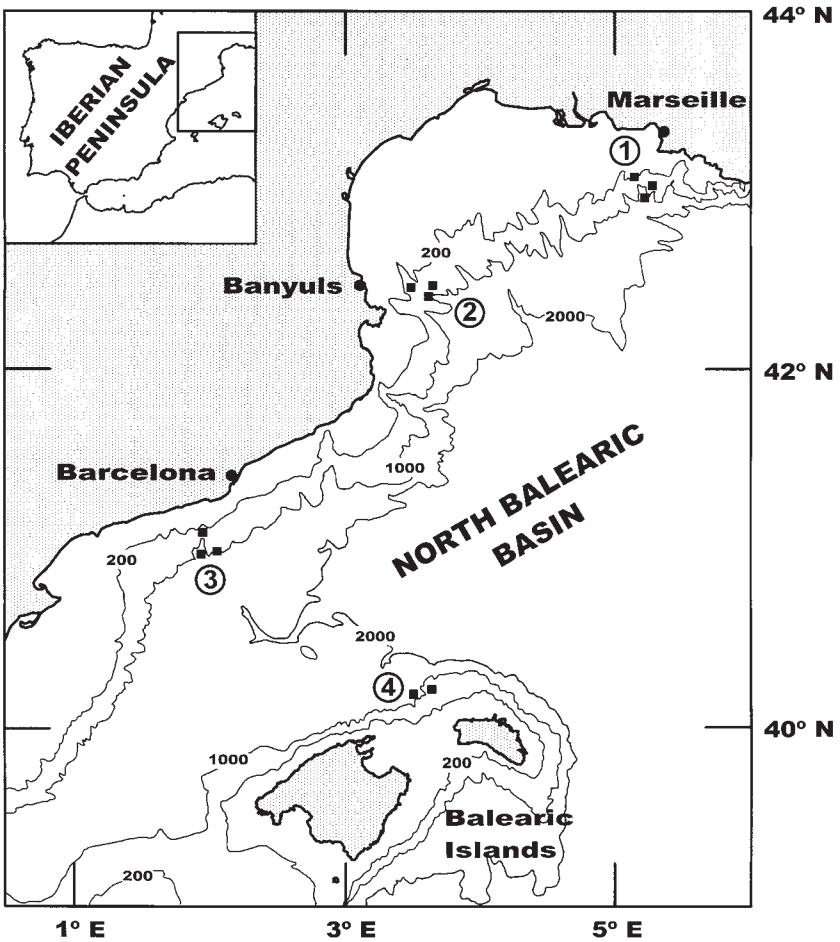

FIG. 1. – Map of the study-area, showing the location of the mooring sites (black squares) during the EUROMARGE-NB experiments. Site 1: Planier canyon; site 2: Lacaze-Duthiers canyon; site 3: Foix canyon; site 4: Balearic slope.

5% formaldehyde solution in filtered seawater to prevent degradation of organic matter between collection and the time that the traps were taken up. The samples were processed in the laboratory and swimmers were removed by hand-picking under a dissecting microscope. Gelatinous swimmers had been preserved in excellent condition, which facilitated taxonomic investigations of the hydromedusae.

RESULTS

Material collected and species descriptions

ANTHOMEDUSAE
FILIFERA
Family PANDEIDAE Haeckel, 1879

Leuckartiara brownei Larson and Harbison, 1990
(Fig. 2)

Material examined: 1 specimen, 7.0 mm high, 15-31 October 1996, 500 m depth.

This is the first record of this species in the Mediterranean. It was previously recorded only in the Southern Ocean, in surface waters of the Ross Sea (Larson and Harbison, 1990) and in the Weddell Sea where several specimens were collected from the surface down to the 720-450 m depth range (Pagès and Schnack-Schiel 1996; Pagès unpublished data).

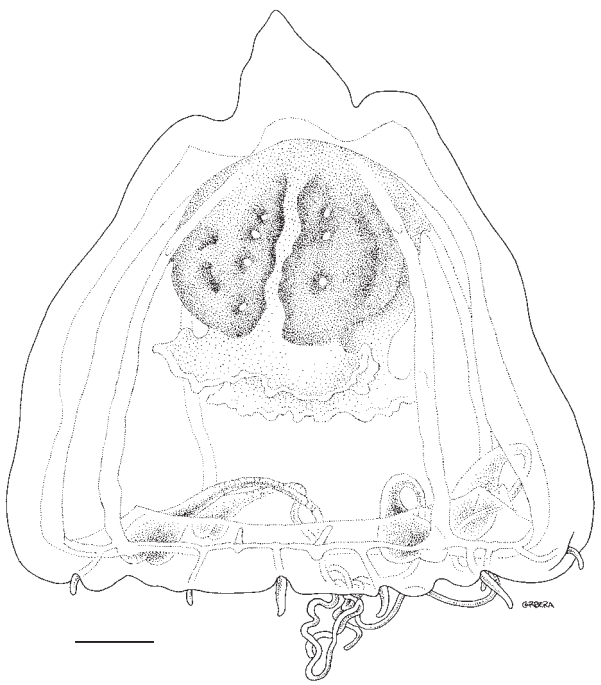

FIG. 2. – *Leuckartiara brownei*. Scale: 1 mm.

CAPITATA
Family EUPHYSIDAE Haeckel, 1879

Euphysa aurata Forbes, 1848

Material examined: 1 specimen, 3.3 mm high, 16 March-1 April 1996, 500 m depth.

Family ZANCLEIDAE Russell, 1953

Zanclea sp.

Material examined: 1 specimen, colour deep red, 3.6 mm high, 1-15 May 1996, 500 m depth.

LEPTOMEDUSAE
Family LAODICEIDAE Agassiz, 1862

Diagnosis: Leptomedusae with marginal cordyli with or without cnidocysts; with 4, 8, or more simple or branched radial canals; gonads on radial canals, on radial canals and lobes of manubrium or in manubrial pouches; marginal tentacles hollow; with or without marginal cirri; with or without adaxial ocelli; without statocysts.

Guillea gen. nov.

Etymology: This genus is dedicated to Prof. Alain Guille for his outstanding activities as Director of the Observatoire Océanologique de Banyuls (Laboratoire Arago) and for his relevant contribution to the knowledge of the Mediterranean marine fauna. Prof. Alain Guille is one of the founders of the *Laboratoire European des Sciences de la Mer* (LEA), within whose framework this and previous works related to medusae in submarine canyons have been carried out.

Diagnosis: Laodiceidae with club-shaped cordyli; with marginal cirri; with adaxial ocelli; with 4 simple radial canals; manubrium with perradial pouches, with gonads developing in numerous dorso-lateral lamellar folds (gonadal diverticulae) extending from the proximal part of manubrium into the manubrial pouches and out onto the proximal portions of the radial canals.

Remarks: This new genus has the basic characters of the family Laodiceidae. It is close to the genus *Laodicea* by virtue of its club-shaped cordyli, marginal cirri and ocelli; it also shows affinities with the genus *Ptychogena* by the presence of manubrial pouches and gonadial diverticulae. However, the genus *Ptychogena* has been classically defined as deprived of cirri and ocelli; therefore the new genus *Guillea* is here proposed. This new genus appears to have characters intermediate between the genera *Laodicea* and *Ptychogena*.

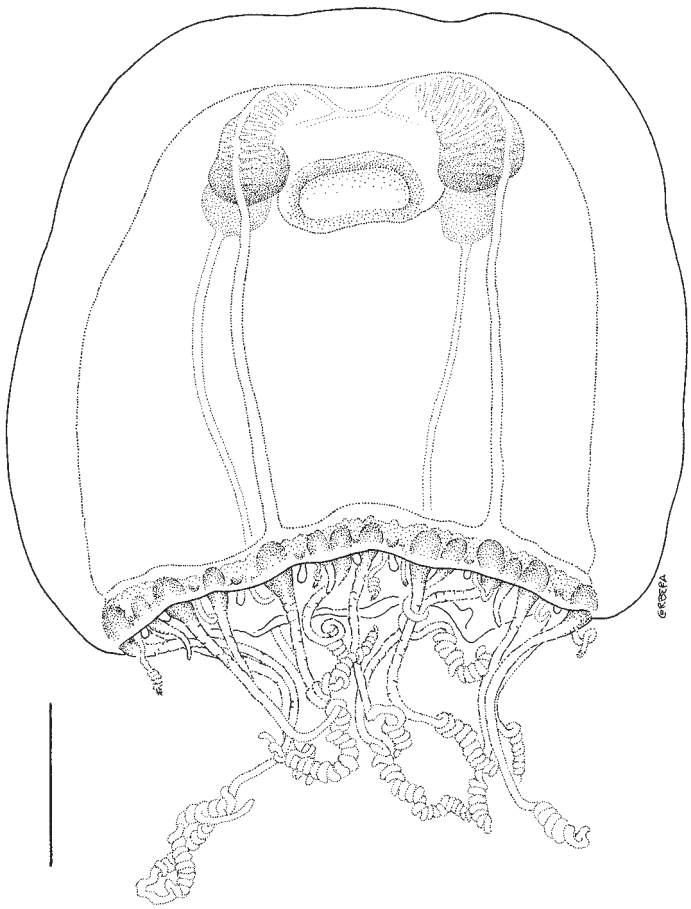

FIG. 3. – *Guillea canyonincolae*. Scale: 1 mm

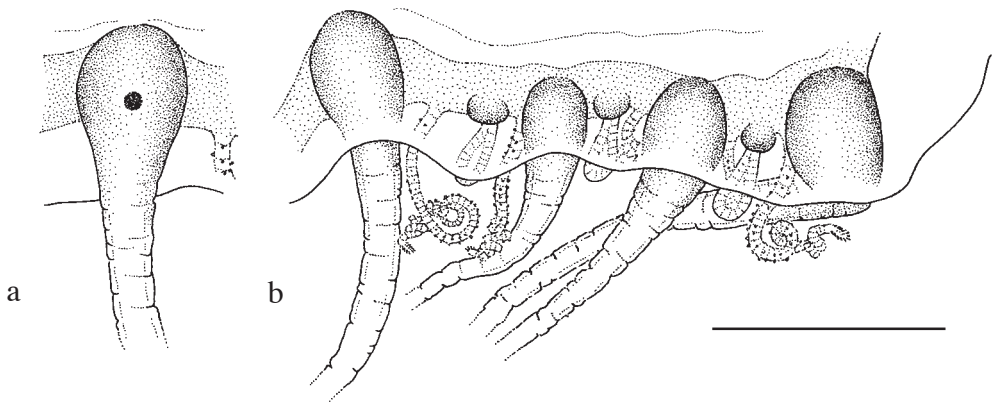

FIG. 4. – *Guillea canyonincolae*, detail of the umbrella margin; (a) adaxial view of the tentacle, showing ocellus; (b) abaxial view, showing club-shaped cordyli and spiral cirri. Scale: 0.5 mm.

Guillea canyonincolae sp. nov.
(Figs. 3 and 4)

Type locality: Lacaze-Duthiers canyon, off Banyuls (France), northwestern Mediterranean.

Material examined: one specimen collected at 500 m depth on 1-16 March 1996 and deposited at the Institut de Ciències del Mar, Barcelona, Spain.

Etymology: *canyonincolae*, inhabitant of canyons.

Description: Umbrella higher than a hemisphere, dome-shaped, 4.5 mm wide, 4.0 mm high; with vertical walls and flatly rounded apex, mesoglea uniformly thick; velum narrow; manubrium quadrangular, short, with four perradial gastric pouches; mouth large, almost circular, without distinct lips but with swollen margin, faintly folded; four simple narrow radial canals not meeting exactly in the center of

manubrial roof; circular canal narrow; four perradial gonads, differentiating into numerous dorso-lateral lamellar folds (gonadal diverticulae), extending from proximal part of manubrium into the gastric pouches to proximal parts of the radial canals; up to 24 marginal tentacles with coiled extremities; marginal bulbs broad, rounded; one club-shaped cordylus without cnidocysts and one to two spiral cirri between successive tentacles; one ocellus on the adaxial side of each marginal bulb.

Diagnosis: Umbrella higher than a hemisphere, dome-shaped, 4.5 mm wide, 4.0 mm high; with rounded apex, mesoglea uniformly thick; manubrium quadrangular, short, with four perradial pouches; mouth large, without distinct lips; four simple narrow radial canals; circular canal narrow; 4 perradial gonads differentiating into dorso-lateral lamellar folds (gonadal diverticulae) from proximal part of manubrium into the gastric pouches to the proximal parts of the radial canals; up to 24 marginal tentacles; marginal bulbs broad, rounded, each with one adaxial ocellus; one cordylus and one to two spiral cirri between successive tentacles.

Family MITROCOMIDAE Torrey, 1909

Foersteria antoniae Gili, Bouillon, Pagès, Palanques, Puig and Heussner 1998

Material examined: All specimens were collected at 500 m depth; 2 specimens, 16 February-1 March 1996; 2 specimens, 1-16 March 1996; 3 specimens, 16 March-1 April 1996; 3 specimens, 1-16 April 1996; 3 specimens, 16 April-1 May 1996; 3 specimens, 1-16 May 1996; 2 specimens, 16 July-1 August 1996; 3 specimens, 1 August-1 September 1996; 3 specimens, 5-6 mm wide, 1-16 September 1996. Size: 4.1-6.2 mm wide.

Family TECLAIIDAE fam. nov.

Diagnosis: Leptomedusae with 4 simple radial canals; with hollow tentacles; with 4 simple lips; with gonads elongated forming linear sacs on radial canals, separated from manubrium; with one to three cordyliform structures between successive tentacles; without ocelli; without cirri; with or without open statocysts.

Parateclaia gen. nov.

Diagnosis: Teclaiidae with open statocysts.

Parateclaia euromarge sp. nov.
(Figs. 5 and 6)

Type locality: Lacaze-Duthiers canyon, off Banyuls (France), northwestern Mediterranean.

Material examined: 3 specimens, 16 July 1996; 2 specimens, 1-16 August 1996; 2 specimens, 1-16 September 1996. All specimens were collected at 1000 m depth. Holotype and paratypes deposited at the Institut de Ciències del Mar, Barcelona, Spain.

Etymology: *euromarge* in acknowledgement of the European Community research program EUROMARGE which made possible the study of the medusan fauna in submarine canyons.

Description: Umbrella to 6.0 mm wide, 4.5 mm high; somewhat flatter than a hemisphere; mesoglea

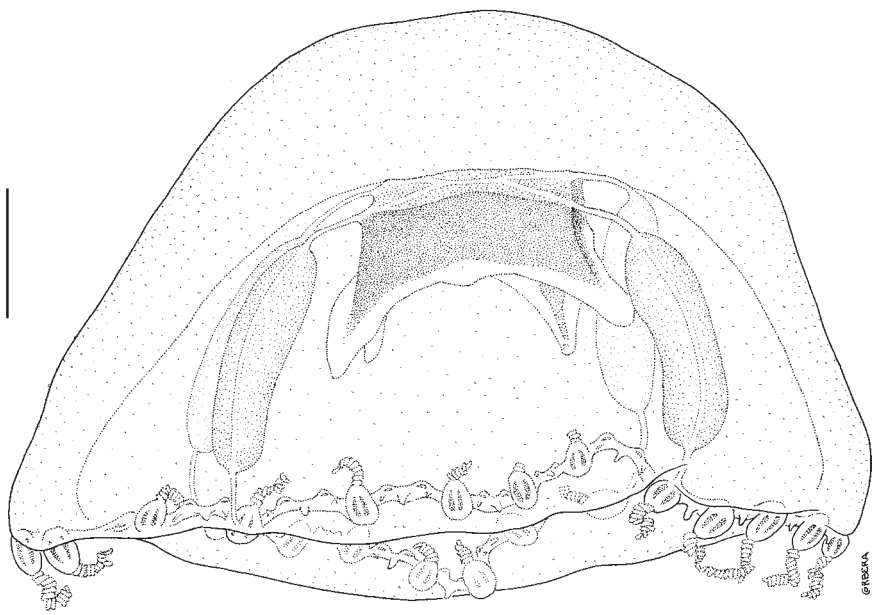

FIG. 5. – *Parateclaia euromarge*. Scale: 1 mm

fairly thick at the apex, thinning towards umbrella margin; exumbrella sprinkled with cnidocysts; velum narrow; manubrium short, square, with large base, about 1/4 of subumbrella cavity height and 1/3 of subumbrella cavity width, without gastric peduncle, colour light brown; mouth with 4 simple groove-like lips, white in colour; as long as manubrium height; with 4 simple radial canals not meeting in the centre of the manubrial roof, circular canal narrow; up to 24 hollow marginal tentacles; with elongated conical marginal bulbs each with two large brown bands; up to three cordyliform conical structures, each with central brown pigment spots and terminal cnidocysts; one to two open statocysts between successive marginal tentacles; gonads elongated, cylindrical, extending along the middle 2/3 of the radial canals and leaving both ends free.

Remarks: *Teclaia recincolae* Gili, Bouillon, Pagès, Palanques and Puig 1999 was tentatively referred to the Laodiceidae by Gili *et al.*, (1999). The discovery of this new species, which is nearly completely identical to *T. recincolae* except for the presence of open statocysts, questions the family position of this genus which is taken out from the Laodiceidae. Only two previously described families of Leptomedusae have open statocysts, the Mitrocomidae and the Tiaropsidae (Bouillon, 1985). The latter exhibits very special compound sense organs formed by the association of ocelli and an open statocyst which excludes it from the present discussion. The Mitrocomidae constitutes a uniform family mainly characterised by the possession of open statocysts and without cordyli. The inclusion of species without statocysts and with cordyliform structures would greatly disturb the definition of this family. We prefer to create a new family for these two very unusual deep-water species. *Teclaia recincolae* has been found in the Foix canyon, Catalán Sea at 1210-1180 m depth (see Table 1). None of the specimens observed (81) had open statocysts. All the specimens of *Parateclaia euromarge* have been found in the Lacaze-Duthiers canyon off Banyuls at 500 m depth and had open statocysts. As stated above these two genera are almost indistinguishable except for the presence/absence of sense organs. They presumably differentiated from a common ancestor, having been isolated by geological events in the above-mentioned canyons. Whether the original species did or did not have open statocysts is an insoluble question, but open statocysts are a character found in many deep-water medusae (see Gili *et al.*, 1998).

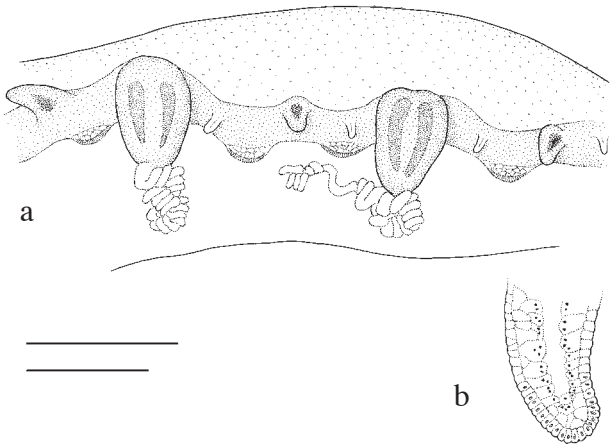

FIG. 6. – *Parateclaia euromarge*, detail of the umbrella margin: (a) abaxial view showing the elongated conical marginal bulbs, each with two large brown bands, the cordyliform conical structures, and the open statocysts between successive marginal tentacles. Scale: 0.5 mm ; (b) microscopic view of the cordyliform structures with terminal cnidocysts. Scale: 0.05 mm.

NARCOMEDUSAE
Family CUNINIDAE Bigelow, 1913

Cunina simplex Gili, Bouillon, Pagès, Palanques, Puig and Heussner, 1998

Material examined: 1 specimen, 1 August-1 September 1996; 1 specimen, 1-16 September 1996; 1 specimen, 1-16 October 1996. Size: 3.7-4.0 mm wide.

Solmissus albescens (Gegenbaur, 1857)

Material examined: 3 specimens, 1-16 January 1996; 1 specimen, 1-16 February 1996; 1 specimen, 16 April-1 May 1996; 1 specimen, 1-16 May 1996; 1 specimen, 1-16 December 1996; all these specimens were collected at 500 m depth. In addition one specimen collected at 1000 m depth on 1-15 December 1995. Size: 2.1-2.8 cm wide.

TRACHYMEDUSAE
Family PTYCHOGASTRIIDAE Mayer, 1910

Ptychogastria asteroides (Haeckel, 1879)

Material examined: 1 specimen, 16 March-1 April 1996; 1 specimen, 1-16 April 1996; 2 specimens, 16 April-1 May 1996; 4 specimens, 16 May-1 June 1996; 3 specimens, 1-16 June 1996; all these specimens were collected at 500 m depth. In addition one specimen collected at 1000 m depth on 1-16 October 1996. Size: 1-4 mm high.

Family RHOPALONEMATIDAE Russell, 1953

Homoeonema platygonon Browne, 1903

Material examined: 1 specimen, 1.4 mm high, 15-31 October 1996, 500 m depth.

Persa incolorata McCrady, 1859

Material examined: 2 specimens, 1.8-2.1 mm high, 1-16 June 1996, 500 m depth.

TABLE 1. – Species collected in the three submarine canyons investigated in the western Mediterranean. New species described are in bold. The species collected at stations located outside the canyons and cited in previous studies (Gili et al., 1998, 1999, 2000) are also listed.

	Foix	Lacaze-Duthiers	Planier	Stations outside of canyons
Foersteria araiae Gili, Bouillon, Pagès, Palanques and Puig, 1999	•			
Teclaia recincolae Gili, Bouillon, Pagès, Palanques and Puig, 1999	•			
Barcino foixensis Gili, Bouillon, Pagès, Palanques and Puig, 1999	•			
Solmissus albescens (Gegenbaur, 1857)	•	•	•	
Ptychogastria asteroides (Haeckel, 1879)	•	•	•	
Homoeonema platygonon Browne, 1903	•	•	•	
Calycopsis simplex Kramp and Damas, 1925		•		
Euphysa aurata Forbes, 1848		•		
Cunina simplex Gili, Bouillon, Pagès, Palanques, Puig and Heussner, 1998		•		
Solmaris flavescens (Kölliker, 1853)		•		
Leuckartiara brownei Larson and Harbison, 1990		•		
Guillea canyonincolae gen. nov., sp. nov.		•		
Parateclaia euromarge gen. nov., sp. nov		•		
Foersteria antoniae Gili, Bouillon, Pagès, Palanques, Puig and Heussner, 1998		•	•	
Persa incolorata McCrady, 1859		•	•	
Zanclea spp.			•	
Haliscera racovitzae (Maas,1906)			•	
Arctapodema australis (Vanhöffen, 1902)			•	
Sminthea eurygaster Gegenbaur, 1857				•
Amphinema rubra (Kramp, 1957)				•
Modeeria rotunda (Quoy and Gaimard,1827)				•
Cunina globosa Eschscholtz, 1829				•
Haliscera bigelowi Kramp,1947				•

GENERAL REMARKS

Recent investigations carried out in four western Mediterranean submarine canyons have shown that the specific composition and abundance of the hydromedusa populations differ between each canyon (see Table 1). These differences may be related to environmental factors which are summarized as follows:

1) The seasonal fluxes observed inside submarine canyons possibly enhance species abundance and the geomorphological structure of each canyon appears to have a great influence on its faunal composition. For instance, the narrowest canyons (such as Foix), having less communication with the open sea, appear to favour species isolation and so seemingly have induced greater speciation over evolutionary times.

2) Flux of biogenic components varies according to location and period of the year and it increases downstream (from Planier to Foix canyons), following the Northern Current. The number and abundance of endemic species also appears to increases from Planier to Foix canyons.

3) Seasonal distribution of the most abundant canyon hydromedusae, all of which are meroplanktonic, reflects the probable existence of a polyp or other resting stages, and a life-cycle adapted to the environmental fluctuations inside the canyons.

The highest number of species and individuals have been collected from March to mid-June in the traps located nearest the sea floor. However, the global number of medusae in Mediterranean canyons appears to remain quite constant, because some specimens have also been collected during summer in the intermediate-depth traps (Fig. 7). *Foersteria antoniae*, a species only reported from the Lacaze-Duthiers and Planier canyons (Gili *et al*. 1998) was present in the bottom trap during spring and early summer, while *Parateclaia euromarge* was present only during summer in the intermediate-depth traps of Lacaze-Duthiers canyon. The medusae collected during autumn and winter were common species like *Solmissus albescens* and *Ptychogastria asteroides* that have been recorded in all the Mediterranean canyons investigated (Gili *et al*. 1998, 1999, 2000).

In order to study relationships between environmental factors and species occurrence, temporal evolution of total mass fluxes inside the Lacaze-Duthiers canyon during 1996 was studied (Heussner unpublished data) (Fig. 7). The total mass fluxes (Fig. 7a) and the opal (biogenic silica) percentage (Fig. 7b) (derived from biological activity from the surface waters on the shelf) are higher in the spring when the number of both species and individuals of hydromedusae increased in the trap samples. The spring total flux peak (potential food supply to the

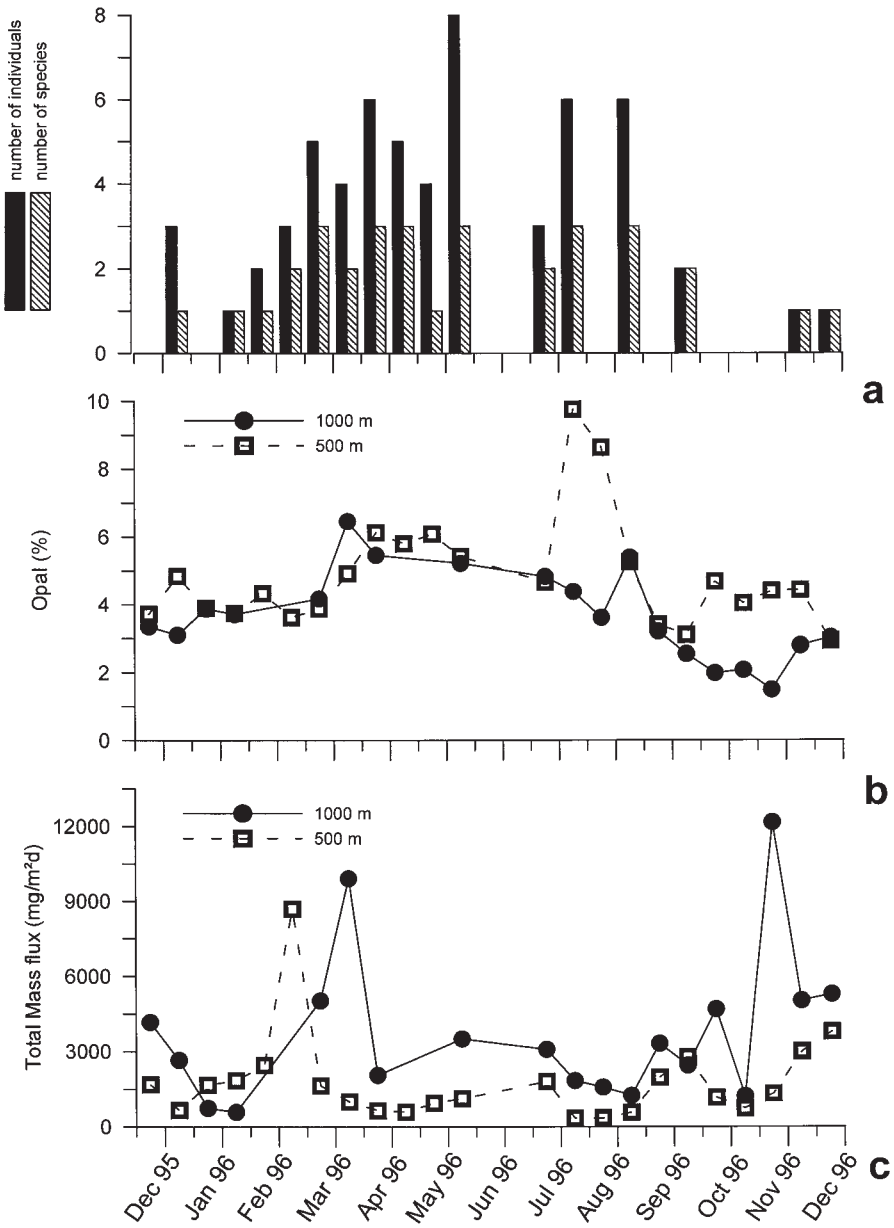

FIG. 7. – Lacaze-Duthiers canyon, 1996: (a) temporal evolution of both the number of individuals and the species of hydromedusae collected during the year; (b) time series of biogenic opal contents; (c) total mass fluxes of settling particulate matter traped 30 mab and 500 mab at the Lacaze-Duthiers canyon on 1000 m bottom depth.

canyon bed) appears to serve as a threshold episode which triggers medusa population growth. After the spring, medusan populations remain quite constant until early autumn, when both medusa abundance and opal flux decrease. The rather small increase of fluxes during the summer at the intermediate level coincides with the occurrence of *Parateclaia euromarge* in the traps. These general trends agree with previous observations in the Foix canyon (Gili *et al.* 2000), where the higher number of individuals and species appears after a peak of biogenic opal caused by the spring discharge of rivers. The observations at the Lacaze-Duthiers canyon seem thus to support the previously mentioned hypotheses concerning environmental control of the biodiversity of medusae inhabiting canyons

In general, the biological and environmental features of the submarine canyons in the north-western Mediterranean lead to the postulation that these habitats shelter a high and perhaps novel faunal diversity, known so far mostly by new species of hydromedusae. The endemic hydromedusan species of the Planier, Lacaze-Duthiers, and Foix canyons are closely related (see Table 1). For instance, *Foersteria antoniae* occurs

in low numbers in two canyons, Planier (with very few individuals) and Lacaze-Duthiers canyon, which could mean that both canyons are and/or were connected by currents. Both of those canyons are quite isolated from the southernmost canyon (Foix) where *F. araiae* is the endemic dominant species. More investigations in additional, and still-unstudied canyons located between Foix and Lacaze-Duthiers (near Blanes and Palamós) are needed to define possible biogeographical borders and whether their existence could be related with environmental constraints. The isolation of species could be related to the hypothesis mentioned in previous papers that this fauna appears to be characterised mostly by species that develop their entire life-cycle inside a canyon. These life-cycles seem to be governed by external factors such as transport of organic debris to the sea floor (related to river inputs, storms, etc.), interaction and circulation of water masses along with the canyon's topography, and biological production throughout the water column. These observations have led us to consider submarine canyons as key habitats for the understanding of biodiversity in the shelf and slope zones.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

The life cycle of *Halimedusa typus*, with discussion of other species closely related to the family Halimedusidae (Hydrozoa, Capitata, Anthomedusae)*

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SUMMARY: The little-known Anthomedusa *Halimedusa typus* has been collected from several locations in California, Oregon, and British Columbia on the Pacific coast of the United States. The adult medusa is redescribed based on new observations of living material and is found to have capitate tentacles. Polyps of *H. typus* were raised several times after spawning field-collected medusae in the laboratory; the cultures on one occasion lived for more than a year. The capitate polyp is solitary and very tiny, emerging from a basal perisarc measuring 200–300 μm in diameter. One cultured polyp produced a medusa, which is described. The taxonomic positions of several other morphologically-similar Anthomedusae in the Capitata are compared and discussed here. *Tiaricodon coeruleus* and *Urashimea globosa* are moved from the Polyorchidae to the Halimedusidae, and the similarity of *Boeromedusa auricogonia* (Boeromedusidae) to all of these medusae and to the genera *Polyorchis*, *Scrippsia* and *Spirocodon* of the family Polyorchidae is considered. The group of species under consideration is basically restricted to the Pacific Ocean, except for *T. coeruleus* and *U. globosa*, which have also been collected in the south Atlantic and south Atlantic/Antarctic. It is noted that medusae of the Halimedusidae are typically found quiescent near the surface, whereas those of the Polyorchidae either rest on the bottom or must continue pulsating to stay up in the water column, indicating a basic underlying difference in buoyancy and resultant behavior between the medusae in these two families.

Key words: *Halimedusa, Tiaricodon, Urashimea, Boeromedusa, Polyorchis, Spirocodon, Scrippsia.*

INTRODUCTION

Halimedusa typus Bigelow, 1916 is a little-known species whose medusa has only infrequently been collected and whose polyp has not been described. Its taxonomic position has been uncertain because so few live medusae had been observed. Until recently (Wrobel and Mills, 1998), the only images available in the literature (Bigelow, 1916, reproduced in Kramp, 1968; Arai and Brinckmann-Voss, 1980) were drawings of highly contracted, preserved specimens that were of limited value in recognizing live medusae or for understanding the taxonomic placement of the species. This paper derives from observations of live material collected in central and northern California; polyps were grown several times in the laboratory from field-collected medusae and one time produced a medusa.

The monotypic northeast Pacific genus *Halimedusa* was originally placed, with some trepidation, in the family Pandeidae by Bigelow (1916), and remained in this catch-all family (Kramp, 1961, 1968) until it was removed and assigned its own family in the suborder Filifera by Arai and Brinck-

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mann-Voss (1980; Brinckmann-Voss and Arai, 1998). It has been mentioned only occasionally in the literature since its original description except in summary lists, with only a little new information (McCormick, 1969 as "moerisiid"; Rees, 1975; Arai, 1987). *Halimedusa* and the Halimedusidae are here moved to the suborder Capitata, within the Anthomedusae, based on new information about morphologies of both the medusa and polyp phases of its life cycle and the cnidom.

Also considered here is the southern Atlantic and Pacific *Tiaricodon coeruleus* Browne, 1902. This monotypic genus was originally placed in the Anthomedusae, family Tiaridae (Browne, 1902), was then moved to the Anthomedusae, Codonidae (Mayer, 1910), and later to the Limnomedusae, Moerisiidae (Browne and Kramp, 1939; Kramp, 1959, 1961; Zhang, 1982). *Tiaricodon* has most recently been moved to the Polyorchidae (Petersen, 1990; followed by Schuchert, 1996). In the present paper, *Tiaricodon* is moved once more, to the family Halimedusidae, based on similar characters of the medusae.

Urashimea globosa Kishinouye, 1910, another monotypic Pacific genus, apparently endemic to Japan, is also moved here into the Halimedusidae. This genus was originally placed in the Anthomedusae, Cladonemidae (Kishinouye, 1910), but was eventually moved into the catch-all Pandeidae by Uchida and Nagao (1961). *Urashimea* remained for many authors in the Pandeidae within the Filifera (e.g. Kubota, 1998), but has also been subjected to other taxonomic relocation several times, most recently having joined *Tiaricodon* and others in the family Polyorchidae (Bouillon, 1995 - which gives the full history).

The relationships of all of the above species to the Polyorchidae (which will henceforth contain only *Polyorchis*, *Scrippsia* and *Spirocodon*) are discussed.

MATERIALS AND METHODS

Medusae of *Halimedusa typus* were collected in Bodega Bay and Bodega Harbor, California, and in Yaquina Bay, Oregon in the 1970s, and in Humboldt Bay, California in 1991. In Bodega Harbor, *H. typus* medusae were collected at both Mason's Marina and at the Coast Guard Station floating docks. In Humboldt Bay, they were collected off the floating docks of the Woodley Island Marina, Eureka, California. The medusae were individually hand-collected using glass bowls or beakers, or were taken in short plankton tows.

The *H. typus* medusae were transported back to the laboratory and were set up in bowls of fresh seawater for spawning, incubated at ambient seawater temperature of about 11°C. Medusae were removed from the bowls after spawning, and planulae were allowed to settle either directly onto the surface of the glass bowls or on glass slides placed on the bottom of the bowls. Polyps that developed from the settled planulae were maintained in these bowls, incubated in a running seawater table at ambient temperature (9-12°C), and offered a variety of potential prey.

Nematocysts were examined in squash preparations of live material, with measurements taken of undischarged capsules at 1000 times magnification.

RESULTS

Systematic account

Class HYDROZOA
Order ANTHOMEDUSAE-ATHECATA
Suborder Capitata Kühn, 1913
Family HALIMEDUSIDAE Arai and Brinckmann-Voss, 1980

Halimedusa typus Bigelow, 1916

Description of the adult medusa (Fig. 1A). Up to 16 mm tall and 13 mm wide, jelly thick, especially at the apex. Manubrium suspended from a broad, low peduncle; cruciform and extending along each radial canal until the point where the radial canals turn down the subumbrellar wall; typically hanging about half-way down the subumbrellar cavity. Mouth small and quadrate, studded with a row of tightly-packed round nematocyst batteries around its rim. Gonads cover the entire surface of the manubrium, with flat, not folded, surface, typically whitish in color, with a dark brown horizontal stripe, which parallels the base a bit down from the top, sometimes coalescing into a nearly black spot at the intersection of each radial canal. With 4 straight, smooth-sided, narrow, radial canals and four characteristic interradial "peaks" in the jelly between radial canals, rising up above the level of the radial canals. With 4 hollow perradial tentacles and 4 interradial groups of up to 10–11 smaller hollow tentacles, all

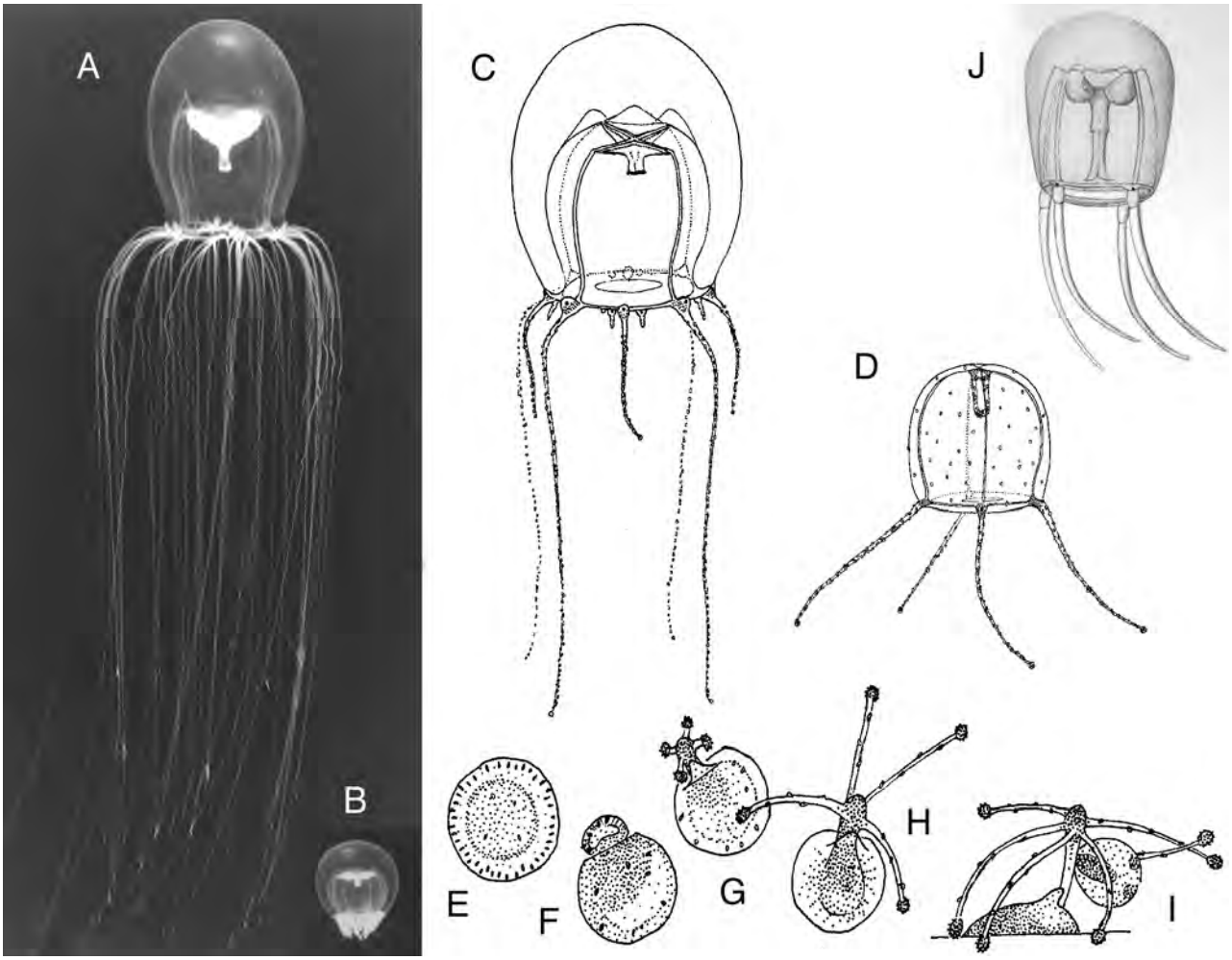

FIG. 1. – A-I *Halimedusa typus*. J *Tiaricodon coeruleus* (reproduced from Browne and Kramp, 1939, with the permission of Cambridge University Press). A, mature medusa; B-C, developing medusa stages; D, newly-released medusa; E, settled planula; F and G, primary polyps; H and I, mature polyps; I, polyp with developing medusa bud; sizes given in text.

with red-orange to purple-black ocelli on the outer, abaxial side of their basal bulb, but without the additional splash of red pigment typical of the bulbs of the Polyorchidae; the core of each tentacle bulb is blackish-brown. Tentacles are covered with scattered nematocyst batteries which begin a short distance below the bulb and terminate in a small round cluster that is only evident when the tentacles are relaxed. Bell very transparent in life.

Nematocysts of the medusa (Fig. 2A–C). Desmonemes, stenoteles, and microbasic mastigophores were observed in the tentacles of four adult medusae measuring 8–13 mm in bell height. Nematocysts on the tentacles are arranged in spherical batteries that are scattered unevenly over the length of the tentacles. Desmonemes (Fig. 2B), the most common nematocysts, measured undischarged 7.6–10.5 μm tall x 3.8–5.0 μm wide (n=15); stenoteles (Fig. 2A) were uncommon and measured undischarged 12.0–14.5 μm tall x 9.0–11.4 μm wide (n=15); microbasic mastigophores (Fig. 2C) were uncommon and measured undischarged 9.0–12.3 μm tall x 7.0–9.9 μm wide (n=15). The tentacle bulbs contained primarily stenoteles (not measured), with a few desmonemes and microbasic mastigophores. Lips of the manubrium were lined by a row of spherical nematocyst batteries, which contained only stenoteles that measured undischarged 10.4–12.4 μm tall x 8.6–9.5 μm wide (n=10) in a medusa that was 13 mm tall. Stenoteles were also scattered in the tissue of the manubrium itself.

Remarks. This report differs in several important respects to Bigelow's original description (1916), which was reiterated by Kramp (1961, 1968).

Bigelow's preserved specimens had contorted so as to fold and twist the manubrium and gonad region, and Bigelow noted the irregularity of these folds; in fact all living specimens had smooth, unfolded gonads. The tentacles of Bigelow's preserved specimens were highly contracted, so that he was unable to see the nematocyst clusters throughout the tentacle length and the small terminal nematocyst cluster, visible only on very relaxed tentacles (Fig. 1A,C,D).

Distribution and behavior of medusae. Known from the Queen Charlotte Islands and the west coast of Vancouver Island in British Columbia, and bays on the outer coast of Oregon and northern to central California, in the summer and autumn. In all cases, the medusae were collected near the surface, where they are typically quiescent, bell upright, with tentacles outstretched in a fishing posture. All known collections are cited below, from north to south on the Pacific coast:

Masset Inlet, Queen Charlotte Islands, British Columbia
12–19 June 1984, four, surface plankton tow, Arai, 1987.
Amphitrite Point, west coast of Vancouver Island, British Columbia
12 Sept. 1914, three, at the surface, Bigelow, 1916.
Yaquina Bay, Oregon
18 August 1967, one (as "moerisiid"), McCormick, 1969
10 July 1968, several (as "moerisiid"), near the surface, McCormick, 1969
25 July 1968, one? (as "moerisiid"), near the surface, McCormick, 1969
Sept.-Nov. 1971, several, near the surface, R.J. Larson, collector
12 July 1973, near the surface, R.J. Larson, collector
July 1974, near the surface, R.J. Larson, collector
Bodega Bay, California
25 July 1972, one, surface plankton, J.T. Rees, collector
27 July 1973, one, surface plankton, J.T. Rees, collector
Bodega Harbor, California
11 Oct. 1974, one, at the surface, with *Bythotiara stilbosa*, C.E. Mills, collector
15 Oct. 1974, one, at the surface, with *Bythotiara stilbosa*, C.E. Mills, collector
20 Nov. 1978, two, at the surface, with *Polyorchis penicillatus* and *Eutonina indicans*, C.E. Mills, collector
23 Nov. 1978, 22, at the surface, with *Polyorchis penicillatus*, *Aglauropsis aeora* and *Eutonina indicans*, C.E. Mills, collector
Humboldt Bay, California
25 Oct. 1991, ~ 50 at the surface, with *Polyorchis penicillatus* C.E. Mills, collector

Development. Female medusae spawned 1–3 hours after dark, producing about 500–700 eggs per female. The eggs were 110–120 μm in diameter and colorless. The eggs and planulae initially floated high near the water surface, but eventually the planulae sank (many hours later). The planulae were elongate, about 225–250 x 100–120 μm, and completely ciliated, without nematocysts. Planulae began settling in glass bowls after two days; most settled individually, with little clumping. The flat, settled planular disks were 200–300 μm in diameter (Fig. 1E). About 50 nematocysts (of the isorhiza/anisorhiza type, below) developed in each settled planular disk, primarily near the edge and oriented in a radiating pattern. Within two days of settlement, the central portion of the settled disk thickened and a slight bulge containing two types of nematocysts became evident on the side of each disk as the primary polyp began to form (Fig. 1F). In all cases, the primary polyp emerged out of the side of the disk; 3–4 small capitate tentacles developed within hours of the emergence of this polyp (Fig. 1G), about 3 days after settlement. A small, blunt thecal spine formed over the base of the polyp. The "footprint" of the fully-formed solitary polyp remained the same as that of the original settled planula.

Description of the polyp (Figs. 1H, 1I). The polyps remained very small and solitary, with a small circular thecal base that had a short, fingerlike, protective perisarcal extension, or spine, above the polyp. Most polyps had four capitate tentacles, but occasionally had three, or up to eight tentacles; the tentacles were arranged in a single whorl just below a rounded-cone shaped hypostome. Each tentacle had a capitate tip with terminal nematocyst cluster and also had a few nematocysts scattered along its length. The hydranth was 150–200 μm in length and the most robust specimens had tentacles about twice that length (Fig. 1I). Within the basal perisarc, the central portion contained the opaque base of the polyp, with a transparent outer "rim" area characterized by fine, radiating fiber-like structures.

Nematocysts of the polyp (Figs. 2D–E). Nematocysts were not seen in 17-hour old swimming planulae viewed with a compound microscope, but were first evident around the border of settled planulae several days after fertilization. These elongate nematocysts looked like the isorhiza/anisorhizas found later in the polyps, but were not seen discharged; undischarged they measured 5.7–7.6 μm tall x 1.9–2.8 μm wide (n=10). The polyps contained 2-3 types of nematocysts in their tentacles. Stenoteles (Fig. 2D) were the most common and measured undischarged 6.2–7.6 μm tall x 4.3–5.7 μm wide (n=10). There were also a few elongate nematocysts (Fig. 2E) that were either basitrichous isorhizas or anisorhizas or both; these measured undischarged 6.6–7.6 μm tall x 1.9–2.4 μm wide (n=10) and 4.7–5.7 μm tall x 1.9–2.4 μm wide (n=6).

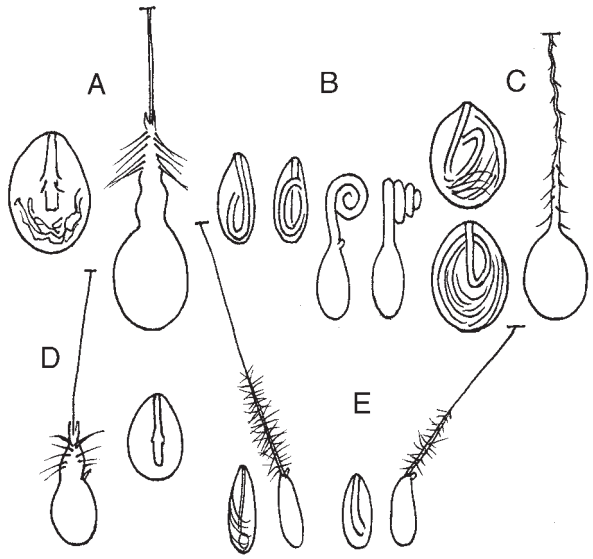

FIG. 2. – Nematocysts of *Halimedusa typus*; A–C, medusa; D–E, polyp. A and D, stenoteles; B, desmonemes; C, microbasic mastigophores; E, basitrichous isorhizas or anisorhizas; drawn to scale, sizes given in text.

Polyp behavior and ecology. The polyps ate small nematodes and rotifers in the culture dishes. They were too small to feed on *Artemia* nauplii or harpacticoid copepods, typical foods for many cultured hydroids; they also did not capture very small (80 μm) sea urchin or polychaete eggs that were offered. When a polyp was disturbed, it contracted slightly, rolling its tentacles inward, but it could not withdraw entirely within the basal perisarc. The longest-lived polyps persisted in the laboratory for more than one year. The polyps are so small and colorless that the possibility of finding them in the field is very slight.

Medusa development. Seven months after settlement, in late June and early July, two polyps each developed a single medusa-bud, attached just below the whorl of tentacles. One of these buds matured, reaching a diameter of about 250 μm (Fig. 1I); the newly-released medusa was 0.55 mm in both bell height and diameter (Fig. 1D). This medusa had scattered nematocysts over its entire bell-surface and had four tentacles, each about as long as the bell height, with scattered nematocysts along the length and a round terminal nematocyst cluster. The manubrium and mouth also had scattered nematocysts on them. The medusa was an active swimmer and had a strong crumple response when disturbed, pulling the tentacles inside its contracted bell. It did not feed and died within 4 days of its release. A small *Halimedusa typus*, 4 mm in bell height with 8 tentacle bulbs, was collected in the plankton in late July; this specimen developed 16 tentacles and was 5 mm tall after 10 days of culture in the laboratory. (Arai [1987] reports very small *H. typus* medusae in early July in British Columbia, and the earliest collections of *H. typus* medusae in Yaquina Bay were also in July [Larson, see above]). A small 16-tentacled medusa that was 5 mm in bell height was collected in November (Fig. 1C), indicating that medusa bud production may take place over a prolonged period in the summer and early fall.

Tiaricodon coeruleus Browne, 1902

Description of the medusa. The abbreviated description of *T. coeruleus* from Kramp (1968, p. 103) reads as follows: "24 mm wide, 25 mm high, jelly thick, bell-shaped; stomach nearly to velar level, four lips distinct, crenulated; radial lobes sac-like, on peduncle only; four perradial tentacles, stout, tapering, nematocysts in proximal part in small, rounded warts, in middle part forming transversal clasps, in distal part rings; an abaxial ocellus. Polyp unknown." Browne and Kramp (1939, p. 312) add that "The gonads occupy the lobes of the stomach and also extend about half way down the manubrium." Many more details are given by Browne and Kramp (1939), whose illustration (Fig. 1, Plate XVIII) is reproduced here as Figure 1J. The nematocysts of *T. coeruleus* medusae are reported to be stenoteles, desmonemes and heteronemes (Schuchert, 1996).

Distribution of medusae. This species occurs primarily in the southern hemisphere, having been collected (details follow) in the South Atlantic at the Falkland Islands and the coast of Argentina, in the South Pacific in several locations in South America, China and New Zealand, and in the Weddell Sea in the Southern Ocean; it is also present in at least two NW Pacific locations in China. The collection data imply that it may occur nearly year-round, but is especially abundant in the austral summer (even when collected in northern hemisphere China): Stanley Harbor, Falkland Islands, S Atlantic, November–March, 1898–1902, numerous specimens (Browne, 1902; Browne and Kramp, 1939); Weddell Sea, S Atlantic/Antarctic, 2 February 1902, three specimens (Kramp, 1948); Straits of Magellan, Chile, SE Pacific (Vanhöffen, 1913); Callao, Peru, SE Pacific, September 1883, one specimen (Vanhöffen, 1913); Valparaíso Bay, Chile, SE Pacific, 15

August 1958, one specimen (Kramp, reported by Schuchert, 1996); coast of Argentina, SW Atlantic, no date given (Kramp, 1968); Xiamen Harbour, China, NW Pacific, Dec. 1980–April 1981, numerous specimens (Zhang, 1982; Lin and Zhang, 1990); Shandong Province, Huanghai Sea, China, NW Pacific, no date given (Zhang, 1982); Wellington Harbor, New Zealand, S Pacific, 15 June 1994, 1 specimen (Schuchert, 1996).

Behavior and ecology of medusae. Like *Halimedusa typus*, *T. coeruleus* is typically seen oriented upright, a few inches below the surface, quiescent with tentacles streaming out very far; adult specimens were collected in November, with young stages appearing along with other size classes in January (observations from the Falkland Islands, reported in Browne and Kramp, 1939).

Remarks. I propose to move *Tiaricodon coeruleus* from its most recent resting place in the Polyorchidae (Petersen, 1990; Schuchert, 1996) to the Halimedusidae. The rationale for this move is presented in the Discussion, below. In order to accommodate *Tiaricodon coeruleus* in the Halimedusidae, the description of this family must be modified (see below).

Urashimea globosa Kishinouye, 1910

Description of the medusa. The abbreviated description of *U. globosa* from Kramp (1968, p. 52) reads as follows: "Up to 16 mm high, slightly higher than wide, bell-shaped or globular; exumbrella with numerous (up to about 36) meridional lines of nematocysts; manubrium short, four-sided, four frilled lips with nematocysts; gonads 8-16 sac-like protruberances in walls of stomach; four long tentacles with numerous stalked nematocyst knobs in their entire length; with abaxial ocelli." Additional details are given by Kishinouye (1910) and Uchida and Nagao (1961), who both collected specimens 17 mm high. The nematocysts of *U. globosa* have not been published.

Distribution of medusae. This species has been collected in many locations from central to northern Japan, including Hokkaido, and on Sakhalin Island (Kishinouye, 1910; Kramp, 1961; Uchida and Nagao, 1961). It was also reported in Amoy, China (Chiu, now Qiu, cited by Kramp 1961), but this location was not confirmed by Zhang (1982). Forty-five *U. globosa* medusae were collected in plankton samples from in St. Helena Bay, South Africa in 1991 and 1993 (Buecher and Gibbons, 2000). Whether this species is indigenous or recently introduced to South Africa cannot be determined, but the likelihood of an introduction there via shipping or other human-mediated processes is possible, if not probable.

Description of the polyp (from Uchida and Nagao, 1961). Polyp lacking periderm emerges from thin, circular membrane measuring 0.2 mm diameter. Polyp measures 0.2-0.3 mm in height, with cone-shaped hydranth not distinctly demarcated from the hydrocaulus; with 4 or 5 filamentous tentacles, each terminated by a small but distinct nematocyst knob, but without nematocyst rings along the shaft.

Remarks. I propose to move *Urashimea globosa* from the Polyorchidae (Bouillon, 1995) to the Halimedusidae. The rationale for this move is presented in the Discussion, below. In order to accommodate *Urashimea globosa* in the Halimedusidae, the description of this family must be modified (see below).

Family HALIMEDUSIDAE

The family Halimedusidae Arai and Brinckmann-Voss, 1980 (p. 62) was originally described, including only *Halimedusa typus*, as follows: "Anthomedusae with four radial canals; with subumbrella protruding into stomach giving a peduncle-like appearance; mouth cruciform with row nematocysts; with four perradial tentacles and four interradial groups of tentacles, each hollow and lacking adhesive organs; with marginal bulbs with abaxial ocelli."

The revised family Halimedusidae is now modified in order to also accommodate *Tiaricodon coeruleus* and *Urashimea globosa* and described as follows: Anthomedusae with four radial canals; with low peduncle and with distinct interradial peaks in jelly above base of the manubrium; gonads extending out from the manubrium as lobes below the upper portions of the four radial canals, but without mesenteries; quadratic mouth with lips lined by a row of sessile nematocyst clusters; with four perradial hollow tentacles or with four perradial tentacles and four interradial groups of hollow tentacles; with cylindrical marginal bulbs each with an abaxial ocellus.

DISCUSSION

Bigelow (1916), with some hesitation placed *Halimedusa* in the Pandeidae because of its hollow tentacles with abaxial ocelli. He noted the peculiar row of nematocyst knobs at the margin of the mouth as different from other pandeids, but failed to see the relationship to the Capitata on his distorted and somewhat damaged preserved specimens. Arai and Brinckmann-Voss (1980) removed *Halimedusa* to its own family in order to better define the family Pandeidae, noting the structure of the lips and clustered interradial tentacles of *Halimedusa* as diagnostic to this new family, the Halimedusidae. Its capitate nature was still not evident. Observation in the present study of both polyps and living medusan morphology, as well as the cnidom, reveals that this genus belongs in the Capitata.

Tiaricodon fits much more naturally into the same family with *Halimedusa* than it does with *Polyorchis*, *Scrippsia* and *Spirocodon* (see Table 1). The gonad of *Tiaricodon*, on the peculiar sac-like extensions of the gut out onto the radial canals is very similar to the (unpouched) gonad and manubrium arrangement in *Halimedusa*, but is different from gonads in the Polyorchidae, which are suspended from the peduncle (see below). Similarly, both *Halimedusa* and *Tiaricodon* have a low, gelatinous peduncle with distinctive interradial "peaks" in the adjacent jelly that rise above the level of the radial canals, unlike the very well-defined peduncle and unmarked jelly above the subumbrella characteristic of the Polyorchidae. *Halimedusa* and *Tiaricodon* both have abaxial ocelli, on otherwise unpigmented tentacle bulbs; whereas additional red pigment accompanies the dark abaxial ocelli of all of the Polyorchids. Both *Halimedusa* and *Tiaricodon* have a quadratic manubrium and mouth with four slightly crenulated lips lined with a row of nematocyst clusters; the lips of *Polyorchis* are lined with a band of nematocysts. *Halimedusa* and *Tiaricodon* both have scattered nematocysts on the exumbrella of newly released medusae, whereas newly-released polyorchids have distinctive clusters of nematocysts arranged in eight vertical adradial rows on the exumbrella. Finally, both *Halimedusa* and *Tiaricodon* medusae are typically found fishing quiescently at the surface with tentacles outstretched (Browne and Kramp, 1939; this paper), whereas the polyorchids are primarily benthic in habit, sometimes swimming up and then sinking passively again (Mills, 1981; Arkett, 1984). Polyorchids can not rest passively at the surface without sinking (Arkett, 1984, 1985). Their distinctly different intrinsic buoyancies lead to very different habitats and behaviors between these two families.

Placement of *Urashimea globosa* into the Halimedusidae is a bit less certain, but I have resisted the temptation to create a separate family for *Urashimea*. With the genus *Urashimea*, some novel characters are added to the Halimedusidae, specifically the presence of numerous meridional nematocyst tracks on the exumbrella, and the unusual tentacles with stalked nematocyst knobs along their lengths. Neither of these characters, however, affect the family diagnosis. Like *Halimedusa* and *Tiaricodon*, *Urashimea* medusae also have characteristic interradial jelly peaks above the radial canals, and the manubrium extends out onto the radial canals carrying saclike swollen gonads (as figured by Hartlaub, 1913, and Uchida and Nagao, 1961) in a manner very similar to *Tiaricodon coeruleus*. The lips of *Urashimea* are a bit more crenulated than those of *Halimedusa* and *Tiaricodon*, but they are also lined by a row of nematocyst knobs. The tentacles have abaxial ocelli. There are no behavior descriptions for *U. globosa* medusae in the literature. The solitary, capitate polyps of *U. globosa*, reared by Uchida and Nagao (1961), look very similar to those described here for *H. typus*, except the *U. globosa* polyps are shown with nematocysts only at the tips of the tentacles and not also scattered along their lengths, as occurs in the polyps of *H. typus*.

The Halimedusidae (*Halimedusa*, *Tiaricodon* and *Urashimea*) and the Polyorchidae both have similar hollow tentacles with large cylindrical bulbs, that are attached to the margin of the umbrella with a narrow base and do not protrude into the substance of the umbrella. But the Polyorchidae are also characterized by unusual tubular gonads that are attached to the radial canals on the peduncle (rather than on the manubrium), large tentacle number, many times branched or diverticulate radial canals, and newly-released medusae bearing highly characteristic adradial rows of nematocyst clusters (newly released *Scrippsia* have never been seen). This suite of characters unites only *Polyorchis*, *Scrippsia* and *Spirocodon* into a fairly solid morphologically-defined family, the Polyorchidae. The cnidom of all of these polyorchids includes stenoteles and desmonemes in the adult medusae (Rees and Mills, in preparation); microbasic mastigophores were also found in the exumbrellar nematocyst clusters of newly released *P. penicillatus* medusae, along with

TABLE 1. – Comparison of some characters of medusae in the capitate genera *Halimedusa*, *Tiaricodon*, *Urashimea*, *Boeromedusa* and the polyorchids *Polyorchis*, *Spirocodon* and *Scrippsia*. Characters that stand out as particularly out of line with the others are noted with an asterisk and set in italics.

Family *Genus*	Nematocysts	Mouth	Gonads	Radial canals	Tentacles and tentacle bulbs	Interradial jelly "peaks"	Exumbrellar nematocysts new medusae	References
HALIMEDUSIDAE *Halimedusa*	stenoteles, desmonemes, microbasic, mastigophores, isorhizas *or* anisorhizas	quadratic with 4 lips, lined by a row of spherical nematocyst knobs	on manubrium with smooth lobes extending out onto radial canals (not folded or pendant sacs)	4 smooth, unbranched	4 perradial hollow tentacles *and *4 clusters of interradial tentacles*, all with cylindrical bulb with abaxial ocellus, and scattered clusters of nematocysts	4 present in mature specimens	scattered	Bigelow, 1916 This paper
HALIMEDUSIDAE *Tiaricodon*	stenoteles, desmonemes, heteronemes	quadratic with 4 lips, lined by a row of spherical nematocyst knobs	on manubrium with lobes extending out onto radial canals as swollen pouchlike sacs	4 smooth, unbranched	4 perradial hollow tentacles, each with cylindrical bulb with abaxial ocellus, and scattered clusters of nematocysts	4 present in mature specimens	scattered	Browne, 1902 Browne and Kramp, 1939 Schuchert, 1996
HALIMEDUSIDAE *Urashimea*		quadratic with 4 lips, lined by a row of spherical nematocyst knobs	on manubrium with lobes extending out onto radial canals, as undulating swollen sacs	4, with **numerous minute abaxial processes*, unbranched	4 perradial hollow tentacles, each with cylindrical bulb with abaxial ocellus, and **stalked nematocyst knobs*	many present in mature specimens	not known	Kishinouye, 1910 Uchida and Nagao, 1961 Bouillon, 1995
BOEROMEDUSIDAE *Boeromedusa*	stenoteles, desmonemes, **microbasic euryteles*	**circular, surrounded by an inconspicuous ring of nematocysts*	not on manubrium, but as pendant, flattened, sacs suspended from junction of radial canals and manubrium	4 smooth, unbranched	4 perradial hollow tentacles, each with cylindrical bulb **without ocelli*, and scattered clusters of nematocysts	4 present in mature specimens	not known	Bouillon, 1995
POLYORCHIDAE *Polyorchis* *Scrippsia* and *Spirocodon*	stenoteles, desmonemes, microbasic mastigophores, **or microbasic euryteles*	quadratic with 4 lips, lined by a distinct marginal band of nematocysts	*as *pendant tubes suspended from junction of radial canals and manubrium or from radial canals along peduncle*	4 with **many blind branches or diverticulae*	numerous hollow tentacles not in clusters, all with cylindrical bulb with abaxial ocellus, and scattered clusters of nematocysts	**not present*	**in clusters, in 8 adradial rows*	Itô and Inoue, 1962 Kramp, 1968 Mills, 1976 J.T.Rees and C.E. Mills, unpublished

stenoteles and desmonemes (Mills, 1976 and unpublished). The halimedusids *Halimedusa* and *Tiaricodon* have stenoteles, desmonemes, and at least one other type of nematocyst, variously identified as isorhizas, anisorhizas or heteronemes (Table 1).

Boeromedusa auricogonia Bouillon, 1995 is the sole representative of another (south) Pacific monotypic genus placed in its own family in the Capitata, the Boeromedusidae, and shows certain distinctive morphological similarities to the species being considered here (see Table 1). It has been collected only near New Zealand (Bouillon, 1995; Schuchert, 1996). Like *Halimedusa, Tiaricodon* and *Urashimea*, *Boeromedusa* has distinctive interradial peaks in its jelly between, but above the 4 radial canals, which are solid rather than hollow extensions of the subumbrella (Schuchert, 1996). Like *Halimedusa* and *Tiaricodon*, *Boeromedusa* has hollow tentacles with scattered clusters of nematocysts along the length, terminating with a nematocyst cluster. Like *Tiaricodon*, it has saclike gonads that develop from extensions of the manubrium out along the tops of the radial canals; but in *Boeromedusa*, as in the polyorchids, the gonadal material is restricted to the region just beyond the manubrium and does not cover the manubrium itself. One might postulate a progression of gonad shape from *Halimedusa* to *Tiaricodon* to *Urashimea* to *Boeromedusa* and the polyorchids. Further differentiating *Boeromedusa*, its tentacle bulbs lack the ocelli found on all other species mentioned here and are of a different shape that those of the Halimedusidae and the Polyorchidae. Its cnidom is also somewhat different, comprising stenoteles, desmonemes and microbasic euryteles (Bouillon, 1995).

All of the species considered here are basically of Pacific distribution, with one species, *Tiaricodon coeruleus*, also occurring in the South Atlantic and Southern Ocean. The recent discoverey of *Urashimea globosa* off South Africa seems likely to represent a recent introduction. It seems evident that members of the Halimedusidae (*Halimedusa*, *Tiaricodon* and *Urashimea*) , the Boeromedusidae (*Boeromedusa*), and the Polyorchidae (*Polyorchis*, *Scrippsia* and *Spirocodon*) are closely allied within the Capitata, but are still separated by small differences in cnidoms and morphologies. Of all of these medusae, only the polyps of *Halimedusa typus* and *Urashimea globosa* are presently known; complete knowledge of all of the life cycles would be very helpful in better understanding their relationships, as would molecular studies.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Nerve net differentiation in medusa development of *Podocoryne carnea**

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SUMMARY: The phylum Cnidaria is the most primitive phylum with a well-developed nervous system. Planula larvae and polyps display a diffuse nerve net (plexus), which is densest in the polyp hypostome. In contrast, the nervous system of the medusa is more complexly structured and reflects the anatomical needs of a well differentiated non-sessile animal. We analyzed the nervous system of two life stages of the hydrozoan *Podocoryne carnea*. Nerve nets of both polyps and developing medusae were examined in whole mounts and gelatin sections by using antibodies and vital staining with reduced Methylene Blue. In the polyp, both RFamide-positive nerve cells and tyrosine-tubulin containing nerve cells form an ectodermal plexus. However, apical neuronal concentration is stressed by a particular nerve ring formed by tyrosine-tubulin positive nerve cells in the hypostome above the tentacle zone. This apical nerve ring is not detected with antisera against RFamide. In developing medusa buds, the earliest detected RFamide positive nerve cells occur at stage 4 at the location of the prospective ring canal. The nerve net of the developing medusa is fully differentiated at bud stage 8. Similar results were obtained with the anti tyrosine-tubulin antibody. Strikingly, two different nerve nets were discovered which connect the medusa bud with the plexus of the gonozoid, suggesting neuronal control by the polyp during medusa bud development. Vital staining with reduced Methylene Blue (Unna's) identified not only nerve cells at the ring canal but also bipolar cells within the radial canal. These cells may fulfill sensory functions.

Key words: differentiation, nerve cells, Hydrozoa.

INTRODUCTION

The phylum Cnidaria is subdivided into four classes, the Hydrozoa, Scyphozoa, Cubozoa and Anthozoa (Schuchert, 1993; Bridge *et al*., 1995). Aside from the planula larva, the polyp and medusa represent the main structural morphs. During the 17th and 18th Centuries, polyps and medusae were not recognized as animals but rather were referred to as plants. Pioneering anatomical analysis in the 19th Century revealed that these species belong to the animal kingdom and posses a well developed nervous system (Hertwig and Hertwig, 1879). For example in 1850, Louis Agassiz first described marginal nerve rings in the hydromedusae *Sarsia* and *Bougainvillia* (reviewed in Mackie, 1989). Later, Hadži (1909) among others, carefully described the nervous system of *Hydra*. In the last nine decades, the *Hydra* nervous system has been intensively explored. In the ectoderm, 2 to 7 times more nerve cells are found compared to the endoderm and 11 different types of nerve cells have been morphologically distinguished (Epp and Tardent, 1978). In contrast to the uniformly distributed nerve cells in the endoderm, the ectodermal nerve cells are concentrated at the hypostome and in the foot of *Hydra* (Kinnamon and Westfall, 1981; Bode, 1996). Further evidence for a degree of adoral (i.e. uppermost

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part of hypostome) nervous centralization was provided using an antiserum against the C-terminal fragment of neuropeptides (arginine-phenylalanine-amide, RFamide) which showed a nerve ring in *Hydra oligactis* but not in three other *Hydra* species (Grimmelikhuijzen, 1985). Moreover, RFamide positive nerve cells are not randomly distributed in *Hydra* but display a gradient like distribution in the body column with highest density in the hypostome (Grimmelikhuijzen, 1985; Bode *et al*., 1988). The nerve cells are synaptically and electrically connected and form a diffuse nerve net, which is called a plexus (Mackie, 1999). A plexus is also found in planula larvae (Tardent, 1978). The nerve cells derive from large interstitial cells (I-cells), which first divide into differentiation intermediates. When cell division is completed, they elaborate the morphological characteristics of either sensory cells or ganglion cells. This organized architecture rather than a random distribution is remarkable, since all cells continuously change their location within the animal and are lost by sloughing at the foot, head and tentacles (Campbell, 1967). Similar observations were obtained from vital staining experiments with polyps from *Podocoryne carnea* (Bravermann, 1969). Therefore, the constantly -moving cells abruptly switch their gene expression pattern and differentiation status in a region dependent manner.

Investigations of the medusa nervous system during the last 130 years have revealed a higher degree of complexity than that of polyps or planulae. Among others, *Polyorchis penicillatus* and *Aglantha digitale* are the best studied species (Spencer and Arkett, 1984; Arkett and Spencer 1986; Mackie and Meech, 1995a and b). In these species, the nervous system consists of a diffuse ectodermal plexus in the tentacles, manubrium and subumbrella, with concentrations below the radial canals and at the margin of the bell. But endodermal nerve rings were also reported close to the margin of the bell (Jha and Mackie, 1967; Mackie and Singla, 1975; Singla, 1974, 1975). Although ocelli have been described in the polyp of the scyphozoan *Stylocoronella riedli* (Blumer *et al*., 1995), well-developed sense organs only exceptionally occur in polyps. Nevertheless, many medusae have evolved sense organs such as ocelli and statocysts, which in hydromedusae are located on the exumbrellar surface of the tentacle bulb. The margin of the bell is the place where peripheral pathways converge and neurons receive input from sense organs and where activity patterns are generated (Mackie, 1990). Thus, the whole morphology of the nervous system of polyps and medusae differs considerably and reflects various behavior repertoires (Spencer, 1974, 1975), although most genes are expressed in both life stages (Bally and Schmid, 1988; Aerne *et al*., 1996). We analyzed the nervous system of the hydrozoan *Podocoryne carnea* which displays a full life cycle. Polyp colonies of *Podocoryne* produce medusae by asexual budding. The epimorphic medusa budding is a *de novo* differentiation and is

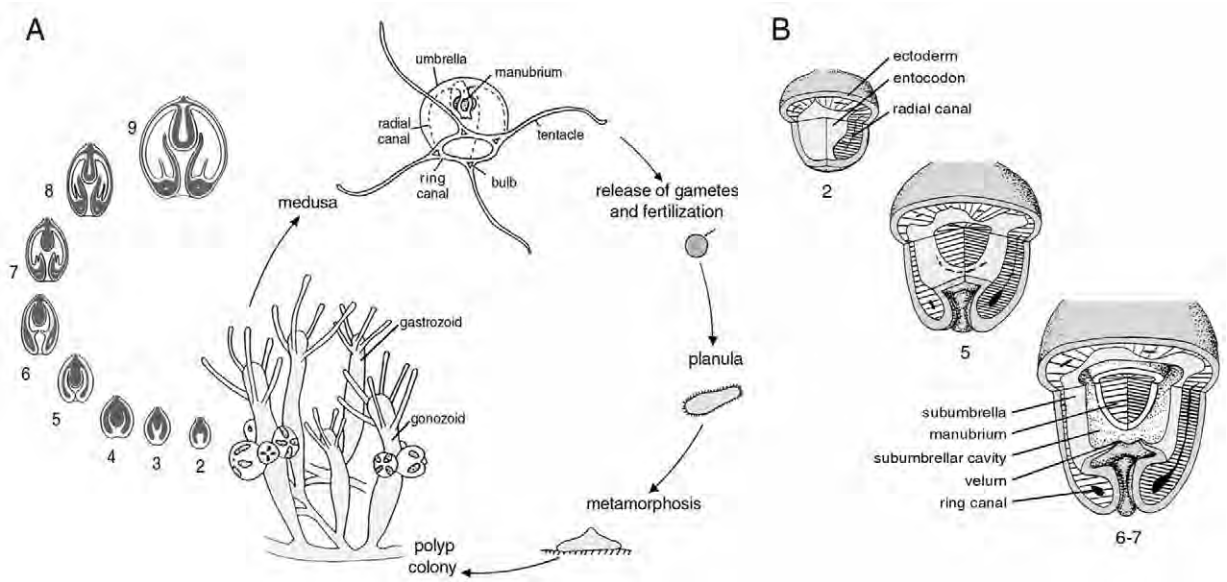

FIG. 1. – The life cycle of *Podocoryne carnea* and medusa bud development. (A) Schematic drawing of the life cycle. Gametes, planula and metamorphosing polyp are not proportionally drawn. On the left, the development of a medusa bud is schematically shown and the numbers indicate the bud stage according to Frey (1968). (B) Three dimensional sketch of bud stages after Boelsterli (1977). The numbers refer to the bud stage.

characteristic for the class Hydrozoa (Tardent, 1978). The bud of an Anthomedusa develops from a diploblastic evagination of the polyp (gonozoid) to the fully differentiated medusa within 7 days (Fig. 1). When *Podocoryne* medusae are released from gonozoids, they swim actively and shed gametes. Upon fertilization, planula larvae develop, settle on the appropriate substrate and metamorphose into primary polyps, the founders of new polyp colonies.

To approach the structure of the nervous system, we applied different methods. A vital staining procedure with reduced Methylene Blue by Unna's method (Pantin, 1948) can be used alternatively to silver staining experiments on whole mounts and reveals both the nerve net and sensory system (Batham *et al.,* 1960). The use of antisera against RFamides has demonstrated that these neuropeptides are widely found in the phylum Cnidaria (Grimmelikhuijzen and Westfall, 1995; Grimmelikhuijzen *et al*., 1996). RFamides are produced by a plexus of neurons and thus, antisera against RFamide can be used in addition to silver or vital stainings. Since not all nerve cells produce RFamides and not all cells stain well with reduced Methylene Blue or with silver (Batham *et al*., 1960), the use of additional markers is indispensable to uncover the nervous system in its entirety. We used a monoclonal antibody against tyrosine-tubulin, which is immunospecific for C-terminal tyrosine of mouse α-tubulin and crossreacts with many tyrosine-tubulin, including that of yeast (Kreis, 1987). The processes of neural elements (neurites) contain microtubules composed of tubulin (Thomas and Edwards, 1991), therefore the use of anti-tyrosine-tubulin antibody is suitable to visualize neurites.

Our data show the similarities between the *Podocoryne* and *Hydra* polyp nerve net structure. Moreover, we describe pattern formation and morphogenesis of the nervous system in the developing medusa bud which form the more complex and more highly- organized nervous system of the Anthomedusa *Podocoryne carnea*.

MATERIALS AND METHODS

Animals

Polyp colonies of *Podocoryne carnea* were cultured in artifical seawater as described by Schmid (1979). Medusae were obtained daily from polyp colonies by overnight-sampling. Medusa buds were staged according to Frey (1968) with bud stage 1 being the youngest and 10 being the oldest stage.

Immunocytochemistry on whole mount preparations

All steps were carried out at room temperature unless otherwise stated. Apart from planulae, the animals were relaxed (1 part seawater and 1 part 7% $MgCl_2*6H_2O$ in distilled water) for 5 minutes (medusae) or 15 minutes (polyps). Fixation was performed in phosphate-buffered saline (PBS) with 4% paraformaldehyde for 90 minutes, followed by three washes with PBS plus 0.1% Tween 20 (PBST), each for 30 minutes. Incubation with RFamide antiserum (rabbit antibody diluted 1:2000 in PBS; kindly provided by Dr. C.J.P. Grimmelikhuijzen) or with a monoclonal anti-tyrosine-tubulin antibody (a mouse antibody, clone TUB-1A2, Sigma; diluted 1:2500 in PBS containing 10% fetal calf serum) was accomplished at 4°C for 12 to 18 hours. Unbound antibodies were removed by three 30 minute washes in PBST. Subsequently, specimens were incubated at 4°C for 12 to 18 hours with either fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (Sigma; diluted 1:200 in PBST) or with FITC-conjugated goat anti-mouse IgG (Sigma; diluted 1:200 in PBST). For double staining, either tetramethylrhodamine isothiocyanate (TRITC)-conjugated goat anti-mouse IgG (Sigma; diluted 1:80 in PBST) or Alexa-conjugated anti-rabbit IgG (Alexa-568, Molecular Probes) was applied according the manufacturers' recommendations. Three washes in PBS for 30 minutes were followed by mounting the specimens in DABCO (2,5 g 1,4- diazabicyclo-[2.2.2.] octane triethylenediamine (Sigma) in 10 ml PBS and 90 ml glycerol) which retards photobleaching. Prior to DABCO mounting, nuclei were stained by incubation with DAPI (stock solution: 1 mg 4'6-diamidino-2-phenyl-indole ·2HCl (Serva) in 20 ml H_2O; working dilution: 30 μl stock solution in 4 ml seawater) for 30 seconds. The preparations were examined with either a Photomikroskop III (Zeiss) or with a laser confocal microscope (DMRXE, Leica). Confocal images were given false colours to match the fluorescent emission colours.

Immunocytochemistry on gelatin sections

Unless stated differently, all steps were performed at room temperature. Polyps and medusae were fixed upon relaxation as described above.

After three washes for 30 minutes in PBST, specimens were placed in a small trim block. Gelatin was dissolved to a final concentration of 8% by heating in water. This solution was cooled down to approximately 70°C and carefully pored in the trim block. The gelatin was deaerated by incubation in a vacuum oven (VD-23, Binder) at 65°C and 100 mbar for 40 minutes. Subsequently, gelatin was allowed to polymerize and specimens were oriented under the dissecting microscope. After 20 minutes at 4°C, the gelatin block was removed from the trim block and fixed in PBS with 4% paraformaldehyde at 4°C overnight. The gelatin block was trimmed and cut into sections of 50 μm thickness with a vibratome (Vibratome Series 1000, Technical Products International, Inc.). These free-floating sections were pooled in PBST, stained with antibodies, and processed as described above.

Vital staining with reduced Methylene Blue by Unna's method

Vital staining using reduced Methylene Blue was performed at room temperature as described by Batham *et al*. (1960). Briefly, animals were relaxed in narcotizing seawater (1 part seawater and 1 part 7% $MgCl_2$*$6H_2O$ in distilled water) for 5 minutes (medusae) or 15 minutes (polyps). About 20 specimens were kept in a volume of 4 ml and stained by addition of 8 drops of reduced Methylene Blue. Reduction of Methylene Blue was performed as follows: 50 mg Methylene Blue (Polychromes Methylenblau Unna, Ciba) were dissolved in 10 ml narcotizing seawater and filtered after the addition of 10 μl 24% HCl. 240 mg Rongalit (sodium formaldehyde sulfoxylate dihydrate, Fluka) were dissolved in 2 ml narcotizing seawater and added to the dissolved Methylene Blue with constant stirring and gentle warming. The mixture was set aside to cool with constant stirring until the color turned to pale yellow, and was finally filtered. Methylene Blue from various suppliers had to be tested for this protocol.

RESULTS

The nerve net of polyps (Fig. 2)

Since the processes of neural elements (i.e. neurites) contain microtubules, antibodies against this highly conserved structural component can be used to visualize the nervous system. Best results were obtained with a mouse anti-tyrosine-tubulin antibody. Whereas an antibody raised against mouse anti-α-tubulin also cross-reacted with flagellae, a mouse anti-β-tubulin antibody did not recognize any epitope in *Podocoryne carnea* (data not shown).

In *Podocoryne carnea*, the mouse anti-tyrosine-tubulin antibody binds to neurites and nematocysts. The neurites form a nerve net, which is regularly dispersed all over the ectoderm of the polyp column and forms a plexus in the tentacles as well. The neurites are mainly bipolar and run in a proximodistal direction in the body column and in the tentacles (Fig. 2A). Remarkably, just above the tentacle zone a few neurites run perpendicularly to the others and form a unique ring, which is interconnected with the proximodistal-oriented nerve net. Similarly, the RFamide-positive nerve cells form also a plexus along the body column but these neurites run exclusively in a base to apex orientation. Fewer RFamide-positive nerve cells are detected within the tentacles. RFamide expressing nerve cells occur predominantly in the hypostome and display a gradient-like distribution along the body column. No subhypostomal ring is formed by RFamide positive nerve cells (Fig. 2B). Double-staining confirmed differences in the distribution of nerve cells expressing RFamide and tyrosine-tubulin positive cells (Fig. 2C, D). Nevertheless, both nerve cell populations are localized in the ectoderm.

Two different types of neurons were detected with the anti-tyrosine-tubulin antibody. This is best demonstrated on gelatin sections of the body column. Large nerve cells which are oriented perpendicularly to the mesoglea and which project a cilium at the ectodermal surface are sensory cells. Smaller cells project their neurites parallel to the mesoglea and are termed ganglion cells. (Fig. 2E).

The nervous system in developing medusa buds (Fig. 3)

The developing bud is covered with a protective envelope, the perisarc. Since it interferes with penetration and diffusion, the technique of gelatin sections is an appropriate tool to investigate bud development.

RFamide-positive cells are first detected from stage 4 at the place of the prospective ring canal (Fig. 3A). RFamide expression in this region persists through all stages. In addition, RFamide-positive nerve cells are present in the developing

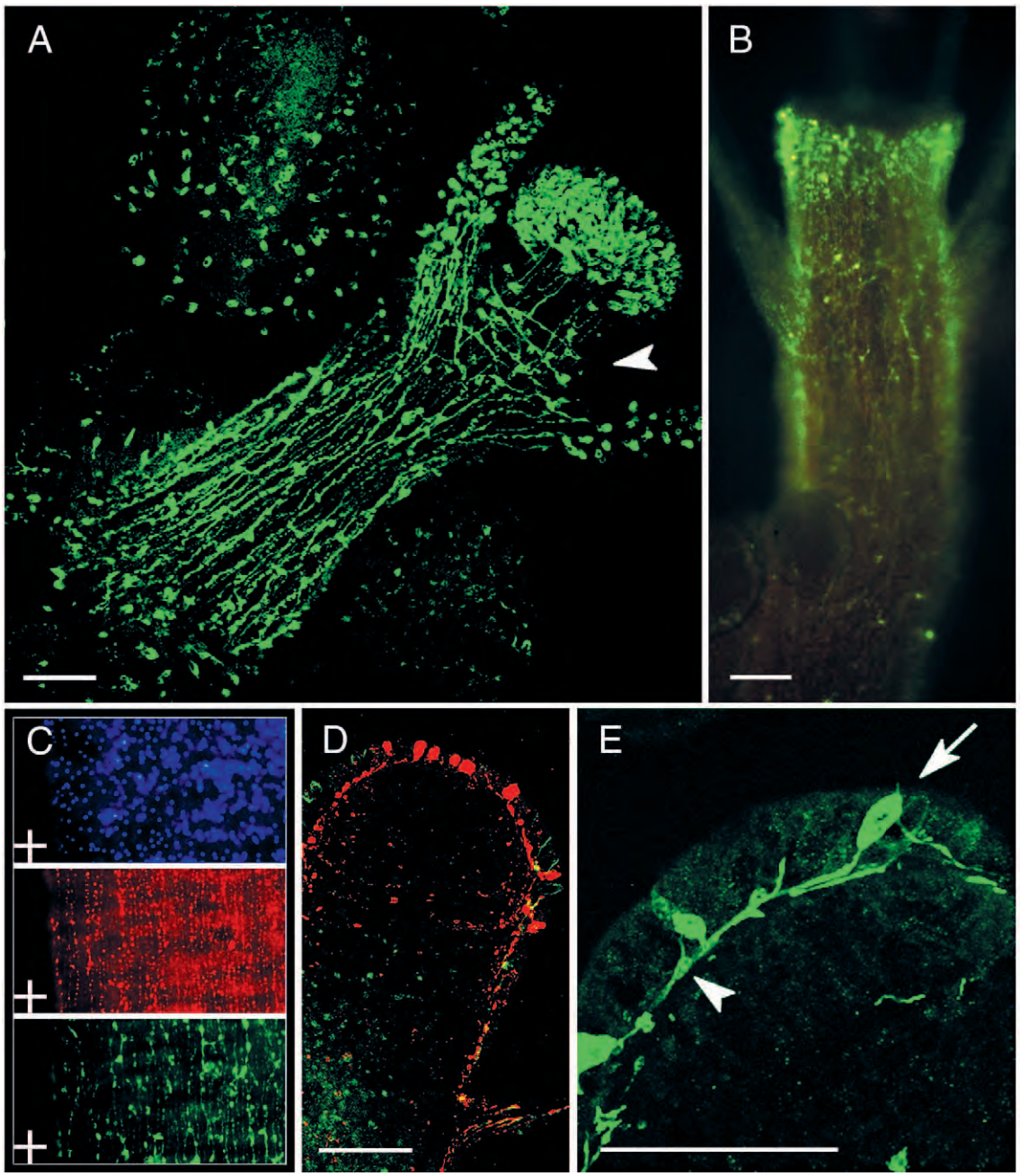

FIG. 2. – The nervous system of the polyp. Fluorescent signals were detected either with a laser confocal microscope (A, D and E) or with a conventional photomicroscope (B and C). (A) Nerve cells stained with anti-tyrosine-tubulin antibody in a gonozoid. The arrowhead points to the hypostomal nerve ring. (B) RFamide-positive nerve cells in a gonozoid display a gradient-like distribution, with the highest concentration in the hypostome. (C) Detail of DAPI-stained (visualization of all nuclei) and double-labeled body column. On top DAPI-labeled nucleotides in the body column are shown in blue, in the middle the tyrosine-tubulin positive net is shown in red, and at the bottom the RFamide positive plexus is shown in green. The cross on the left refers to the same position in all pictures. (D) Double-labeling of a hypostome in a gelatin section shows RFamide-positive nerve cells (red) and tyrosine- tubulin positive cells (green). (E) Cross section of body column. Arrow points to a sensory cell, arrowhead to a ganglion cell. Bars equal 100 μm.

manubrium from stage 5 to 6 on. Afterwards, nerve cells at the radial canal start to express RFamide (Fig. 3B). When tentacles develop (stage 7 to 8), the neurons at the radial canal connect the manubrial plexus to the nerve rings close to the ring canal (Fig. 3C). Interestingly, projecting neurites from the subradial nerve cells immigrate into the developing tentacle bulbs (Fig. 3D). Thus, ectodermal neurites project into the bulb endoderm. Similarly, RFamide-positive neurons from the polyp ectoderm project from early bud stages on through the stalk, penetrate the mesoglea and project to the endodermal portion

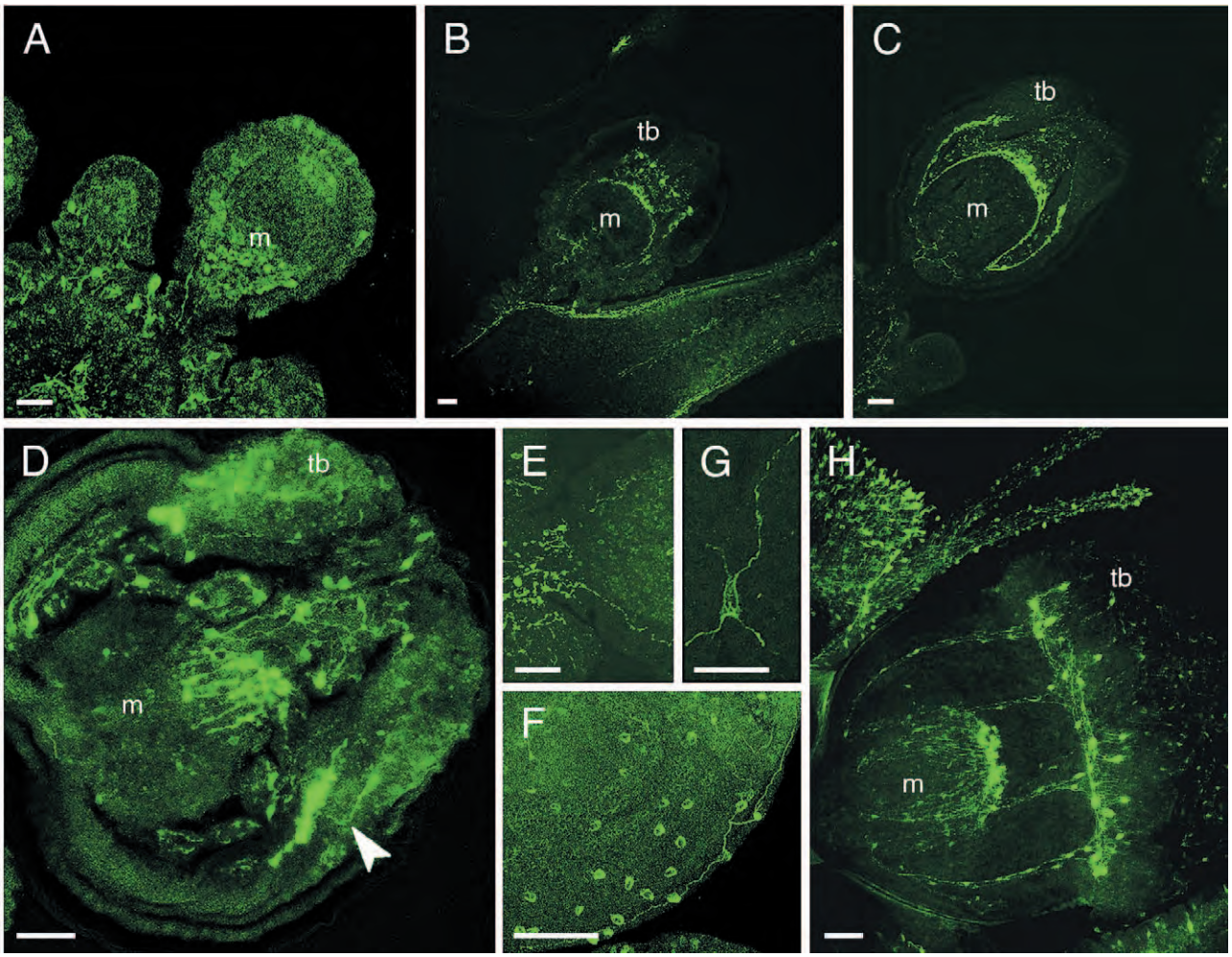

FIG. 3. – The developing nervous system in medusa buds. A-G are laser confocal images; H was done with a conventional photomicroscope. A-D and H are stained with antiserum against RFamide, the others with anti-tyrosine-tubulin. (A) Cross section of buds of stages 2 and 4 with RFamide expression in the apical part of the entocodon. (B) Section of a gonozoid with a bud of stage 5-6. RFamide -positive cells are detected at the developing ring canal, manubrium, and below the radial canals. Note the neurites which project through the stalk. (C) Cross section through bud stage 7-8. (D) Cross section of bud stage 8. The arrowhead points to the neurites, which project at right angles from the lower margin of the subumbrella into the tentacle bulb. (E) Projected neurites from the polyp (left) through the stalk into the bud (right). (F) Several, but not all nematocytes are connected to an ectodermal nerve net. (G) Multipolar nerve cell in the ectoderm of a medusa bud. (H) Whole mount of bud stage 8. Note that the developed nerve cells in the tentacles are not stained in this whole-mount preparation. m, manubrium; tb, tentacle bulb. Bars in A-F an H correspond to 100 μm, in G to 50 μm.

of the developing manubrium (Fig. 3A-C). When the tentacles are completed at stage 8, the nerve plexus is established (Fig. 3D).

The antibody against tyrosine-tubulin stained the developing nervous system in the bud similarly to the RFamide antiserum. Remarkably, tyrosine-tubulin positive neurites project through the stalk in the bud ectoderm corresponding to the prospective exumbrella (Fig. 3E,F). These neurites make contact with some, bud not all, nematocytes. Therefore, the bud communicates with the polyp by at least two different nerve nets. One is localized in the prospective exumbrella, connects the polyp through the stalk ectoderm and can be identified with the anti-tyrosine-tubulin antibody. The other nerve net is RFamide positive and contains neurites, which are projected through the endoderm of the stalk. Fig. 3G depicts a multipolar nerve cell detected with the tyrosine-tubulin antibody. Not only the neurites but even the cell body is stained. The RFamide- positive nerve net is developed from bud stage 8 on, however, the RFamide-positive nerve cells of the tentacle are not labeled in whole mounts (Fig. 3H).

The nerve net of the mature medusa (Fig. 4)

The proportions of the fully developed nerve net are similar to those from bud stage 8 on. Four nerve

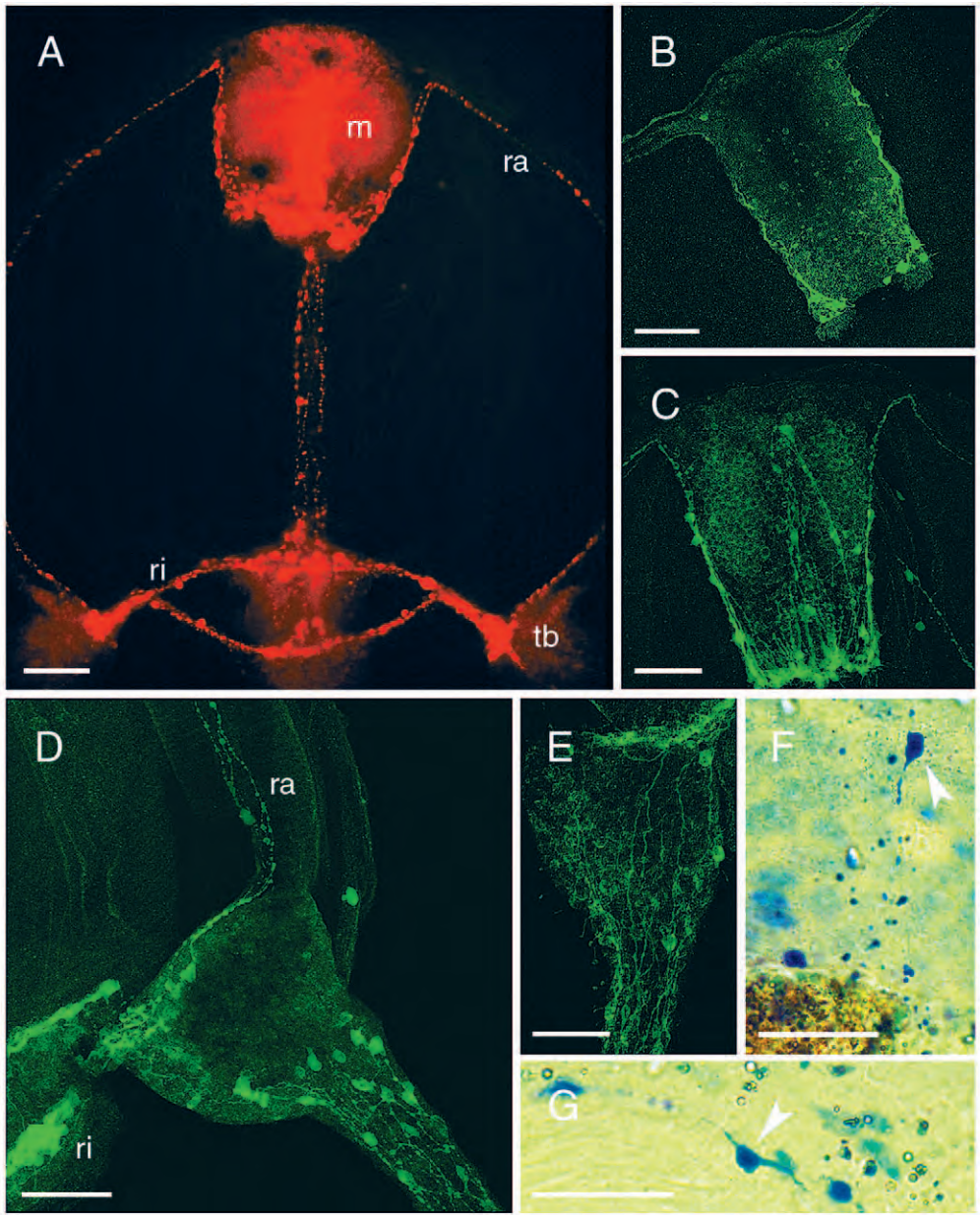

FIG. 4. – Nervous system of the mature *Podocoryne carnea* medusa. A to E are laser confocal images; F and G were done with a conventional lightmicroscope. A and B stained with antiserum against RFamide; C-E tyrosine-tubulin; F and G vital stainings with reduced Methylene Blue (Unna's). (A) Whole mount of a medusa. The picture is over exposed to visualize the outline of the medusa. (B) Cross section of a manubrium. (C) Whole mount shows projected neurites from the manubrial plexus to the subumbrellar fascicles. (D) Magnification of the bell margin with a tentacle bulb. (E) Tentacle bulb viewed from the inner side. Several neurites project side by side from the ring canal to the tentacles (F) Vital staining of a large cell with processes within the radial canal (arrowhead). (G) Vitally -stained bipolar cell at the ring canal (arrowhead). m, manubrium; ra, radial canal; ri, ring canal; tb, tentacle bulb. Scale bar = 100 μm.

fascicles, which lie in the subumbrellar ectoderm connect the nervous system of the manubrium with the nerve rings at the ring canal and the four nerve plexi of the bulbs and tentacles (Fig. 4A). RFamide-positive nerve cells of the manubrium surround the mouth opening and mingle with the four nerve fascicles in the subumbrellar ectoderm (Fig. 4B). This is also the case for tyrosine-tubulin positive nerve cells. Nevertheless, only the tyrosine-tubulin antibody stains nematocytes at the manubrial lips. Both nerve cell-types project their neurites additionally into the whole lower part of the manubrial ectoderm towards the base of the gonads (Fig. 4C). The neurites of the subumbrellar ectoderm connect to the ring canal nerves and to the plexus of bulb and tentacles (Fig. 4D). Most neurites enter the bulb at the inner ectoderm whereas the lateral and outer part seem not to be invaded (Fig. 4D,E). We detected no

immunoreactivity of nervous elements in the velum. Thus, the subradial neurites cross the mesoglea in the tentacle bulbs to connect to the nerve rings at the ring canal.

Interestingly, vital staining using reduced Methylene Blue revealed labeled cells within the radial canal (Fig. 4F). These large cells have processes similar to bipolar nerve cells at the ring canal that are detected with the same method (Fig. 4G). Strikingly, cells of the radial canal that are oriented to the periphery have been found to express a *Pax* gene (Gröger *et al.*, in preparation) and might fulfill a sensory function.

DISCUSSION

The structure of the nervous system of the hydrozoan *Podocoryne carnea* has been elucidated by means of vital staining and immunocytochemistry on both whole mounts and gelatin sections. Whereas both polyp and planula have a diffuse nerve net, the polyp shows a clear tendency for adoral concentration in the hypostome (Fig. 2A,B). The medusa nerve net, however, displays a clearly higher degree of organization typical for hydromedusae (Mackie *et al.*, 1985).

Polyps display a diffuse nerve net similar to planulae

Although nervous systems of both polyps and planulae are organized as a plexus, the polyp nervous system of *Podocoryne carnea* displays a tendency for concentration close to the mouth opening. Particularly, the tyrosine-tubulin positive nerve ring above the tentacles is exclusively found in the hypostome. Nowhere else in the body column can such ring-like connections be detected. If we superimpose the gradient-like distribution of RFamide -positive nerve cells with its highest concentration in the hypostome, a clear apical neural concentration becomes evident. Although no sense organs are found in *Podocoryne* polyps, recent data show that a few cells expressing a *Pax* gene are concentrated in the hypostome (Gröger *et al.*, in preparation). This *Pax* gene is probably involved in differentiation pathways of sensory cells and the apical localization of the *Pax* -expressing cells displays further evidence for a clearly apical sensory nerve cell concentration. As mentioned above, nerve cells, like epithelial cells, continuously move towards the end of the body column (Yaross *et al.*, 1986). Thus, the polyp nerve cells exhibit a fascinating position-dependent morphogenetic plasticity within the body column.

Pattern formation and morphogenesis in the developing medusa bud

The use of gelatin sections combined with immunocytochemistry improved the study of the developing nervous system of the medusa. At bud stage 8, nervous system formation is completed and coincides with the realization of the structural differentiation of the subumbrellar striated muscle cells (Boelsterli, 1977). It has been previously demonstrated that from bud stage 8 on, experimentally isolated buds develop into a medusa while younger isolated bud stages regenerate polyp structures (Schmid, 1972). These results indicate that accomplished morphogenesis at bud stage 8 is a prerequisite to maintain the medusa stage. The earliest RFamide positive nerve cells are found in bud stages 4 where the ring canal will develop. Boelsterli (1977) reported that bud stages 3-4 begin to harbor myofilaments in the entocodon. If we superimpose the detection of RFamide-positive nerve cells at stage 4 with the appearance of myofilaments it becomes apparent that nerve and muscle differentiation both begin synchronously around bud stage 3 to 4.

Striking observations include the invading neurites, which travel through the stalk of the bud. Spencer (1974) analyzed the responses of attached medusa buds to colonial excitation in *Proboscidactyla flavicirrata* and argued that ectodermal conduction causes crumpling while an endodermal conducted colonial pulse leads to swimming behavior. Our data demonstrate a neuronal interface bridging polyp and medusa in *Podocoryne*. Although, we found no evidence that these processes of the polyp nerve system connect to the medusan nervous system, these results indicate that the polyp - at least in part - may supervise medusa bud development by neuronal control. These findings may contribute to our understanding of why isolated buds can fulfill medusa development exclusively from stage 8 on. The ectodermal neurites, which project from the gonozoid into the prospective exumbrellar layer, make contact to many, but not all nematocytes. In the mature medusa, nematocytes are also found scattered all over the exumbrella, although we never observed neurites connecting them. Whether this network is temporally restricted to the developing medusa bud remains to be resolved. In *Hydra*,

synapses were reported between neurons, between neurons and epitheliomuscular cells and between neurons and nematocytes (Westfall, 1973; Kinnamon and Westfall 1984). But it seems unlikely that these synaptically-connected nematocytes in the medusa bud serve as mechano-receptors or may be discharged upon neuronal stimuli, as the perisarc firmly wraps the whole medusa bud.

The nerve system of the adult medusa displays higher organization

The organization of the *Podocoryne* medusa nervous system is in complete agreement with data from other hydromedusae (Mackie *et al*., 1985). Cnidarians lack a predominant nervous center, a brain. However, above the inner side of the four tentacle bulbs, the main nervous junctions are made. At the tentacle bulbs, stimuli from tentacles and the manubrium (and sense organs, if present) are passed to the ring canal nerves, which represent the processing center. Spencer and Arkett (1984) argued that a radially symmetrical animal should have a ring-shaped central nervous system. Furthermore, the nerves at the ring canal are morphologically concentrated in different bundles. In general, an outer nerve ring, which is more marginally positioned, is distinguished from an inner nerve ring. Nerves within the nerve bundles are further differentiated, such as the giant axons for rapid conduction in *Aglantha digitale* (Mackie and Meech, 1995a,b). Moreover, an endodermal nerve ring has been reported in the hydromedusae *Sarsia*, *Euphysa* and *Stomotoca* (Jha and Mackie, 1967; Mackie and Singla, 1975). Our results show that the *Podocoryne* nervous system begins to develop at the prospective umbrella opening and only later develops in the manubrium, below the radial canals and finally in the tentacles. The high degree of morphological differentiation and the early onset of differentiation imply that the ring canal nerves represent the central nervous system of hydromedusae.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Sarsia marii n. sp. (Hydrozoa, Anthomedusae) and the use of 16S rDNA sequences for unpuzzling systematic relationships in Hydrozoa*

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SUMMARY: A new hydrozoan species, *Sarsia marii,* is described by using morphological and molecular characters. Both morphological and 16S rDNA data place the new species together with other *Sarsia* species near the base of a clade that developed, "walking" tentacles in the medusa stage. The molecular data also suggest that the family Cladonematidae (*Cladonema, Eleutheria, Staurocladia*) is monophyletic. The taxonomic embedding of *Sarsia marii* n. sp. demonstrates the usefulness of 16S rDNA sequences for reconstructing phylogenetic relationships in Hydrozoa.

Key words: *Sarsia marii* n. sp., Cnidaria, Hydrozoa, Corynidae, 16S rDNA, systematics, phylogeny.

INTRODUCTION

Molecular data and parsimony analysis have become powerful tools for the study of phylogenetic relationships among extant animal taxa. In particular, DNA sequence data have added important and surprising information on the phylogenetic relationships at almost all taxonomic levels in a variety of animal groups (for references see Avise, 1994; DeSalle and Schierwater, 1998). We may not forget, however, that inferring phylogenetic relationships from molecular data is neither trivial nor a final solution to systematics, since the molecular option can be prone to a large number of pitfalls and misunderstandings (for refs. see Miyamoto and Cracraft, 1991; Schierwater *et al*., 1994; Swofford *et al*., 1996). Nevertheless molecular data are especially useful or even indispensable in groups where other characters, like morphological data, are limited or hard to interpret.

One classical problem to animal phylogeny is the evolution of cnidarians and the groups therein (Hyman, 1940; Brusca and Brusca, 1990; Bridge *et al*., 1992, 1995; Schuchert, 1993; Schierwater, 1994; Odorico and Miller, 1997). Particularly the systematic relationships within the Hydrozoa represent one of the most difficult and long-standing problems to evolutionary biologists. In lack of fossil records and a sufficient number of unambiguous morphological characters, neither the phylogenetic relationships between nor within the orders have been understood and are still subject of controversial debates (see for

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example, Petersen, 1979, 1990; Cunningham and Buss, 1993; Schuchert, 1996). Historically, hydroid systematics has been hindered by problems of deciding the importance of polyp versus medusoid characters (Rees, 1957; Naumov, 1960; Boero, 1980; Bouillon, 1981; Boero and Bouillon, 1987). Since in most hydroids the medusa stage has been reduced to sessile gonophores, reconstructions of phylogenetic relationships have mainly been based upon morphological characters of the hydroid stage and the assumption of a progressive reduction of the medusoid stage (Werner, 1984; Boero and Sarà, 1987). Here, the arrangement of tentacles and the inventory of nematocysts have been two of the most important characters. The morphological simplicity and plasticity of hydroids, however, makes morphological homoplasy likely to be common in this group. We suggest that molecular characters must be added to morphological and life cycle characters in order to resolve hydrozoan systematics.

By applying molecular data to previously undescribed species we might in many cases be able to resolve their relative taxonomic position even in the absence of morphological or developmental data. This approach promises valuable results if molecular data from closely related taxa are available for comparative analyses. We present an example from a new athecate hydroid species which we assign to the genus *Sarsia* based on both morphological and molecular data.

MATERIALS AND METHODS

Animal material

Based on morphological characters we used the following closely related species of the infra-order Capitata (Werner, 1984) for embedding the new species into a taxonomic framework: *Stauridiosarsia producta* Wright (1858), *Sarsia tubulosa* Sars (1835), *Sarsia reesi* Vannucci (1956) (all members of the family Corynidae), *Staurocladia bilateralis* Edmondson (1930), *Staurocladia oahuensis* Edmondson (1930), *Cladonema radiatum* Dujardin (1843) (Cladonematidae), and *Eleutheria dichotoma* Quatrefages (1842) (Eleutheriidae). The Filifera *Thecocodium quadratum* Werner (1965) served as an outgroup. Animal material of *Staurocladia bilateralis* and *S. oahuensis* were provided by Yayoi Hirano (Awagun, Japan), of *Stauridiosarsia producta* and *Sarsia tubulosa* by Gerhard Jarms (Hamburg, Germany), of *Sarsia reesi* by Alvaro Esteves Migotto (São Sebastião, Brazil), and of *Thecocodium quadratum* by Stefan Berking (Cologne, Germany).

Hydroids of *Eleutheria dichotoma*, *Cladonema radiatum* and *Sarsia marii* n. sp. were collected by the authors from the green alga *Ulva lactuca,* obtained 10-50 cm under the water surface at the rocky shore of the Laboratoire Arago, Banyuls sur Mer, France. Single polyps were isolated from the algae and grown in small finger bowls in the lab using our standard culturing conditions for hydrozoans (Schierwater *et al*., 1992; Schierwater and Hadrys, 1998). Polyps were maintained in artificial seawater (30‰ salinity) at 19-21°C and fed *ad libitum* two times weekly on 3-4 day old brine shrimp larvae (*Artemia salina*). Under these conditions polyps of all three species, including *Sarsia marii*, grew into colonies of polyps connected by a horizontal net of stolons (the latter grew attached to the glass surface of the finger bowl). While medusae of *Eleutheria dichotoma* and *Cladonema radiatum* are found regularly year round, *Sarsia marii* medusae were found only once in spring 1998. Since the latter stayed alive just for a few days, morphological traits could be observed on subadult medusae only.

The original polyp was found on a thallus of the green alga *Ulva lactuca* collected on 5 April 1997 at the Laboratoire Arago, Banyuls sur Mer, France, 42°29'N, 3°08'E.

DNA extraction, amplification and sequencing

Small samples of hydroid tissue were homogenized in HOM buffer (100mM Tris-HCL, 10mM EDTA, 100mM NaCl, 0.5% SDS, pH 8.0), and extracted once with phenol-chloroform-isoamyl alcohol (25:24:1). DNA was precipitated by addition of two volumes ethanol and 1/10 volume 5M NH_4Ac, and after a single centrifugation and washing step resuspended in TE buffer (1mM Tris-HCL, 0.1mM EDTA, pH 7.5) (for details see Schierwater and Ender, 1993). For PCR amplification of part of the 16S rRNA mitochondrial large subunit, hydrozoan specific primers were used (Cunningham and Buss, 1993). The amplified region corresponds to the 5´-end of the 16S rRNA gene and revealed between 588 bp (*Eleutheria dichotoma*) and 595 bp (*Cladonema radiatum*). Amplification reactions were carried out with 10-20ng of DNA in a total volume of 25 μL using a 9.600 thermocycler from Perkin Elmer and the following temperature profile:

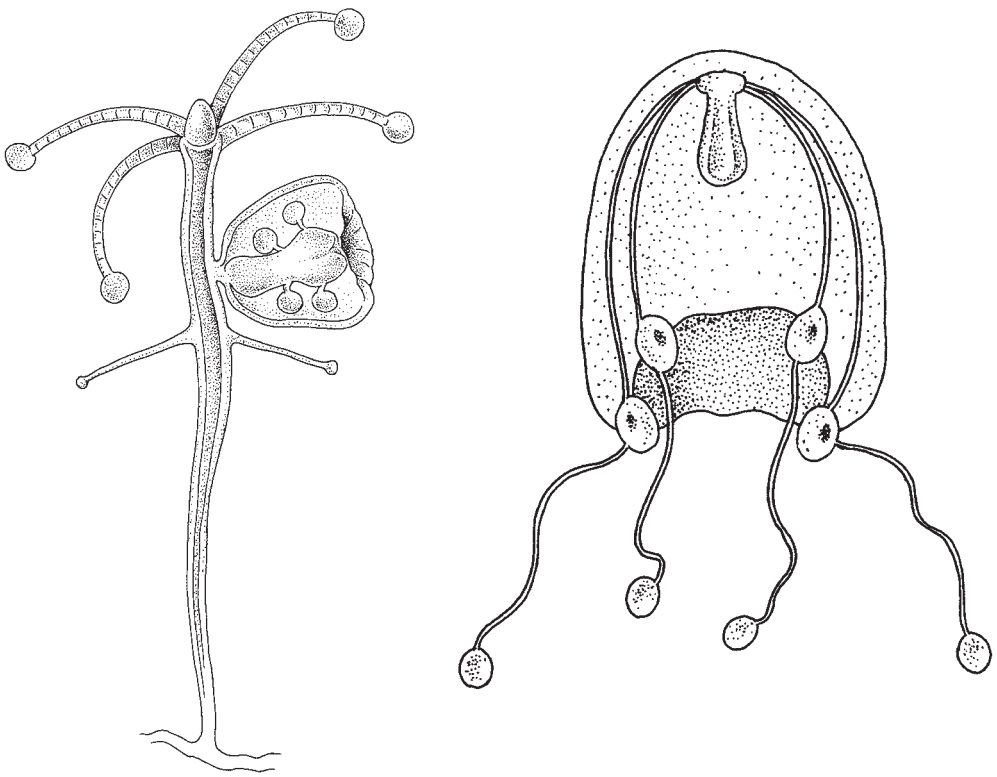

FIG. 1. – Drawings of live specimens of *Sarsia marii* n. sp. raised in the laboratory. A polyp with a well developed medusa bud above the aboral tentacle whorl and a four-day-old medusa are shown. Explanations are given in the text.

10 cycles (92°C/50 s, 45°C/50 s, ramp 3 s/1°C, 72°C/1 min) followed by 40 cycles (92°C/50 s, 50°C/1 min, ramp 3 s/1°C, 72°C/1 min); finally fragments were elongated at 72°C for 5 min. DNA cloning and sequencing were performed as described in Ender *et al*. (1996).

DNA sequence alignment and phylogenetic analyses

16S rDNA sequences were aligned with the aid of CLUSTAL (Higgins and Sharp, 1989) and ambiguous regions (often surrounding variable loops) were deleted. Since computer based alignment programs did not reveal consistent alignments, sequences were controlled by eye and compared to a secondary structure model of the *Eleutheria dichotoma* 16S rRNA molecule (Ender, 1997). A detailed description of the 16S rDNA data collection and analyses will be given elsewhere (together with a larger hydrozoan tree; Ender and Schierwater, in prep.). For tree inference by maximum parsimony or neighbor-joining data were analyzed using PAUP 3.1 (Swofford, 1993) and PHYLIP 3.75c (Felsenstein, 1995), respectively.

RESULTS

Sarsia marii n. sp.

The morphology of *Sarsia marii* polyps and medusae (Fig. 1) suggests relationships to the Cladonematidae and Corynidae (cf. Werner, 1984; Petersen, 1990). The polyp of *S. marii* looks rather similar to a *Cladonema radiatum* polyp and exhibits in addition to the oral whorl of capitate tentacles a second whorl of 4 filiform sensory tentacles. The number of capitate tentacles was always four, with each 10-11 endodermal cells (Table 1). The medusa looks like a typical *Sarsia* medusa and lacks any *Cladonema* typical tentacle branching patterns, suggesting that *Sarsia marii* is related to the genus *Sarsia*. Here the new species appears to be very similar to *S. reesi* (synonym: *Dipurena reesi* Vannucci, 1956; Brinkmann-Voss and Petersen, 1960), both in the polyp and medusa generation (Table 1). The typical long manubrium of *S. reesi* medusae, however, was not found in the immature medusae of *S. marii*. The most striking difference between *S. marii* and *S. reesi* medusae is found in the catching tentacles, which are capitate in *S. marii* and filiform in *S. reesi*.

TABLE 1 – Distinguishing morphological traits for *Cladonema radiatum*, *Sarsia reesi* (Brinkmann-Voss and Petersen, 1960; Schuchert, 1996) and *Sarsia marii* n. sp.

Characters	*Cladonema radiatum*	*Sarsia marii*	*Sarsia reesi*
Hydroid			
number of endodermal cells in capitate tentacles	7-8	10-11	18-20
number of capitate tentacles	4-5	4	3-7
number of filiform (sensory) tentacles	4-5	4	4-6
location of filiform tentacle	0.3/0.7	1/1	1/1
knob of capitate tentacles	cone shaped	cone shaped	button shaped
Medusa			
number of radial canals	7-10	4	4
length of manubrium	short	short	long
tentacle branching	present	absent	absent
type of defense tentacle	filiform	capitate	filiform
number of cnidocyst clusters of defense tentacle	50-100	1	up to 100

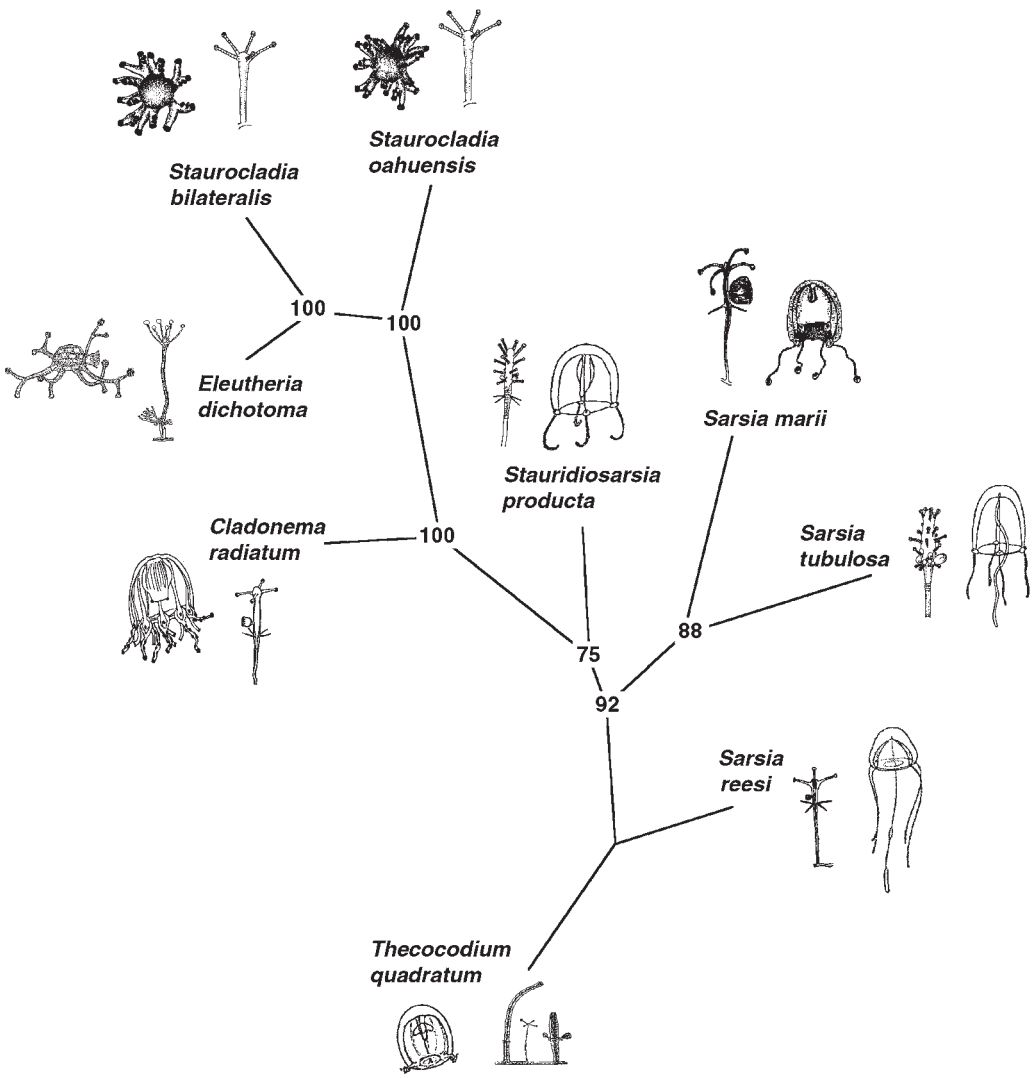

FIG. 2. – Most parsimonious 16S rDNA phylogram for *Sarsia marii* n. sp. and 7 related species of capitate athecates. The tree is rooted to the outgroup *Thecocodium quadratum* (Athecata, Filifera). Shown is the single most parsimonious tree (PAUP, Branch-and-Bound search, 500 bootstrap replicates, reweighting of characters RC=0.734, CI=0.847, RI=0.867). Note, the shown clade is a monophyletic clade within a more comprehensive tree that includes 27 hydrozoan species (Ender and Schierwater, in prep.). The number on nodes corresponds to the bootstrap value and branch lengths are drawn proportionally to the number of changes on each branch. The tree supports a close relationship between known Corynidae and the new species *Sarsia marii*, which appears within the *Sarsia* group. Note, that the Corynidae form a paraphyletic clade which varies according to the outgroup used. Please also note that the *Staurocladia* polyp shown is the drawing of a *Staurocladia wellingtoni* polyp (Schuchert, 1996) since polyps of *Staurocladia bilateralis* and *S. oahuensis* have not been found yet.

While it cannot be excluded from organismal observations alone that the observed differences between *S. reesi* and *S. marii* medusae are the result of phenotypic plasticity (we have not seen any mature *S. marii* medusae yet), 16S rDNA sequence data unambiguously identify them as different species.

Taxonomic placement

The 16S rDNA phylogram shown in Figure 2 supports the placement of *S. marii* within the genus *Sarsia*. The given branching patterns are the result of a branch and bound search using character reweighting according to the rescaled consistency index, and using bootstrap analyses with 500 resampling replicates. In the single most parsimonious tree (Fig. 2) *S. marii* clusters as a sister species to *S. tubulosa*. The genus *Sarsia* forms a paraphyletic group, depending on the outgroup used. The latter reflects the diversity - and consequently also the classification difficulties - of genera within the Corynidae, which are well known for their considerable morphological variability (including the number, placement and type of tentacles of hydroids; cf. Boero and Bouillon, 1987). For instance, the genera *Sarsia* and *Dipurena* were distinguished by a single character (gonads in one ring or gonads in two or more rings; Kramp (1961), and the genus *Stauridiosarsia* from *Sarsia* solely by the presence or absence, respectively, of aboral filiform tentacles (Bouillon, 1985).

The genus *Sarsia* appears near the base of a clade that gave rise to the invention of walking (Fig. 2). The derived genera *Cladonema*, *Staurocladia*, and *Eleutheria* all exhibit branched tentacles with specialized tentacles for attaching the medusae temporarily to a substrate. The tree supports a placement of *Stauridiosarsia* close to the family Corynidae. It is noteworthy that the above conclusions are supported by high bootstrap values and also neighbor-joining analysis (not shown), which revealed the same branching patterns as shown in Figure 2.

DISCUSSION

Hydroid taxonomists have traditionally faced difficulties many other taxonomists have not, including controversies over the relative importance of polypoid versus medusoid characters and the lack of one of the two bauplans in many groups. Furthermore, the independent gains and losses of morphological features in any one generation may produce high levels of morphological homoplasy (convergence), especially since the number of morphological characters in Hydrozoa is severely limited (cf. Boero, 1980; Boero and Bouillon, 1987; Petersen, 1979, 1990). The main advantages of adding molecular data to phylogenetic analyses of Hydrozoa include that molecular characters are independent of any developmental or life cycle stage and can be generated at *cum grano salis* unlimited numbers. Our example, in which we describe a new species, *Sarsia marii*, demonstrates the usefulness of 16S rDNA sequence data for verifying the taxonomic placement for a new species and simultaneously resolving its phylogenetic relationships to closely related species.

Both the morphology and the 16S rDNA sequence data provide unambiguous evidence that *Sarsia marii* n. sp. must be regarded as a member of the Corynidae, closely related to *Sarsia tubulosa*. Based on morphological data alone this taxonomic placement would be less clear, especially since we are yet lacking knowledge of the morphology of sexually mature medusae. However, it was the morphology which allowed us to identify closely related species for comparison in a molecular tree. The tree itself is widely congruent with our expectations from morphological data, and seems to be well supported by the high bootstrap values, and the independence of the tree topology from the tree building algorithm used. Furthermore, the clade discussed here also remains stable in a larger scale phylogeny, which includes a total of 27 hydrozoan species (Ender and Schierwater, in prep.). Nonetheless we would like to note that any molecular phylogeny is sensitive for example to the selection of taxa, to the choice of the outgroup, to the algorithm used for tree reconstruction, and in case of DNA sequence data, particularly also to the alignment of putative homologous nucleotide positions (for refs. see DeSalle and Schierwater, 1998). While future progress is to be expected – and needed – with respect to finding the most robust means for analyzing DNA sequence data, it seems clear that 16S rDNA data provide informative characters for unpuzzling phylogenetic relationships in Hydrozoa at different taxonomic levels.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Interannual variation in the composition of the assemblages of medusae and ctenophores in St Helena Bay, Southern Benguela Ecosystem*

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SUMMARY: The assemblages of medusae and ctenophores were examined from samples collected each winter from St Helena Bay, over the 10-year period 1988-1997. A total of 50 hydromedusae, 1 scyphozoan and 2 ctenophore species were identified from 243 samples. Although the data set is characterised by great interannual variability, two main assemblages could be identified each year. These were characterised by either holoplanktonic medusae (e.g. *Liriope tetraphylla, Aglaura hemistoma*) or meroplanktonic medusae (e.g. *Mitrocomella millardae*, *Chrysaora hysoscella*) and ctenophores (e.g. *Pleurobrachia pileus*). The holoplanktonic medusae were typical of samples at the southern edge of the Bay, and were positively associated with both depth and temperature. Their abundances tended to increase during warm years (1992, 1993 and 1997) as warm surface water flooded the Bay. The meroplanktonic medusae and ctenophores were typical of samples collected within the Bay, and the density of species tended to be negatively correlated with temperature and depth. In spite of the eurythermal nature of the meroplanktonic species, they were more common during cold years (1990 and 1995). This paper represents the first Bay-wide, interannual study of any zooplankton group, and contributes important base line information on the structure of regional pelagic assemblages.

Key words: zooplankton, jellyfish, diversity, upwelling, South Africa.

INTRODUCTION

Coastal upwelling areas are of ecological interest, as well as economic importance, because of their enhanced primary productivity, which is generated by the vertical transport of nutrients to surface waters. The Benguela system along the southwest coast of Africa (~15°S to ~35°S) is one of the most important wind-driven coastal upwelling areas in the world. The region is bounded by two warm currents, the Angola Current in the north and the Agulhas Current in the south. This highly hydrodynamic area can be considered an unstable environment (Hart and Currie, 1960), where physical events can be evoked to explain the variability in species abundance and distribution (McGowan, 1974).

Cnidarians and ctenophores are thought to be good indicators of water mass movement (Colebrook, 1977; Raymont, 1983). Studies of their spatial distribution in and around the Benguela system have revealed the presence of distinct assemblages associated with both latitudinal and longitudinal gradients (Pagès and Gili, 1991a, 1991b; Pagès *et al.*, 1991; Pagès, 1992; Fearon *et al.,* 1992). Although the boundaries to these assemblages tend to agree with the regional oceanography, they vary

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with upwelling intensity. The diversity of the inner-shelf assemblages of gelatinous zooplankton is universally low (see also Gibbons and Hutchings, 1996). This contrasts with the pattern of numerical abundance, which tends to peak over the shelf and reflects spatial gradients of productivity.

Pelagic cnidarians are considered to be important predators in the pelagos (e.g. Matsakis and Conover, 1991; Purcell *et al.,* 1994). However, our understanding of their precise role in the region is still a product of postulation (e.g. Gibbons *et al.*, 1992). In sheltered bays and harbours, they have been shown to influence the abundance of some other zooplankton species (Fulton and Wear, 1985), and they have also been linked to fluctuations in wider-scale fisheries (Möller, 1984). Given the rapacious appetites of gelatinous carnivores (Costello, 1988; Gibbons and Painting, 1992), these impacts are primarily the result of a coincidence in time and space of predators and prey, which can be fostered by the sheltered nature of the environment. In pulsed and seasonal upwelling ecosystems, such as the Benguela, it is very difficult to disentangle the impact of pelagic cnidarians and ctenophores on zooplankton and fisheries from more general, wider environmental variability. This is because the distribution and abundance of plankton is dramatically altered with each upwelling pulse (Andrews and Hutchings, 1980).

It has recently been shown that inter-annual fluctuations in the abundance of commercial pelagic fishes in the southern Benguela are linked to changes in the environment (including the zooplankton food environment) (Verheye and Richardson, 1998; Verheye *et al.*, 1998). The present paper looks at interannual variability in the composition, abundance and distribution of medusae and ctenophores over a part of the same time period in an effort to improve our understanding of long term dynamics of ecosystems. This study describes the relationship between medusae and ctenophores with the environment, and so might enable us to make more direct links with fisheries in future.

MATERIALS AND METHODS

Study Site

St Helena Bay (~ 31°50'S - 32°50'S) (Fig. 1) is the broad shelf area to the north of Cape Columbine, which is one of the five major upwelling centres along the west coast of southern Africa (Shannon, 1985).

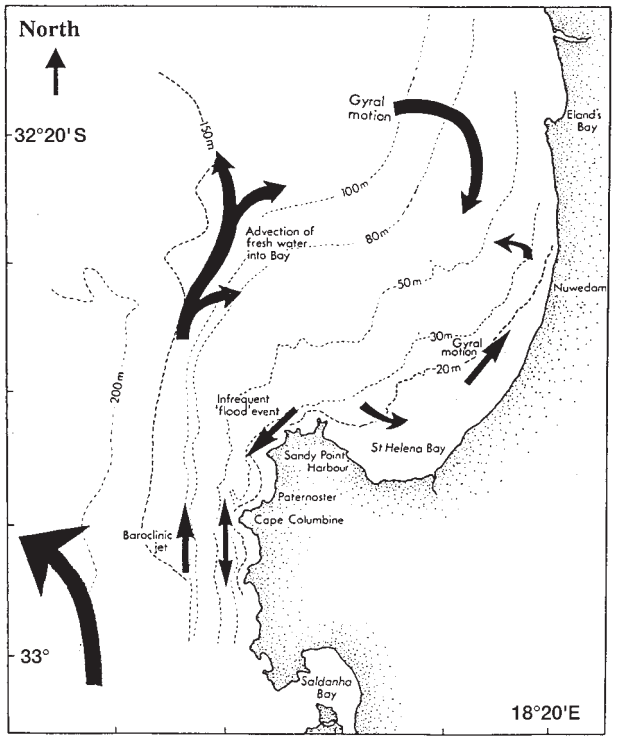

FIG. 1. – Map of the study area, showing generalised patterns of circulation (adapted from Chapman and Bailey, 1991)

Field Sampling

Ten to 44 stations 10 nautical miles apart were sampled each year by either the *FRS Africana* (1988-1994) or the *FRS Algoa* (1995-1997) during May and June from up to fifteen transects perpendicular to the shoreline of St Helena Bay (Table 1). The number of transects and the number of stations per transect varied each year according to the needs of the stock assessment surveys decided by Marine and Coastal Management. All stations were sampled during the day.

Zooplankton samples were collected at each station using paired vertical Bongo nets (57 cm mouth diameter), fitted with a 200 μm mesh (McGowan and Brown, 1966). The nets were lowered to 5 m above the sea floor (maximum depth for the studied area was 398 m in 1991) and hauled vertically at a speed of 1 m.s^{-1}. From 1988 to 1992, the volume filtered by the nets was calculated as a function of the mouth area and the sampling depth. From 1993 - 1997, the volume filtered by the nets was measured using an electronic flowmeter mounted in the mouth of one of the nets. On retrieval, all samples were immediately preserved in 5% buffered seawater formalin.

Crustacean mesozooplankton were identified and counted from replicate 5 ml subsamples. Numbers were converted to dry-weight using the equations of Painting (Painting *et al*., unpublished), prior to summation and the calculation of total mesozooplankton biomass (per m^2).

All medusae (hydromedusae and scyphomedusae) and ctenophores were identified and counted, without subsampling, and the data expressed as numbers per 100 m^3. A total of 243 samples were examined. Less than 0.5% of all the animals collected were too damaged to be identified.

A number of environmental parameters were measured at the same time as the zooplankton samples were collected. The speed and direction of near-surface currents (at 30-35 m depth) were determined on station, using a hull-mounted acoustic doppler current profiler (ADCP). Wind speed and direction were determined at each station using an anemometer mounted above the bridge of the research vessel. Although a conductivity-temperature-depth (CTD) instrument with a 12-bottle rosette was cast from the surface to close to the bottom at each station to provide profiles of salinity, temperature and Sigma-t, only surface values are presented here. Fluorescence profiles were obtained using a Chelsea Instruments Aquatracka submersible fluorometer mounted on a magnum rosette. Water samples were collected at the surface for the determination of size-fractionated chlorophyll *a* (total and > 10 μm), and to analyse the particle size composition of the water. Chlorophyll samples were analysed within 6 h following the method of Parsons *et al*. (1984), while a Coulter multisizer was employed to determine the particle-size composition of the water.

It should be noted that not all environmental parameters were collected across the whole grid each year (see Table 1).

Statistical analysis and data handling

Descriptive and multivariate statistics were used to examine relationships among the samples of medusae and ctenophores each year, in order to see whether there was any horizontal pattern to the assemblages observed. The densities of all species that contributed to greater than 10% of the numeric total in each sample (henceforth known as the dominant species), were root-root transformed and a similarity matrix was constructed between the samples using the Bray-Curtis Index (Field *et al*., 1982). These matrices were used to plot classification diagrams of percentage similarity between samples using group-average sorting. The identified clusters of samples (assemblages) were then superimposed onto maps of the sampling grid (Fig. 2). These analyses were conducted using PRIMER software.

"Indicator species" for each assemblage each year were identified using the SIMPER routine in PRIMER. This exploratory software routine calculates the contribution of each species **both** to the average Bray-Curtis dissimilarity **between** the identified assemblages (sample clusters), and to the average similarity **within** an assemblage. For ease of interpretation, the number of indicator species presented has been restricted to those species which contribute to 80% of the cumulative percentage similarity.

In an effort to determine which of the environmental parameters could "best explain" the pattern observed in the biological assemblages each year, the BIOENV proceedure in PRIMER was used. This software maximises a Spearman rank correlation between the environmental and biological similarity matrices (Clarke and Ainsworth, 1993). The environmental similarity matrices were constructed using normalised Euclidean distance whilst the Bray-Curtis Index was used to construct the similarity matrix for the biological data.

Hitherto, all the analyses have been conducted on the data collected each year. In an effort to detect any common patterns of association between the dominant species (those which were found in more than 10% of samples), Bray-Curtis similarity matrices were constructed from the whole 10-year data set and used to plot a classification diagram of percentage similarity using PRIMER.

Relationships between the abundance of the dominant species, and various environmental characteristics (temperature, depth and chlorophyll *a*) were examined using correlation analyses and multiple regression analyses of the untransformed data. These analyses were conducted using STATISTICA software on the whole 10-year data set.

RESULTS

The sea surface temperature (SST) during winter in St Helena Bay varied from a minimum of 10.9°C (in 1990) to a maximum of 18.9°C (in 1991). SST and sea surface salinity were lower near the coast and in the north (<14.0°C and < 35.0 psu), than offshore and in the south (>16.0°C and > 35.0 psu)

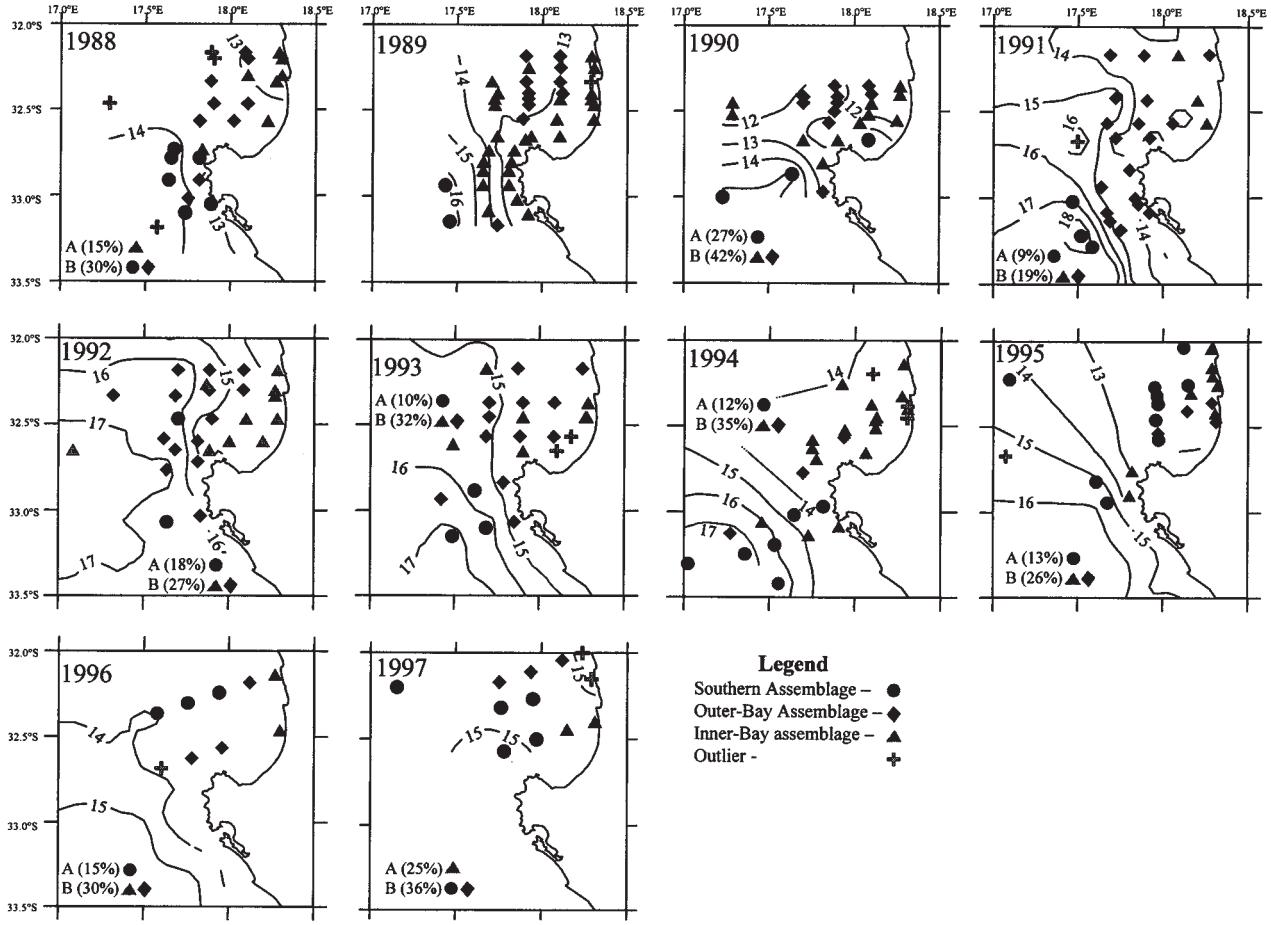

FIG. 2. – The different assemblages of medusae and ctenophores, identified by cluster analysis and superimposed upon maps of the study area each year. Contours represent the position of surface isotherms (°C), while the percentage value indicates the Bray-Curtis level of similarity between the assemblages.

TABLE 1. – List of parameters measured by Marine and Coastal Management during the Recruit Survey, by year: * indicates parameters measured for all samples; # indicates parameters available only for some of the samples. Zooplankton biomass and dry weight were measured for all the zooplanktonic groups, non-crustacean and gelatinous zooplankton.

	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997
Sea surface temperature (SST)	*	*	*	*	*	*	*	*	*	*
Sea surface salinity	*	*	*	*	#	#		#		
Currents at 30-35 m depth (ADCP)			#	#	#	#	#	#	#	#
Sea surface fluorimetry				*	*	*				
Wind speed and direction				*	*	*	*	*	*	
Total Chlorophyll *a*	*	*	*	*	*	*	*	*		
Chlorophyll a < 10µm	*	*	*	*	*	*	*	#		
% Chlorophyll a < 10µm	*	*	*	*	*	*	*	#		
% Chlorophyll a > 10µm	*	*	*	*	*	*	*	#		
Biomass and dry weight zooplankton	#	#	#	#	#	*	*	*	*	
Maximum depth noted during the survey	318 m	365m	216m	398m	313m	202m	201m	202m	166m	148m
Mean temperature	13.3°C	13.1°C	12.2°C	14.3°C	15.4°C	15.6°C	14.3°C	13.0°C	13.6°C	15.3°C
Number of transects	11	15	8	8	9	7	8	9	2	3
Number of samples collected	29	44	27	29	32	25	29	29	10	12

(Fig. 2). The coldest year was 1990, with a mean SST of 12.2°C. By contrast, 1992 and 1993 were the warmest years and the mean SST reached 15.4°C and 15.6°C respectively (Table 1).

A total of 50 hydromedusae species (29 anthomedusae, 13 leptomedusae, 3 limnomedusae, 1 narcomedusae and 4 trachymedusae), 1 scyphomedusa species and 2 ctenophores species (1 tentacula-

TABLE 2. – Species of medusae and ctenophores collected from 1988 to 1997 and the total number of each species collected in the Survey. Species preceded by # are holoplanktonic (totally pelagic), whereas the others are either known, or assumed to be meroplanktonic (with a benthic stage).

Species	Years /occurrences 88	89	90	91	92	93	94	95	96	97	Total number
PHYLLUM CNIDARIA											
CLASS HYDROZOA											
Subclass Hydroidomedusae											
Order Anthomedusae											
Dicodonium sp.	•										1
Dipurena halterata (Forbes, 1846)		•									1
Dipurena ophiogaster Haeckel, 1879				•							1
Sarsia nipponica Uchida, 1927								•			1
Sarsia resplendens Bigelow, 1909								•			1
Ectopleura dumortieri (van Beneden, 1844)		•	•	•			•				14
Euphysa aurata Forbes, 1848	•	•	•	•	•	•	•	•	•	•	763
Euphysilla pyramidata Kramp, 1955	•			•	•		•	•			6
Euphysomma brevia (Uchida, 1947)			•	•			•				3
Euphysora gracilis (Brooks, 1882)	•										1
Euphysora furcata Kramp, 1948				•							1
Hybocodon unicus (Browne, 1902)	•		•		•						4
Pennaria sp.	•										1
Zanclea costata Gegenbaur, 1856			•	•	•		•				5
Zancleopsis gotoi Uchida, 1927					•						1
Zancleopis tentaculata Kramp, 1928								•			2
Staurocladia vallentini (Browne, 1902)							•				1
Turritopsis nutricula McCrady, 1856								•			1
Podocoryne minuta (Mayer, 1900)	•			•		•					3
Podocoryne carnea M. Sars, 1846	•										1
Bougainvillia macloviana Lesson, 1836		•	•	•	•	•	•				125
Lizzia blondina Forbes, 1848					•	•					3
Nemopsis sp.			•								1
Amphinema dinema (Péron & Lesueur, 1809)							•				2
Amphinema rugosum (Mayer, 1900)	•										1
Pseudotiara tropica Bigelow, 1912		•									1
Leuckartiara octona (Fleming, 1823)	•	•	•	•	•	•	•	•	•	•	469
Urashimea globosa Kishinouye 1910				•		•					45
Heterotiara sp.		•									1
Order Leptomedusae											
Krampella sp.	•										1
Staurodiscus sp.		•									1
Mitrocomella grandis Kramp, 1965		•	•		•	•			•	•	149
Mitrocomella millardae Pagès *et al.*, 1992	•	•	•	•	•	•	•	•	•	•	3 089
Obelia spp.	•	•	•	•	•	•	•	•	•	•	1 752
Clytia folleata (McCrady, 1857)								•			4
Clytia hemispherica (Linné, 1767)	•		•	•	•	•	•	•	•	•	67
Clytia simplex (Browne, 1902)			•			•					6
Eucheilota paradoxica Mayer, 1900								•			2
Cirrholovenia polynema Kramp, 1959		•									1
Phialella quadrata (Forbes, 1848)				•	•		•				4
Eirene menoni Kramp, 1953		•			•						2
Aequorea aequorea (Forskål, 1775)	•		•		•	•					5
Order Limnomedusae											
Cubaia aphrodite Mayer, 1894									•		1
Proboscidactyla menoni Pagès *et al.*, 1992	•	•	•	•	•	•	•	•	•	•	547
Proboscidactyla stellata (Forbes, 1846)		•									1
Order Narcomedusae											
#Solmundella bitentaculata (Quoy & Gaimard, 1833)	•	•		•	•	•	•	•	•	•	35
Order Trachymedusae											
#Liriope tetraphylla (Chamisso & Eysenhardt, 1821)	•	•	•	•	•	•	•	•			111
#Aglaura hemistoma Péron & Lesueur, 1809	•	•	•	•	•	•	•	•			790
#Persa incolorata McCrady 1857	•	•	•		•				•		26
#Rhopalonema velatum Gegenbaur, 1856	•	•			•			•			17
CLASS SCYPHOZOA											
Order Semaeostomae											
Chrysaora hysoscella (Linné, 1766) (ephyra)	•	•	•		•	•				•	851
PHYLLUM CTENOPHORA											
CLASS TENTACULATA											
Order Cydippida											
#Pleurobrachia pileus (O.F. Müller, 1776)	•	•	•	•	•	•	•	•	•	•	5 288
CLASS NUDA											
Order Beroida											
#Beroe spp.	•	•	•	•	•	•	•		•	•	290
Number of species collected per year	24	23	21	21	24	19	19	18	12	11	24 512

ta and 1 nuda) were collected over the course of the 10-year survey (Table 2). The maximum number of species recorded in any one year was 24 in 1988 and 1992, and the minimum number observed was 11 in 1997 (Table 2). Many of the species recovered have previously been recorded from the region (Millard, 1975, Pagès *et al.*, 1992; Bouillon, 1999), but there are 21 new records for the southern Benguela. These include *Sarsia resplendens*, *Euphysilla pyramidata*, *Euphysoma brevia*, *Zancleopsis tentaculata* and *Urashimea globosa*.

Although *Euphysa aurata*, *Leuckartiara octona*, *Mitrocomella millardae*, *Obelia* spp., *Proboscidactyla menoni* and *Pleurobrachia pileus* were the only species to be caught every year (Table 2), their abundances were highly variable. For example, the average abundance of *E. aurata* in 1991 was 69.86 ind. m^{-3} (± 248.86), but during 1995 only 0.33 ind. m^{-3} (± 0.75) were recorded.

Mitrocomella millardae was the most abundant species and 5 269.36 ind. m^{-3} were collected in 1994. *Pleurobrachia pileus* was the second most abundant species in St Helena Bay. By contrast, other species such as *Podocoryne carnea* (in 1988) and *Staurocladia vallentini* (in 1994) were recorded only once during the entire survey (Table 2). These and the other uncommon species listed in Table 2 were ignored in all subsequent analyses, which were confined to the dominant 12 species.

Although there was no underlying relationship between total species richness and either temperature or depth (Table 3), an examination of the data by life-cycle reveals two contrasting patterns. The species richness of the meroplanktonic assemblage (comprised of species with an attached polyp stage during the life cycle) was negatively correlated with both temperature and depth, but it was positively correlated with total chlorophyll *a*. By contrast, the richness of the holoplanktonic assemblage of medusae (medusae with a totally pelagic life cycle) was positively correlated with temperature and depth, and negatively correlated with total chlorophyll *a*. *In other words the meroplanktonic species reached peak richness in cold, chlorophyll a-rich shallow water, whilst the holoplanktonic species were more diverse in warm, oligotrophic deep water.*

The total abundance of medusae and ctenophores was negatively correlated with bottom depth and surface temperatures (Table 3). Although the abundance of holoplanktonic ctenophores was also correlated negatively with depth and temperature, the abundance of meroplanktonic medusae was significantly, and negatively, correlated with depth. The abundance of holoplanktonic medusae was positively correlated with temperature but not with depth or total chlorophyll *a* (Table 3).

TABLE 3. – Correlation coefficients between species richness and abundance, and various parameters of the physical environment. Data expressed as totals and subdivided by life-cycle type and taxonomic group. Level of significance indicated: ** $p < 0.05$, * $p < 0.10$.

	Temperature	Depth	Total chlorophyll *a*
Total Abundance	-0.14*	-0.19**	0.02
Total Species Richness	-0.06	-0.07	0.08
Holo-Abundance	0.16**	-0.03	0.06
Holo- Richness	0.51**	0.55**	-0.18**
Mero- Abundance	-0.13	-0.16**	0.08
Mero- Richness	-0.25**	-0.27**	0.18**
Antho- Abundance	-0.06	-0.09	0.08
Antho- Richness	-0.12	-0.05	0.17**
Lepto- Abundance	-0.10	-0.12	0.05
Lepto- Richness	-0.25**	-0.27**	-0.01
Trachy- Abundance	0.15**	-0.04	0.07
Trachy- Richness	0.48**	0.52**	-0.17
Cteno- Abundance	-0.12	-0.15*	-0.03

The results of the cluster analysis suggest that each year two distinct groups of samples could be identified. These clusters roughly correspond to samples collected within, and to the south of, St Helena Bay; their positions have been mapped in Figure 2. The percentage similarity between these two main groups varied between 9% (in 1991) to 27% (in 1990). The samples collected within St Helena Bay (hereafter referred to as "Bay" samples) could usually be further subdivided into two subgroups that corresponded to inshore-and outer-shelf-samples. It is important to note that while all three assemblages could be identified each year, their positions varied from one year to the next (Fig. 2). There were also a number of outlying samples (Fig. 2), which showed a limited degree of similarity with any others and these have been ignored here as uninformative.

The BIOENV procedure suggested that surface temperature and/or water column depth and/or chlorophyll *a* were the environmental variables that best explained the pattern observed each year in the assemblages (Table 4). However, the same variables, or combination of variables, did not always provide the best explanation for the pattern observed amongst the assemblages each year. For example, water column depth and chlorophyll *a* best explained the pattern observed in 1988, whilst temperature alone explained the pattern observed in 1994.

TABLE 4. – Harmonic correlations between environmental parameters which, either singularly or in combination, were significantly correlated (*: $p < 0.05$) with the structure of the medusa and ctenophore assemblages identified by cluster analysis and indicated on Fig. 2. The analysis was conducted using the BIOENV procedure in PRIMER (see text). There were insufficient data during 1996 and 1997 to conduct these analyses.

	1988	1989	1990	1991	1992	1993	1994	1995
Temperature		*	*	*		*	*	*
Depth	*		*	*	*	*		*
Total Chlorophyll	*	*				*		
Correlation factor	0.30	0.40	0.33	0.50	0.20	0.15	0.18	0.37

TABLE 5. – Specific composition of the two main assemblages (Southern and "Bay" assemblages) and of the two sub-groups (inshore-and outer-shelf- assemblages) which compose the "Bay" assemblage. Only species responsible for contributing to 80 % of the assemblage identity are included. The average abundances are indicated in brackets (ind. 100 m^{-3}).

	Southern assemblage	"Bay" assemblage	Inshore assemblage	Outer-shelf assemblage
1988	*P. menoni* (11.6) *Obelia* spp. (109.2) *P. incolorata* (2.6) *P. pileus* (33.2)	No significant species	*C. hysoscella* (1239.9)	*P. menoni* (10.1) *P. pileus* (19.9)
1989	No significant species	*P. menoni* (23.3) *L. octona* (17.7) *P. pileus* (49.9) *Beroe* sp. (14.2)	*P. pileus* (68.7) *P. menoni* (26.3) *L. octona* (16.6)	*L. octona* (20.7) *P. menoni* (15.3) *E. aurata* (5.6)
1990	*P. pileus* (20.7)	*P. pileus* (1275.6) *P. menoni* (25.9) *Obelia* spp. (373.7) *C. hemispherica* (11.7)	*P. pileus* (2318.5) *Obelia* spp. (699.3) *P. menoni* (40.1) *Beroe* sp.(87.1) *C. hemispherica* (19.3)	*P.pileus* (128.4) *E. aurata* (8.4) *P. menoni* (10.3)
1991	*L. tetraphylla* (6.8) *E. aurata* (15.2) *A. hemistoma* (0.8)	*E. aurata* (89.8) *P. menoni* (22.9) *P. pileus* (189.7)	*P. menoni* (23.4) *Obelia* spp. (49.4)	*E. aurata* (104.7) *P. pileus* (221.3) *L. octona* (3.04)
1992	No significant species	*E. aurata* (31.4) *R. velatum* (56.7) *A. aequorea* (16.56) *L. blondina* (9.9) *L. tetraphylla* (66.5)	*A. hemistoma* (97.5) *E. aurata* (36.9) *L. octona* (5.7)	*C. hysoscella* (120.9) *P. menoni* (32.2) *B. macloviana* (90.0) *P. pileus* (185.8) *Beroe* sp. (42.4) *C. hemispherica* (21.5)
1993	A. hemistoma *(4.4)*	*P. pileus* (706.8) *C. hysoscella* (19.1) *Obelia* spp. (292.2) *P. menoni* (6.9) *L. octona* (9.0)	*P. pileus* (128.1) *P. menoni* (8.9) *L. octona* (14.5) *C. hysoscella* (15.8) *E. aurata* (10.6)	*Obelia* spp. (86.4) *P. pileus* (32.5)
1994	*L. tetraphylla* (6.9) *A. hemistoma* (10.9)	*P. menoni* (26.5) *L. octona* (45.6) *E. aurata* (39.0)	*P. pileus* (470.8) *P. menoni* (30.0) *Obelia* spp. (16.5)	*L. octona* (54.4) *P. menoni* (25.8) *E. aurata* (46.0)
1995	*L. octona* (4.9) P. menoni *(4.5)*	*Obelia* spp. (56.3) *M. millardae* (23.3) *P. pileus* (14.6)	*Obelia* spp. (56.3) *M. millardae* (23.3) *P. pileus* (14.6)	*M. millardae* (26.9) *P. pileus* (5.8)
1996	*E. aurata* (7.0) *L. octona* (7.8)	*P. pileus* (88.7) *M. millardae* (927.8) *C. hemispherica* (4.8)	No significant species	*P. pileus* (65.6) *M. grandis* (8.8)
1997	*L. octona* (11.6)	No significant species	No significant species	*E. aurata* (87.1) *L. octona* (5.7)

The dominant species that could be used to identify the different assemblages each year tended to vary (Table 5). For example, during 1988 the southern assemblage was identifiable by relatively high numbers of *Proboscidactyla menoni*, whilst in 1994 it was identifiable by large numbers of *Liriope tetraphylla*. Although many of the species were found in more than one assemblage, some species tended

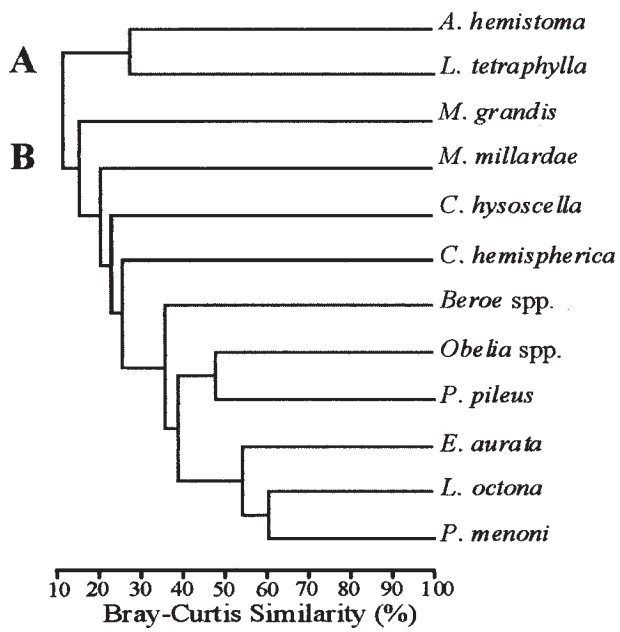

FIG. 3. – Dendrogram of percent similarity (Bray-Curtis Index) between the dominant species of medusae and ctenophores collected during winter in St Helena Bay, over the period 1988-1997. Two clusters can be seen that correspond to warm and deep water species (A), and cooler and shallow water species (B).

to be consistently present in some assemblages but absent in others. For example, *Liriope tetraphylla* was a conspicuous component of the southern assemblage, whilst *Pleurobrachia pileus* and *Mitrocomella millardae* were more characteristic of the Bay assemblages.

The dendrogram of percent similarity amongst the dominant gelatinous zooplankton delimits two main clusters at the 10% level (Fig. 3). These two clusters indicate species-groups whose members tended to be found together in samples, and whose abundances tended to fluctuate in tandem with each other. The greater the similarity between members the more frequently they were found together. The two clusters identified were of very unequal size and correspond to holoplanktonic medusae (group A), and meroplanktonic medusae and holoplanktonic ctenophores (group B). Although the level of similarity between associated species was generally low, these two clusters roughly correspond to the southern and "Bay" assemblages identified previously (Fig. 3, Table 5).

Owing to the fact that generally too few samples of any one species were collected in any one year, it was necessary to look at species-specific responses to the physical environment using the entire data set. The results of the correlation analyses reveal that the abundances of only seven of the 12 dominant species could be linked to the environment. The abundances of the two holoplanktonic species (group A) were both positively correlated with temperature and/or depth. The abundance of group B species was all negatively correlated with either temperature and/or depth, although it should be noted that congruence in the pattern of response was not constant between species. However, from multiple regression analyses done with the species and the environmental parameters (temperature, depth and concentration on chlorophyll *a*), the abundance of only three of the dominant species could be significantly explained (at least at the 0.01 level) by linear combinations of the environment. Of these dominant species, *Liriope tetraphylla* and *Aglaura hemistoma*, group-A members, had their abundances linked to temperature and depth (but not chlorophyll *a*). The remaining species (*Clytia hemispherica*) was from group B, and was negatively affected by temperature.

DISCUSSION

In order to explain the interannual patterns in assemblage composition, abundance and distribution, it is necessary to try to appreciate the environment in which they occur. The western coast of South Africa is subject to SE winds, which quickly result in coastal upwelling. This is particularly pronounced where the shelf-edge lies close to the coastline and in the vicinity of capes (such as Cape Columbine) and peninsulas. Although the newly upwelled water is cold and nutrient-rich, it gradually warms as it moves offshore (by Ekman transport), and phytoplankton populations bloom at the surface. The upwelling front that develops may, if the SE winds are strongly sustained, eventually be coincident with the shelf-edge front that separates coastal waters from warm, chlorophyll-poor oceanic waters. Onshore winds result in the fairly rapid movement of the oceanic water over the shelf, which leads to an increase in SST there and downwelling (Shannon, 1985; Boyd *et al.*, 1992). The system is characterized by much variability, and by rapid changes in the physical environment.

Each year, the assemblages of gelatinous zooplankton could be divided into two groups on the basis of similarities in their specific composition (Fig. 2): a cool, shallow water assemblage (identified as "Bay") and a warmer, deeper water assemblage (identified as "southern"). These groups were

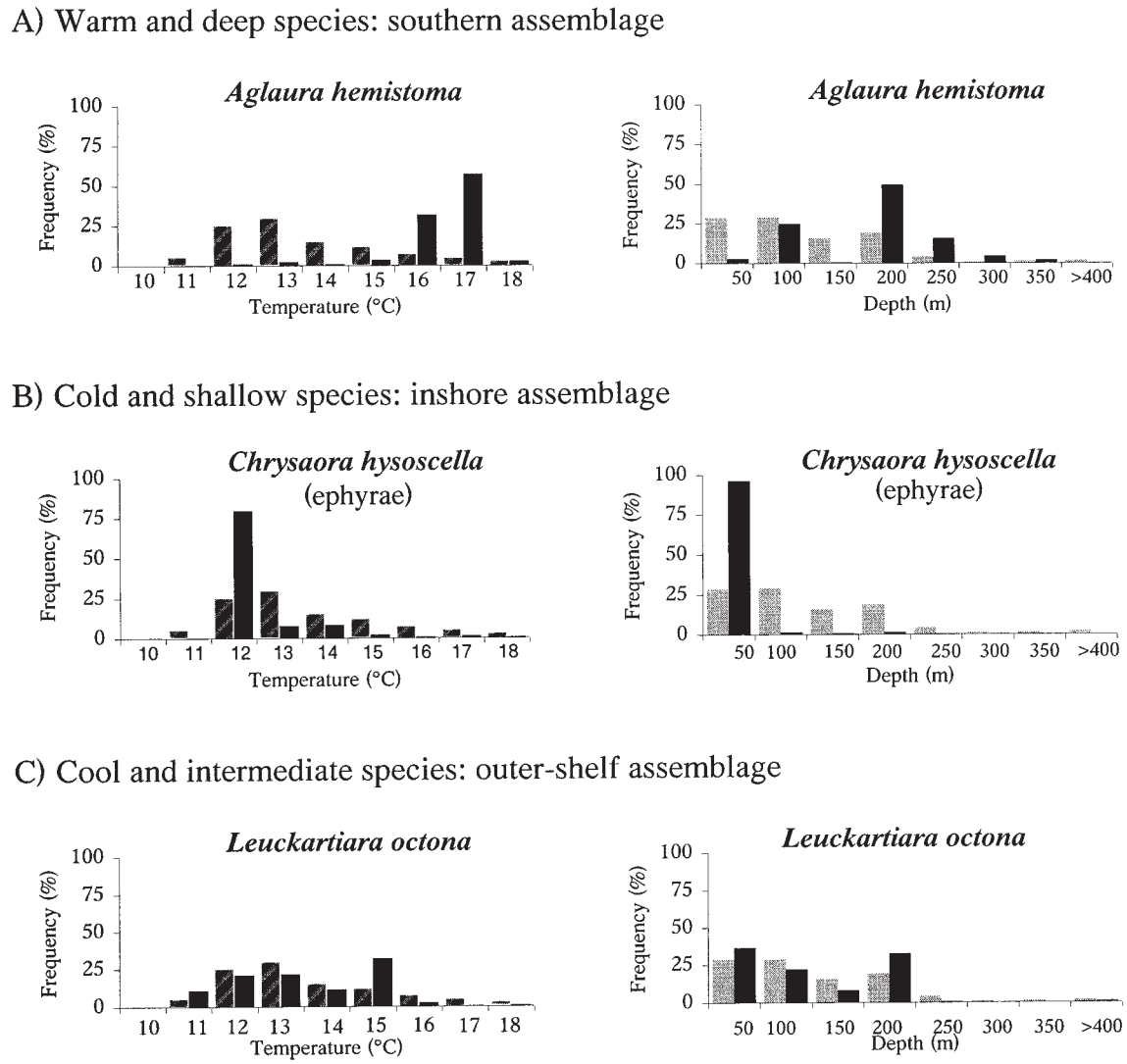

FIG. 4. – Frequency distributions of three species of medusae (black bars) plotted against frequency distribution of temperature (striped grey bars) and depth (light grey bars). Each species is characteristic of one of the three different assemblages identified in St Helena Bay during winter. The frequency distributions were calculated with all the results from the survey. For example: between 1988 and 1997, 24.8% of the samples were collected at temperatures between 10 and 11°C and 79.7% of the ephyrae of *Chrysaora hysoscella* were collected at these temperatures.

"best" explained in terms of the structure of the biophysical environment (temperature, and/or depth, and/or chlorophyll *a*) (Table 4). The spatial limits of the groups varied from year to year, presumably in accordance with the prevailing circulation (Shannon, 1985), and with the water masses present.

The southern assemblage was characterised by holoplanktonic species of medusae, whose richness increased in the warm, chlorophyll-poor offshore waters (Table 2). The dominant species of this assemblage (*Liriope tetraphylla* and *Aglaura hemistoma*) are typical of oceanic waters (Russell, 1953). Their abundance was positively correlated with temperature (Fig. 4) and they tended to be more common during warm years. For example, the mean abundance of *A. hemistoma* in the southern assemblage during 1992 (an El Niño year), was 60.1 ind. m^{-3} but was only 0.1 ind. m^{-3} in 1990. Their greater overall abundance during warm years reflects the greater area of St Helena Bay inundated with warm water then. Pagès *et al.* (1991) described both these species as indicators of oceanic water in the southern Benguela. These authors noted that although they were generally rare, they were occasionally common components of the inshore biota south of Cape Columbine, which was postulated to follow the mixing of shelf and oceanic waters there.

The "Bay" assemblages were less clearly defined in terms of actual species than the southern assem-

blages. However, they were characterised by meroplanktonic medusae and holoplanktonic ctenophores, whose abundance and diversity increased in cold, shallow and chlorophyll-rich waters (Table 5; Fig. 3, Group B). The dominant species were negatively correlated with temperature and tended also to be more abundant during cold years. For example, *Mitrocomella millardae* reached a mean density of 11.1 ind. m^{-3} in 1990, but only 0.5 ind. m^{-3} in 1992.

Meroplanktonic medusae are common in shelf waters (Goy, 1997), because (in part) of their sessile polyp stages which require some sort of substratum for larval settlement. Pagès *et al*. (1991) noted that although shelf assemblages north of Cape Columbine (which largely correspond to the "Bay" assemblage here) were characterised by meroplanktonic medusae, their composition varied with upwelling intensity. These authors also noted that the shelf assemblages in St Helena Bay could be subdivided into two that broadly corresponded to inshore and offshore regions. They found that *Leuckartiara octona* was characteristic of the offshore samples, which is in agreement with the results observed here for that species (Fig. 4).

Although the overall abundance and diversity of the meroplanktonic taxa were negatively linked to temperature and depth (Table 3), correlations between individual species abundance and the physical environment were low. Indeed, it is fair to state that most of the species failed to show any abundance response to the physical environment, and could be found anywhere within the Bay at any time. The lack of observed response at the species level has its origins in a number of factors, enumerated below.

The species (as *Obelia* spp. or the ephyrae of *Chrysaora hysoscella*) could be eurythermal (as opposed to the holoplanktonic species in group A), and showed common responses across the low temperature range observed in the Bay throughout the study period. This is supported by the fact that most of the dominant species recovered here have been widely recorded elsewhere in the world (Kramp, 1961), where they are able to survive and grow in a wide variety of thermal environments.

Alternatively, it should be realised that the occurrence of a medusa in the Bay must reflect the environmental conditions at some stage prior to sampling, while the observed distribution of the species should reflect the oceanographic processes that have taken place in the interim.

The polyp stages of meroplanktonic species have certain, specific environmental cues to which medusa release is the response. These cues include temperature (Werner, 1961) and food (Roosen-Runge, 1970), but may also be light (Costello, 1988) or phases of the lunar cycle (Goy, 1973), or any combination of these (and other) factors (Arai, 1992). However, the precise and relevant cues are not known for most species. Should any factor not be present, then the pelagic stage will not occur in the water column and the species will not be recovered from samples. A species might also be present in low numbers, and not collected by net sampling. However, should the environment have been favourable for medusa release, then although the species blooms (typically in an episodic fashion) the historical cues linking abundance to the environment are missed. Especially in the once-a-year surveys employed here. Pagès *et al*. (1991) encountered a similar problem in their study of the pelagic cnidarians in the Benguela ecosystem and postulated that the overwhelming abundance of some occasional species in St Helena Bay was probably due to an upwelling event prior to the survey that triggered their release.

Tracking the fate of the newly released medusae by backtracking changes in the physical environment in a dynamic environment such as St Helena Bay, is clearly not possible at this stage. However, this very dynamism can account for the poor degree of similarity amongst meroplanktonic associates (Fig. 3), as well as the low correlation coefficients between species abundances and the environment. It can also explain the inter-annual inconsistencies among the environmental parameters that best explain the patterns in assemblage composition (Table 4) and the variable indicator species (Table 5), as well as the variable "boundaries" that were observed to the identified assemblages from one year to the next (Fig. 2).

Comparative studies of interannual variations in the abundance and distribution of zooplankton in the region are sadly lacking. From a long-term study of changes in copepod biomass conducted at a station off the Cape Peninsula, Verheye *et al*. (1998) have recently shown a net increase in the copepods biomass (10 fold) between 1951 and 1996. These authors found a marked increase in the abundance of copepods < 0.9 mm prosome length and a decrease in the numbers of those > 1.0 mm prosome length. It was suggested that these changes in the composition of the communi-

ty might have allowed a regime-shift from sardine to anchovy. These authors postulated that this increase in small-animal biomass could reflect biological responses to the long-term intensification of upwelling in the Benguela ecosystem as well as a reduction in predation pressure due to a decrease in pelagic fish biomass (Verheye and Richardson, 1998).

Long-term studies have begun to show that shifts in the structural composition of pelagic communities might be "normal" for the pelagic marine ecosystem (Russell *et al.,* 1971; Southward, 1980; 1984; Southward *et al.,* 1988). Aside from work on fishes and copepods, there are few pluriannual observations of gelatinous zooplankton. In the Mediterranean Sea, an alternation of gelatinous species has been observed over at least a 17 y period (Morand and Dallot, 1985; Buecher *et al.*, 1997). The species that have replaced each other have the same ecological role but differences in the physical environment (temperature, turbidity) and/or the biological environment (food availability, predation pressure) favours blooming of one species rather than another. Explaining these specific alternations as normal characteristics for the dynamics of gelatinous zooplankton, Boero (1991) described an "internal circannual clock" which either activates some resting stage or stimulates reproduction, and so favours the proliferation of one species over another species. This activation, which may induce pulsations in the abundance of some species (as for *Mitrocomella millardae* in 1994), as well as differential thermal sensitivity, could explain the succession of species observed in St Helena Bay over the 10-year period of observations.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

The effects of exposure to wave action on the distribution and morphology of the epiphytic hydrozoans *Clava multicornis* and *Dynamena pumila**

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SUMMARY: The spatial distribution patterns of two species of epiphytic hydrozoans, *Clava multicornis* and *Dynamena pumila*, on the intertidal alga *Ascophyllum nodosum* were studied in adjacent wave-sheltered and wave-exposed areas. *Clava* were more abundant on the wave-sheltered algae than on the wave-exposed fronds, and in both areas occupied the basal and middle sections of the algae. There was no difference in the abundance of *Dynamena* between the wave-sheltered and wave-exposed areas, but in both areas *Dynamena* were most abundant on the basal and apical sections of the algae than on the central sections. The number of hydranths per colony of *Clava* was higher in the sheltered area than in the exposed area. The hydrocauli of *Dynamena* on the wave-exposed algae bore fewer hydrothecae than those on the sheltered algae. The proportion of *Dynamena* hydrothecae that contained hydranths was close to 100% in the sheltered area, but only 70% in the exposed area. It was concluded that variations in distribution and morphology could be caused by the direct or indirect effects of one or more variables, including wave action, feeding rates, and exposure to solar radiation. Experiments are required to elucidate the specific effects of these variables.

Key words: epiphytic hydrozoans, wave action, distribution, morphology.

INTRODUCTION

The polyp stages of marine hydrozoans may be found on a wide variety of natural and artificial substrata, including seagrasses and intertidal algae (Seed and O'Connor, 1981; Boero *et al.,* 1985; Gili and Hughes, 1995). Some species are found mainly, or only, on specific plant substrata, often in extreme environments, such as the rocky intertidal zone. The epiphytic habit requires the ability to select specific substrata difficult for other species to colonise (Calder, 1991; Hughes *et al.,* 1991a,b; Genzano and Rodríguez, 1998) and may be an adaptation to reduce interspecific competition, so prevalent in epifaunal communities. However, epiphytism may lead to selective pressures to adapt to special circumstances associated with that host and its environment. Marine plants are flexible, often have a high turnover of leaves or fronds, but may grow continuously, providing new substrata. Seagrasses, for example, are usually found in sheltered conditions, but the leaves are short-lived and the specialist epiphytic hydroids have special growth strategies that help maintain their position on the plants (Hughes, 1991a,b; Rossi *et al*., 1997). In contrast, fucoid algae have apical meristems and parts of the plants

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may be several years old, providing more long-lived substrata. The problems faced by fucoid epiphytes are associated with the highly hydrodynamic environment of the rocky intertidal. Hydroids are small, often delicate, animals that may require special adaptations, either of morphology or habit, to withstand the potential damage caused by wave action. Riedl (1971) reviewed some variations in the morphology of hydroids attributable to variations in water movement and more recently Hughes (1992) described some morphological adaptations of *Dynamena pumila* to enable the hydroids to tolerate wave action.

Differences in distributions of epiphytes may occur at several different scales and are more difficult to interpret. For example, the effects of wave action, which are difficult to measure, are usually studied by relative and fairly unspecific comparisons between obviously wave-exposed and obviously wave-sheltered locations. However, if these areas are separated by large distances there is the further complication of studying different populations and gene pools, and noted differences cannot be attributed with certainty to the environmental variations. This may be especially true for species where the dispersal potential is low because of the absence of planktonic stages. There are further difficulties in explaining the causes of small-scale differences in distributions on the algae, because small-scale variations in the environment occur around the host (e.g. O'Connor *et al.,* 1979). For example, some epiphytes are more abundant in the central areas of seaweed clumps where the effects of wave action are reduced (Ott, 1980), but this may be an effect of the water movement rather than a response to it.

The large fucoid alga *Ascophyllum nodosum* is common on relatively sheltered rocky shores of northwest Europe (Vadas *et al.,* 1990) with a large surface area available for colonisation by epiphytes (Peckol 1988). The sessile epiphytic community of *Ascophyllum* consists mostly of hydrozoans, bryozoans, barnacles and algae and shows spatial variations, with height on the shore and with exposure to water movement (Aldrich *et al.,* 1980). This is a study of the differences in distributions and morphology of the hydroids *Clava multicornis* and *Dynamena pumila,* (hereafter referred to only by their generic names), common facultative epiphytes of the seaweed *Ascophyllum nodosum* (see Cornelius, 1979), in relatively wave-exposed and wave-sheltered locations in close proximity (within 50 m) on the Isle of Cumbrae, Scotland.

METHODS

The study site is at the southern end of Ballochmartin Bay on the east coast of the Island of Great Cumbrae, in the Firth of Clyde, Scotland. The shore faces east across the Fairlie Channel to the mainland about 1 km away and has the flora characteristic of a sheltered shore. The wave-exposed area and wave-sheltered area are on the east and west side of an isolated rock promontory. The wave-exposed site receives wave action predominantly when easterly winds blow across the Fairlie channel; the wave-sheltered site is protected from these waves by the rocky promontory.

Whole plants of *Ascophyllum* that bore colonies of *Clava* or *Dynamena* were selected randomly from within a horizontal transect, 1 m wide and 50 m long parallel to the waters edge at the same tidal height in the two areas. The selected plants were either collected for laboratory examination or examined *in situ*. The distributions of the epiphytes were mapped on sections of the *Ascophyllum* plants wich were categorised according to the approximate dichotomous structure of the algae (Fig. 1). The number of colonies of *Clava* in each section were counted. Colonies of *Dynamena* were less discrete because of their extensive hydrorhizal growth and could not be separated. Instead their abundance was measured by counting the number of hydrocauli in each section. The number of hydranths on thirty randomly selected colonies of *Clava* from each area were counted. The number of hydrothecae on thirty randomly

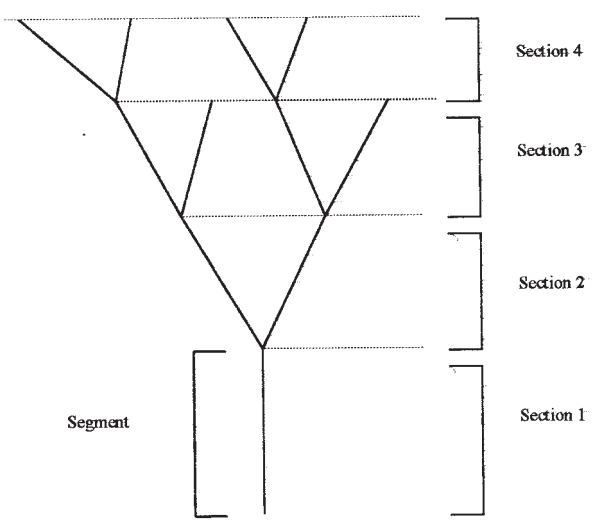

FIG. 1. – Diagram of *Ascophllum* showing how the sections of the algae were categorised.

selected hydrocauli of *Dynamena* from each area were counted and the number of hydrothecae that contained hydranths and were empty were counted on 15 randomly selected hydrocauli fom each area. The distances between adjacent pairs of hydrothecae on 15 randomly selected *Dynamena* hydrocauli from each area were measured.

RESULTS

Clava had a higher density of colonies on *Ascophyllum* in the wave-sheltered area than in the wave-exposed area (Fig. 2a) ($F(1, 247)=18.0$, $p<0.0003$), while *Dynamena* had a significantly higher density of hydrocauli in the exposed area than the sheltered (Fig. 2b) ($F(1, 499)=9.11$, $p<0.005$). In both the exposed and sheltered areas *Dynamena* was more abundant on the basal and apical sections of the algae than on the central sections (Fig. 3b). *Clava* did not show this pattern of distribution and was equally abundant on sections 1-4, rarer on sections 5 and absent on more distal sections. (Fig. 3a).

For both species there were significant differences in size between the exposed and sheltered areas (Fig. 4). The *Clava* colonies had a larger number of hydranths in the sheltered area (22.4±9.13 SD) than in the exposed area (11.7±5.3 SD) ($F(1, 60)=31.6$, $p<0.00001$), while the *Dynamena* hydrocauli bore a larger number of hydrothecae in the sheltered area than in the exposed ($F(1, 53)=17.4$, $p<0.0001$). There was a significant greater proportion of *Dynamena* hydrothecae that contained hydranths in the sheltered area (1±0.14 SD) than in the exposed area (0.7±0.29 SD) ($F(1, 209)=42.79$, $p<0.00001$). There was no significant difference in the distance between adjacent pairs of hydrothecae

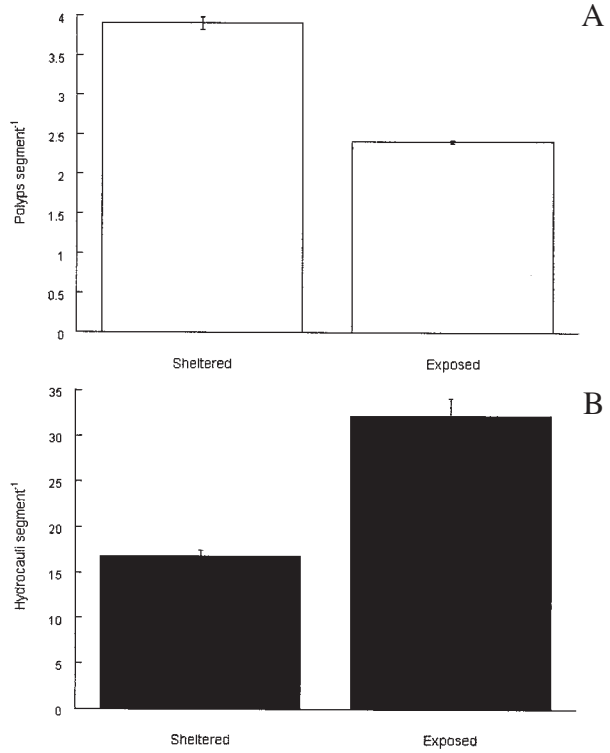

FIG. 2. – The mean number (and standard error) of colonies of *Clava multicornis* (A) and hydrocauli of *Dynamena pumila* (B) on *Ascophyllum* from the wave-sheltered and wave-exposed areas.

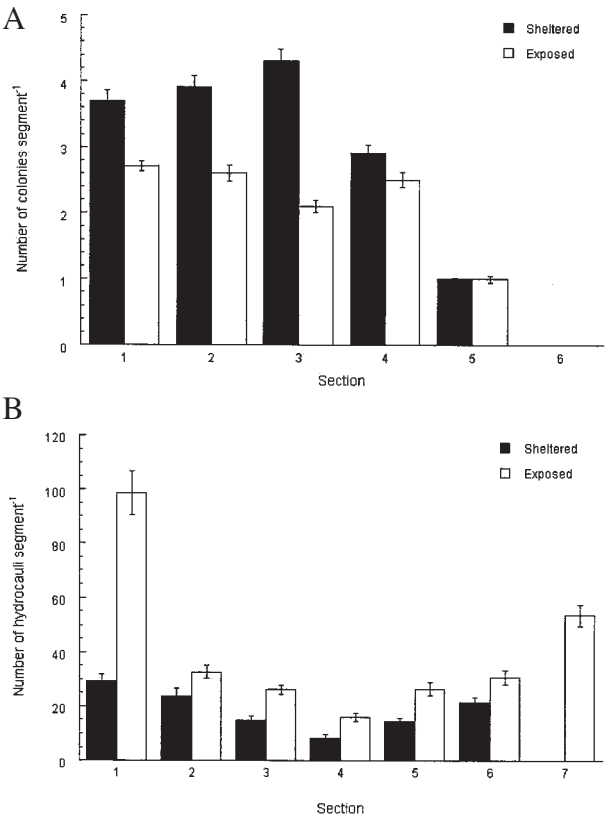

FIG. 3. – The mean number (and standard error) of (A) colonies of *Clava multicornis* (B) hydrocauli of *Dynamena pumila* on each of the numbered sections of *Ascophyllum* in the wave-sheltered and wave-exposed areas.

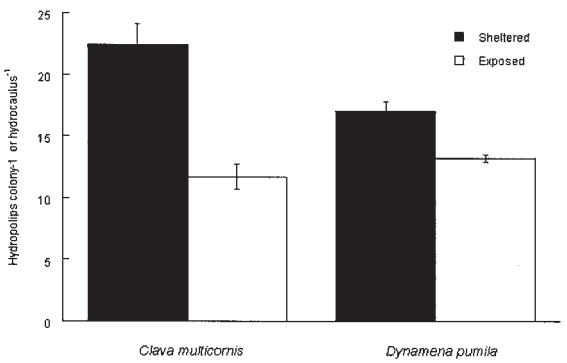

FIG. 4. – The mean number (and standard error) of hydranths per colony of *Clava multicornis* and number of hydrothecae per hydrocaulus of *Dynamena pumila*, on *Ascophyllum* in the wave-sheltered and wave-exposed areas.

of *Dynamena* between the exposed (1.1±0.14 mm SD) and sheltered (1.1±0.23 mm SD) ($F(1, 28)= 0.03$, $p<0.9213$), nor in the distance between adjacent hydrocauli on the hydrorhiza which was consistently close to 3 mm ($F(1, 179)=0.01$, $p<0.9043$).

DISCUSSION

If the abundance of hydroids reflected only the length of time the substrata have been available for colonisation then the epiphytes would be most abundant on the oldest, basal, sections of the algae. However, both *Clava* and *Dynamena* had different distributions, which indicates that other environmental variables affected their distributions. The most significant variables in the environment of a rocky shore that could affect hydroids are the direct and indirect effects of water movement, and the effects of exposure to solar radiation, particularly desiccation and heat stress. Responses to, or the effects of, these variables may explain the distributions of *Dynamena* and *Clava* on the algae. Seed *et al*. (1981) and Seed *et al*. (1983) considered the effects of interspecific competition on the spatial disposition of hydroids and other epiphytes on fucoid algae. In this study the abundance of other epiphytes was much lower than experienced by these authors and no direct affect of competition would have affected the distribution of these hydroids.

Williams (1965) and Orlov (1996a) have shown that the planulae of *Clava* attached mostly on the sheltered parts of *Ascophyllum*, because the larvae were unable to settle when the water movement was too great. Although this may be one explanation for the rarity and absence of *Clava* from the apical sections of the algae, the deleterious effects of wave action subsequent to the settlement of the larvae may also be important because the water movement conditions experienced by planulae, and by recently metamorphosed hydroids, will vary with weather conditions. It is possible that settlement of planulae on calm days may occur on all sections of the algae, and that the most exposed hydroids are removed by subsequent storms. Hydroids are small, delicate passive filter feeders that need to project into moving water to feed. However, on a rocky shore such exposure to moving water makes the hydroids susceptible to damage by breaking waves, particularly athecate hydroids unprotected by an external perisarc. This too may help explain why *Clava* were more abundant on the wave-sheltered shore than on the wave-exposed shore.

That the thecate hydroid *Dynamena*, by contrast, was most abundant on the wave-exposed shore and abundant on the apical sections of the algae may reflect a greater tolerance to wave action, through various morphological adaptations of the perisarc, described by Hughes (1992). *Dynamena*, by surviving in relatively wave-exposed locations, may benefit from feeding in the faster moving tidal currents that sweep across the apical parts of the algae. Moreover, the planula larvae of *Dynamena* tend to be photophilic and not negatively influenced by strong hydrodynamic conditions (Burykin, 1989; Orlov, 1996a). Rapid hydrorhizal growth following settlement (Orlov, 1996b) gives *Dynamena* the potential to colonise new substrata, including the apical sections of *Ascophyllum*, relatively rapidly. This may be interpreted as an opportunistic characteristic as *Dynamena* is a poor competitor in the presence of other epiphytes (Seed and O'Connor, 1981).

Dynamena were less abundant on the central sections of *Ascophyllum* than on the apical and basal sections. The reasons are unknown but these sections of the algae are the most dense and here the proximity to neighbouring algal fronds may reduce feeding opportunities, directly by mechanical abrasion, and indirectly by reducing the water movement around the hydroids. The basal sections of the algae are the oldest and least dense (as there are only a few per alga). When the algae are supported in the water column these basal sections are protected from the worst of the wave action , by the denser more distal parts of the algae, but the tidal currents that flow around them are relatively unrestricted. This, together with the age of these sections, may explain why both species of hydroid were relatively abundant here.

Exposure to solar radiation may also restrict the colonisation of the apical sections of *Ascophyllum,* particularly by the athecate *Clava*. The apical sections are those most exposed to solar radiation when the algae lie flat after the tide has ebbed. Desiccation and heat stress may affect *Clava* more than *Dynamena*, as the hydranths of *Dynamena* may be withdrawn into their thecae which are then closed by operculae. The perisarc may also reduce the amounts of UV radiation reaching the cells of *Dynamena*. Further experiments on the relative susceptibility of the two species to UV radiation, prevention or tolerance of desiccation, and tolerance of heat stress are required to ascertain the importance of these variables in affecting their distributions.

Phenotypic variations in morphology of hydroids have been reported in response to variations in water

movement, food availability and temperature (see review by Gili and Hughes, 1995). Hydroid size is often inversely related to degree of water movement, and this is the case here for both *Clava,* which had fewer hydranths in the wave-exposed area, and *Dynamena*, which had shorter hydrocauli on the wave-exposed algae. Flow velocities may also have an indirect effect on hydroid morphology through feeding rates. Hydroids may feed efficiently in only a narrow range of current velocities, because if the currents are too slow the amounts of food brought to the vicinity of the hydroid is low, and if the currents are too fast the hydranths and the tentacles may be deformed and flattened reducing the surface area presented across the current and reducing feeding efficiency. Further, the potential food particles may be moved past the hydranths too quickly for them to be captured. There is experimental evidence that feeding rates alone may affect hydroid morphology. For example, *Dynamena* and *Gonothyraea* increased hydrorhizal growth and branching in response to increased food supply (Burykin 1980a, b). In *Laomedea* a shortage of food led to shorter and more widely spaced hydrocauli (Berrill, 1950; Crowell, 1957). Thus, water movement may affect hydroid morphology directly and indirectly, and further experiments would be required to ascertain the effects of these different variables. There were no differences between any of the other morphological features evaluated in the two areas, including the distance between hydrocauli of *Dynamena*, which has been postulated to be shorter in exposed areas, thus packing the hydrocauli closer together into a more compact structure (Seed and O'Connor, 1981). Such an effect or response may be seen if samples are taken from shores with a higher degree and range of wave action than those examined here. Alternatively in *Dynamena* the distance between hydrocauli may not be a variable character.

The proportion of *Dynamena* hydrothecae that contained hydranths was lower in the exposed area than in the sheltered area. This may reflect damage to the delicate hydranths by waves, the repair of which may necessitate resorbtion and regrowth of the hydranths. Further observations of the variations in the proportions of empty hydrothecae may provide further evidence, for example by examining hydroids on a wider variety of wave-exposed shores and by examining variations between hydrocauli on different sections of the same alga. Loss of hydranths in other thecate and athecate hydroids has been attributed to an intrinsic cycle of growth, feeding and resorbtion as a mechanism to avoid hydranth ageing. Hughes (1987) argued against this hypothesis, and the almost complete presence of hydranths on the wave-sheltered *Dynamena* in this study supports the view that it is not necessary for hydranths to be resorbed after only a few days to avoid senescence. A difference in food availability may contribute to the difference in hydranth presence between the exposed and sheltered areas, as one response of some hydroids to scarcity of food is for some hydranths to regress, while more peripheral hydranths are generated (see Gili and Hughes, 1995). The amounts of food in the water will be the same because of the proximity of the two areas, but in the exposed area the wave-generated currents may reduce feeding rates (for the reasons outlined above). However, differences in feeding rates are probably not responsible because other data on abundance and distribution do not indicate a relative food scarcity in the wave-exposed area (see above). This work is a good example of how phenotipic variation caused by responses to different water movement may be observed even in a short-space scale. Differences in morphology of hydroids were such that they can be used as indicators of different regimes of water movement (Gili and Hughes, 1995). Hydroids are adapted to high exposed habitats not only by decreasing their size but also by developing structures that either strengthen the hydrocaulus or increase its flexibility, in both cases to prevent fracture of the perisarc or detachment. Such adaptations should be also associated with feeding efficiency and prey availability.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Life history of *Perarella schneideri* (Hydrozoa, Cytaeididae) in the Ligurian Sea*

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SUMMARY: *Perarella schneideri* is a cytaeidid hydroid living in association with the bryozoan *Schizoporella longirostris*. In this study we have distinguished the presence of two kinds of polyps in its colonies: large gastrozooids and very extensible filiform polyps with 4 short tentacles. A study of the relationships between the hydroid and its host indicates a parasitic behaviour of *P. schneideri* on bryozoan adult colonies, and predation on bryozoan larvae. Study of hydroid stomach contents indicates that the trophic strategy of *P. schneideri* depends on two food sources: (i) a benthic one, mainly constituted of nematodes and polychaetes and (ii) a bryozoan source, which is performed in various ways. The *Perarella schneideri* colonies are, in turn, a food source for halacarids which perforate the hydranth wall and, penetrating the gastrovascular cavity, suck the semi-digested prey without evident damage to the polyps.

Key words: Hydrozoa, Cytaeididae, life cycle, trofic strategy, parasitism, commensalism, Bryozoa.

INTRODUCTION

The family Cytaeididae includes the genera *Cytaeis*, *Paracytaeis*, *Perarella* and *Stylactella* (Calder, 1988; Bouillon, 1995). In an earlier review of the family, Rees (1962) included five species in the genus *Perarella*: *P. affinis* (Jäderholm), *P. clavata* (Jäderholm), *P. spongicola* (Haeckel), *P. abyssicola* (Haeckel) and *P. schneideri* (Motz-Kossowska). *Perarella affinis* grows on algae in the Patagonian Sea, its gonophores are unknown and its systematic position is uncertain (Rees, 1962). *Perarella clavata* is found on shells in the Antarctic Ocean at a depth of 360 m (Jäderholm, 1905). Its gonophores have a styloid structure with an apical process and a basal perisarc collar. *Perarella spongicola* and *P. abyssicola* were described by Haeckel (1889) living on sponges in the north and central Pacific at 4200-5300 m depths. The distinction between these two species is based on differences in the shape and arrangement of the hydrorhiza.

Perarella schneideri is one of the two species of the genus recorded in the Mediterranean Sea. It is always associated with colonies of the red cheilostome bryozoan *Schizoporella longirostris* and is characterised by having a gonophore with radial and circular canals, and tentacular bulbs (Motz-Kossowska, 1905). The other species collected in the Mediterranean is *P. propagulata* (Bavestrello, 1987) which is found on shells of the gastropod *Hinia incrassata*, either with living molluscs or inhabited by the hermit crab *Cestopagurus timidus*.

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The association between hydroids and other organisms such as sponges, cnidarians, bryozoans, molluscs, polychaetes, decapods and vertebrates is well-known (for a review see Gili and Hughes, 1995). The symbiosis of hydroids and bryozoans involves several genera including *Zanclea, Perarella, Halocoryne, Hydranthea, Octotiara* and *Zanclella* (Boero and Hewitt, 1992; Piraino *et al*., 1992). The hydrorhiza of *Zanclea*, *Octotiara* and *Halocoryne* is covered by the bryozoan substrate, while in the other genera it grows on bryozoan surfaces in the grooves among the zooecia. Osman and Haugsness (1981) demonstrated that the association of bryozoans and zancleid hydroids increases the survival and the competitive ability of both partners.

The aim of this work has been to define the annual cycle of *Perarella schneideri* in the Ligurian Sea, focusing our attention on the relationships with its bryozoan host.

MATERIAL AND METHODS

The observations were conducted at Cala Niasca in the Portofino Promontory (Ligurian Sea, Western Mediterranean). In this zone the bryozoan *Schizoporella longirostris* grows on natural hard substrata from 1 to 10 m in depth but is also very abundant on discarded glass bottles settled on the sandy sea bottom. On this substratum the bryozoan is confined to

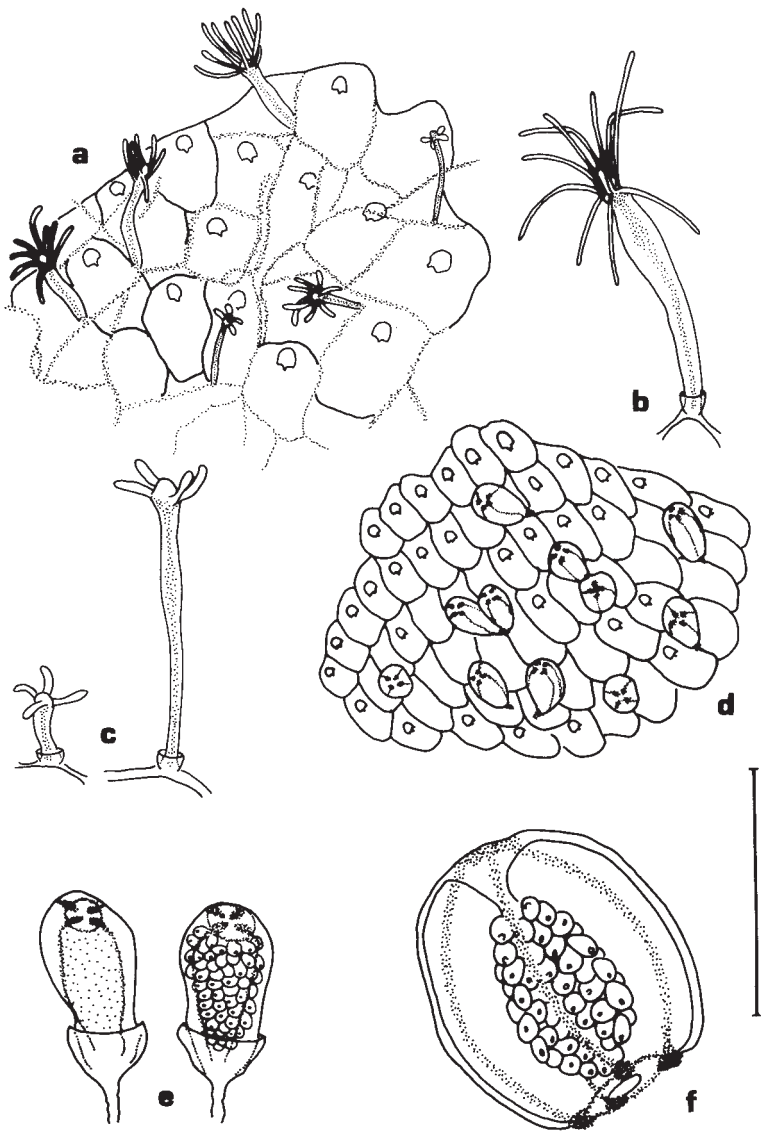

FIG. 1. – *Perarella schneideri*. a) a colony with large and small gastrozooids b) large gastrozooid; c) small gastrozooid, showing its extensibility; d) a summer colony that has lost gastrozooids but retained gonophores; e) male and female gonophores; f) free swimming female medusoid. Scale bar: a,d = 1cm; b,c,e,f = 1mm

a restricted sciophilous belt in direct contact with the bottom. Owing to its manageability, this artificial substratum was remarkably useful for our study. Each month, from July 1997 to July 1998, we collected five bottles. On these bottles all the bryozoan colonies were mapped and their area estimated by a digitizer, distinguishing the living portions from the dead ones. Moreover, for each colony the number of polyps of *Perarella* were counted. Observations on living hydroids were conducted on animals reared in natural seawater aerated by bubbles, and fed by *Artemia* nauplii at 20°C.

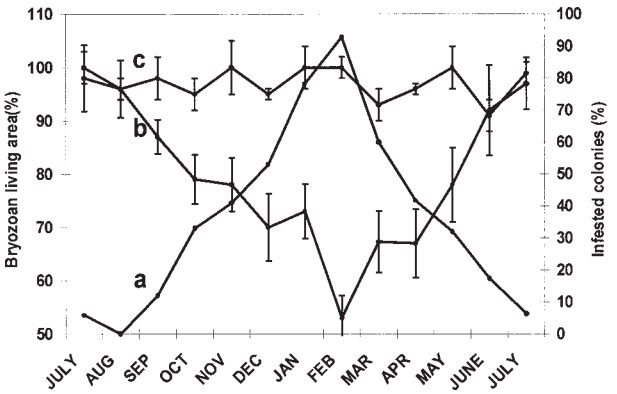

FIG. 2. – Annual cycle of the percent of bryozoan colonies infested by *Perarella schneideri* (a) compared with the annual cycle of the average percent of living bryozoan surface in infested (b) and not infested (c) colonies.

RESULTS

Hydroid morphology

The colonies of *Perarella schneideri* possess two distinct types of polyps (Fig. 1a): i) long and tubular hydranths with a whorl of 8-14 filiform tentacles around a conical hypostome (Fig. 1b) and ii) very extensile filiform hydranths with four short tentacles (Fig. 1c). Both kinds of polyps have bases surrounded by a perisarc collar. Nematocysts are microbasic euryteles (8.1 x 3.1μm) and desmonemes (6.3 x 3.6 μm).

The gonophores of both sexes are medusoids (Fig. 1d-e), 1 mm in length, surrounded at the base by a collar of perisarc with four distinct radial canals, four tentacular bulbs, and a circular canal. The female gonophores contain about 80 eggs, 80-100 μm in diameter, which surround a non-functional manubrium (Fig. 1f). They detach from the colonies and swim for five days until the ejection of the eggs. The complete ejection of the eggs lasts about one hour. Two days after spawning, the medusoids degenerate. No swimming activity was observed in male gonophores.

The colonies of *Schizoporella* are generally composed of a living area and a non-living one. The two areas are easily distinguishable by their colour: vermilion red and white, respectively. The polyps of *Perarella* are located on the living areas of bryozoan, and are almost absent on the non-living areas.

Annual cycle

The annual cycle of the hydroid has been evaluated as the percent of infested *Schizoporella* colonies (Fig. 2). In the Ligurian Sea, *P. schneideri* shows a typical winter seasonality: in February almost all the colonies of the bryozoan are infested, while in July-August no polyps are evident. The reproductive period has been observed between the second half of May and the end of June.

During the annual cycle the ratio between living and non-living areas in the colonies of *S. longirostris* is affected by the presence of the *Perarella* polyps. In non infested bryozoan colonies the living area is always greater than 95%, while in infested colonies the average monthly percent of living area is negatively correlated with the cycle of the hydroid (Fig. 2).

Colonies of bryozoans exhibited the presence of embryos in all the months of the year. We estimated the bryozoan recruitment as the percent of colonies less than 0.5 cm^2 (8-10 zooids). The trend of this parameter is negatively related with the abundance of the hydroid (Fig. 3) which demonstrates direct involvement of *Perarella* in the successful recruitment of bryozoans.

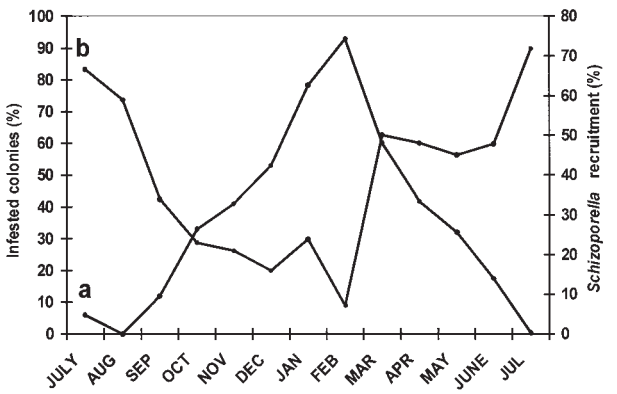

FIG. 3. – Annual cycle of the percent of bryozoan colonies infested by *Perarella schneideri* (a) compared with the annual cycle of the bryozoan recruitment estimated as the percent of colonies less than 0.5 cm^2 (8-10 zooids) (b).

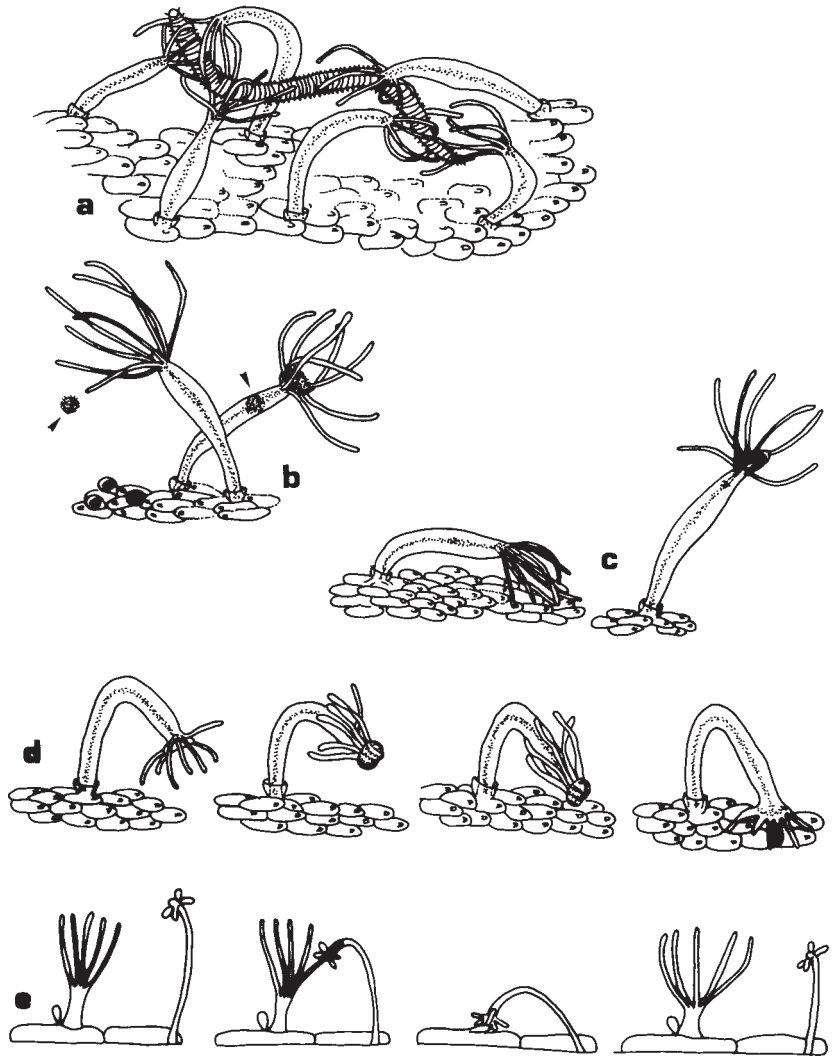

FIG. 4. – Trophic strategies of *P. schneideri*. The large gastrozooids feeding on meiobenthic organisms (a); the capture of newly released bryozoan larvae (arrows) (b); tentacles pick up the organic matter distributed on the bryozoan surface and introduce it, one at a time, into the mouth, sucking the material adhering to them (c); protrusion of the gastrovascular wall that adheres to the bryozoan epidermis (d). The filiform gastro zooids with four tentacles are specialised in engulfing the tip of a single lophophoral tentacle (e).

Trophic strategies

Perarella schneideri has two kinds of gastrozooids that are related to different trophic strategies. While the small filiform gastrozooids are very specialised, the large gastrozooids are involved in the exploitation of different food sources. The latter feed on meiobenthic organisms, particularly nematodes and polychaetes, but are also easily fed with *Artemia* nauplii under laboratory conditions. If the prey is very large many polyps participate in the capture. In this case the prey is ingested by a cluster of surrounding polyps (Fig. 4a). Another food source for large gastrozooids is newly released bryozoan larvae. As these larvae are caught in tentacles, they are quickly ingested: each polyp can engulf two or three larvae at the same time (Fig. 4b).

Sometimes the tentacles of the polyps pick up organic matter on the bryozoan surface and then introduce the matter, one piece at a time, into the mouth which removes the material adhering to them (Fig. 4c). There is often a protrusion on the gastrovascular wall of the gastrozooids that, for several minutes, adheres to the bryozoan epidermis (Fig. 4d). It is not clear whether this behaviour is harmful to the bryozoan though the coelenteron of the gastrozooids is always filled with a red material resembling the color of the bryozoan epidermis.

The filiform gastrozooids with four tentacles are specialised for engulfing the tip of a single lophophoral tentacle of the bryozoan. These exten-

sile zooids wait for lophophore evertion, then prudently approach the lophophore and touch its tentacles very softly. Then the polyp engulfs the distal portion of a single lophophoral tentacle for several minutes. When the lophophore retracts, the polyp is dragged into the bryozoan orifice for several seconds (Fig. 4e). Unlike *Halocoryne epizoica* (Piraino *et al*., 1992) the hydroid never breaks portions of bryozoan tentacles. The polyp seems to suck the lophophoral tentacles, feeding on the food particles caught by the bryozoan.

Commensalism

Perarella colonies are a food source for halacarids, which climb up the hydranth and penetrate the coenosarc with the proboscis for several minutes. The halacarids probably feed on the semi-digested contents in the gastral cavity of the hydroid (Fig. 5). During the perforation process the polyp is motionless. The hole caused by the penetration is visible immediately after the retraction of the proboscis, but it then disappears whereupon the polyp begins to move and to feed. The halacarids feed only on large gastrozooids, never utilising the smaller polyps. This selective behaviour is probably due to the different kinds of trophic resources exploited by the two kinds of polyps.

The behaviour of the halacarids is like that of pycnogonid larvae which infest the hydroid *Halocordyle wilsoni* (Staples and Watson, 1987). The pycnogonid larvae settle on the hydranth, which is grasped by the cheliphores while the proboscis penetrates the coenosarc.

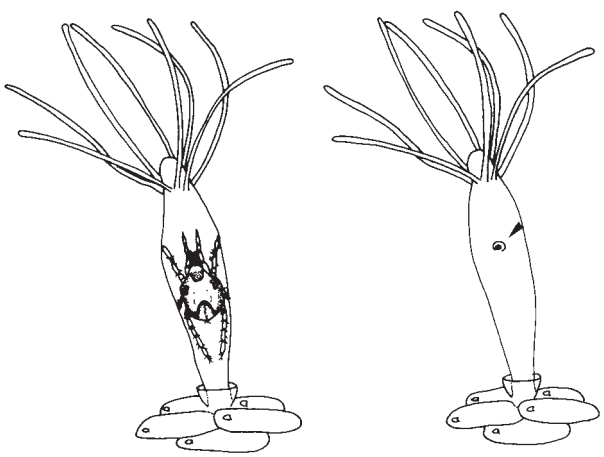

FIG. 5. – *Perarella* polyps are a food source for halacarids which penetrate the hydranth with their proboscis, whereupon they suck the semi-digested content of the gastral cavity. After the halacarid has detached itself, a hole (arrow) is evident on the hydranth.

DISCUSSION

The family Cytaeididae, belonging to the superfamily Bougainvillioidea (Petersen, 1979), also shows a resemblance to Hydractiniidae, particularly in the non-ramified colonies composed of naked polyps arising from a creeping hydrorhiza (Millard, 1975; Bavestrello, 1987). The main features traditionally considered common in the cytaeidid and in other bougaivillioid families are: (i) gonophores arise directly from the hydrorhiza (or hydrocaulus or hydrocladia) rather than from specialised polyps; (ii) oral tentacles occur in medusae of *Cytaeis* and bougainvillids; (iii) the absence of polymorphism.

Our observations indicate that there is polymorphism in the genus *Perarella,* with the simultaneous presence of two gastrozooid types that are well-differentiated from both a morphological and a behavioural point of view. The filiform shape of one of these polyps, with their extensibility and shortened tentacles suggests that they may have a relationship with the dactylozooids of the hydractiniids. Bavestrello (1987) described in *P. propagulata* some cylindrical portions of naked coenosarc surrounded by a perisarc collar that were interpreted as propagules. In light of the present data these structures may be descendants of the small gastrozooids of *P. schneideri* which have lost their very short tentacles.

In our observations of the swimming activity of female medusoids of *P. schneideri*, described earlier, we found a trend of medusa reduction within the family: *Cytaeis* spp. with free medusae; *P. schneideri* with free medusoids; *Perarella* spp. with styloid gonophores. The same is seen in many families of hydroids, and species which have abolished the medusa stage are usually assumed to be descendants.

Piraino *et al*. (1992) demonstrated, as a provisional conclusion, that the symbiosis between hydroids and bryozoans originates from simple epibiosis and leads into parasitism. Our data indicate a parasitic behaviour of the *Perarella* colonies that highly affects the biological cycle of its host. Although the reproductive period of *S. longirostris* occurs throughout the year in the Ligurian Sea, an appreciable recruitment of young colonies is evident only in summer, which corresponds to the phase of regression of the hydroid colonies. This phenomenon is directly related to the active predation of the hydroid on the larvae of its host. A similar behaviour was observed in colonies of *Podocoryne exigua* which actively feed on the larvae of the hermit crab host (Bavestrello, 1985).

More difficult to understand is the way in which *Perarella schneideri* damages and kills the colonies on which it lives. The trophic behaviour of both large and small gastrozooids on lophophores and on the bryozoan surface probably produces negative effects on the *Schizoporella* colonies. It is possible that the grazing of the hydroid on the bryozoan epidermis, as evidenced by the red content of the gastrozooid coelenteron, exposes *Schizoporella* zooecia to infection by microrganisms. A feeding activity by the gastral cavity protrusion has already been observed in *Hydractinia vallini* and *H. ingolfi* living epibiothically on starfish in polar waters (Svoboda *et al*., 1997).

Bavestrello *et al*. (1996) confirmed the presence of a cleptocommensalistic strategy in the relationships involving hydroids and caprellids. The case of *Perarella* is a new and interesting example of this strategy: the hydroid exploits the organic matter collected by the bryozoan lophophore and is, in turn, exploited by the halacarid. This complex pattern suggests that cleptocommensalism is probably widely diffused in trophic marine invertebrate relationships.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Strobilation in a species of Bougainvillioidea (Cnidaria, Hydrozoa)*

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SUMMARY: In november 1994 several specimens of an unknown hydroid were collected in a tank of the Genoa Aquarium (Italy). The polyps, characterised by filiform tentacles around a proboscis, displayed unusual asexual reproduction, leading to a linear chain of three elements (strobilation) joined by alternate oro-aboral polarity. This hydroid is very similar to another, unidentified hydroid collected in 1954 in San Francisco Bay. The hydroid of the Genoa Aquarium released free medusae characterised by four marginal tentacles, four radial canals and four oral tentacles around a tubular mouth. These features indicate that the species belongs to the superfamily Bougainvillioidea.

Key words: Bougainvillioidea, strobilation, asexual reproduction, polyp, medusa.

INTRODUCTION

In November 1954 Hand and Jones (1957) collected two live hydroid specimens from a core sample taken at 30 feet depth off Pt Richmond (San Francisco Bay, California). The bottom was composed of grey mud covered with about 5 mm of loose debris from which the hydroid was taken.

These hydroids, a single polyp and a pair of polyps united at their base, had filiform, very extensible tentacles arranged in a single whorl surrounding a proboscis. They were characterised by a peculiar asexual reproduction, leading to a linear chain of three elements (Fig. 1). Unfortunately all individuals died some months after the collection, and though more than 1200 core samples were taken

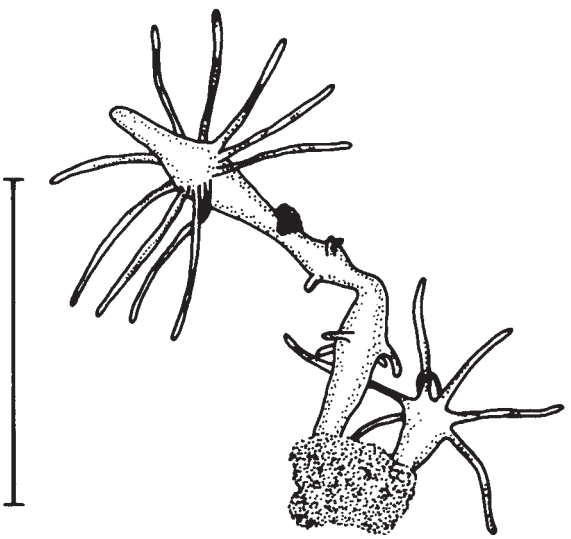

FIG. 1. – A triple and a solitary polyp as figured by Hand and Jones (1957). Scale bar = 1 mm

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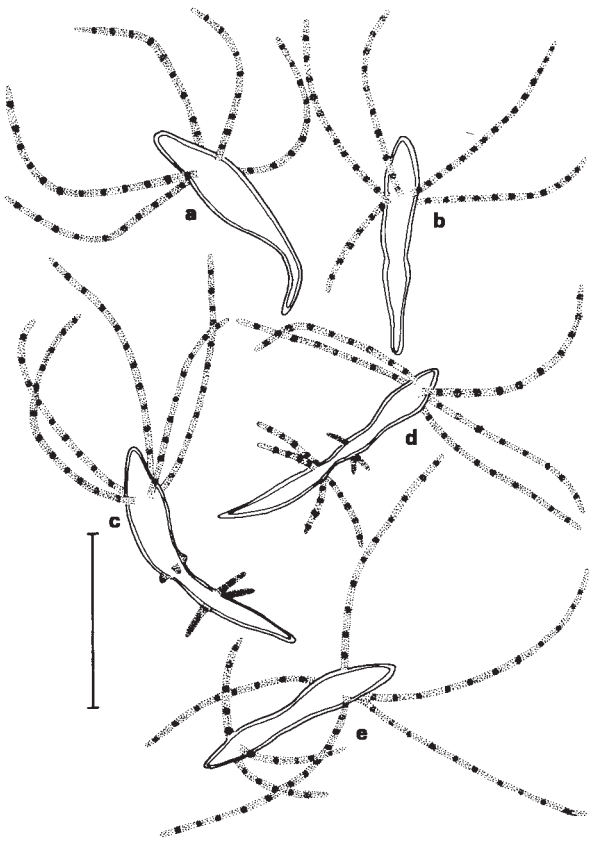

FIG. 2. – Different phases of asexual reproduction of the hydroid from the Genoa Aquarium. On the body of a solitary polyp (a), a constriction develops (b), two whorls of tentacles arise immediately above and below this narrowing, producing a chain of three elements (c) (d), fragmentation of a triple polyp, giving rise to a normal solitary polyp and a double one (e). Scale bar = 1 mm

from the original area of collection, none has been found since (Hand and Jones, 1957).

Exactly forty years later, in November 1994, several specimens of a similar hydroid were taken from a tank of the Genoa Aquarium. We reared this hydroid and observed its morphology, asexual reproduction and release of medusae.

RESULTS

The morphology of the polyp varies with the reproductive phase. The solitary polyp consisted of a sub-cylindrical, naked body ending in a conical hypostome, surrounded by 4-5 filiform tentacles (Fig. 2a). The basal region was free and had no contact with the substratum, but was sometimes attached to particles of sand.

Asexual reproduction of the polyp started with the development of a constriction in the middle of the body (Fig. 2b), followed by the growth of two whorls of tentacles, one immediately above and one below this narrowing, resulting in a chain of three elements (Figs 2c, d, 3a). The hypostome of the first element was connected to the hypostome of the second element, whose opposite, aboral portion was attached to the aboral portion of the third. This chain had a free aboral part on one end and a free hypostome on the other. During this phase all the elements were able to feed. If *Artemia* nauplii were collected by the elements joined at the hypostome, their mouths might partially detach, become functional and re-fuse after the ingestion of the prey.

After some days these triple polyps fragmented and produced a normal, solitary polyp and a double one. The latter had a functional hypostome and a whorl of tentacles at each extremity (Figs. 2e, 3b). The solitary polyps constituted the reproductive individuals, while the double polyps generally remained unchanged in their condition. Perisarc structures were totally absent in each phase of the cycle. Under laboratory conditions, the sequence of the steps in this unusual asexual mode of reproduction was very constant.

Medusa buds were produced in verticils, 2-5 per polyp, during each step of the asexual reproductory process; the buds arose from the body of the initial hydranth of the chain, immediately under the tentacular whorl (Fig. 4a).

Newly released medusae were bell-shaped, 2 mm in diameter, with four radial canals, four tentacular bulbs with ocelli, and a circular canal. From each bulb a group of two tentacles arose. The manubrium was cylindrical with four unbranched oral tentacles around a simple, tubular mouth (Fig. 4b). After five

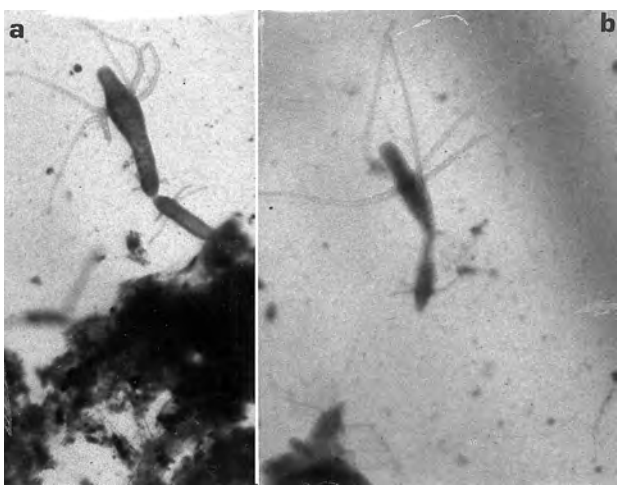

FIG. 3. – Photomicrographs of a chain of three elements, showing the whorls of tentacles (a) and of a double polyp floating in water

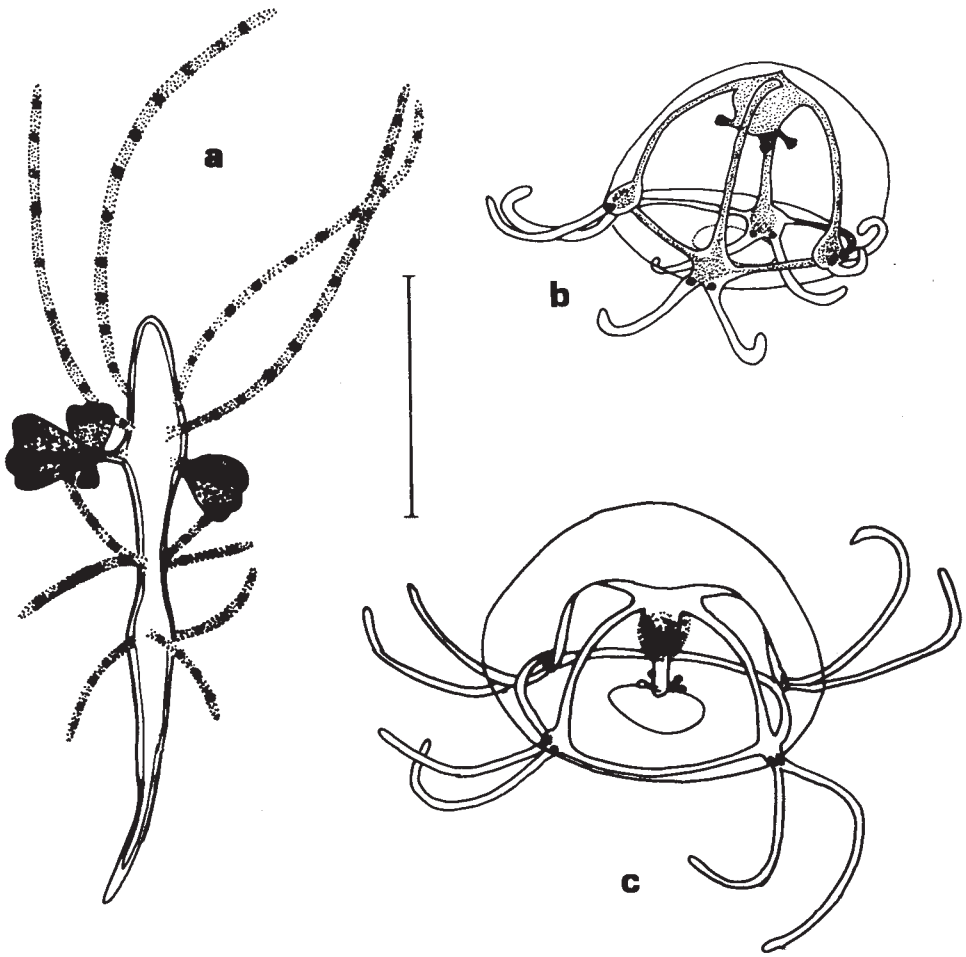

FIG. 4. – a, medusa buds on the body of a triple hydranth. b, one-day-old medusa. c, five-day- old medusa. Scale bar = 1 mm

days of rearing, gonads appeared on the manubrium (Fig. 4c).

The nematocysts were microbasic euryteles (9-10 x 3 μm) and desmonemes (4-5 x 3-4 μm) in both the polyps and the medusae.

After two weeks of rearing the polyps showed a dramatic regression, producing sub-spherical masses of cells that remained in this condition for several months.

Under rearing conditions the polyps lived in the sediment or, under strong water movement, passively fluctuate in the water currents keeping their tentacles expanded.

DISCUSSION

The shape of the polyps and, particularly, their peculiar reproductive strategy indicate that the hydroid of the Genoa Aquarium and that recorded by Hand and Jones (1957) at Pt Richmond are closely related. Hand and Jones indicated a slightly different pattern of asexual reproduction, resulting in the production of two pairs of polyps. In the process of development described, adhesion of the polyps to bottom sediment is important for chain formation, whereas our specimens complete the entire reproductive cycle floating in the water without any contact with particles from the bottom (Fig. 3b).

Unfortunately, the dramatic degeneration of the polyps in culture did not allow us to preserve material for a formal species description. Nevertheless, our data give some indications concerning the systematic position of this hydroid. In fact, the features of the medusa and the nematocyst complement indicate that it is related to the superfamily Bougainvillioidea Petersen, 1979. The main features traditionally linking the species of this taxon are: (i) the absence of polymorphism; (ii) the presence of oral tentacles and a simple, tubular mouth in the medusae; (iii) gonophores arising directly from the hydrorhiza, the pedicels or specialised

polyps. Our species does not share this last characteristic, producing medusa buds directly on the body of a normal gastrozooids as, however, also happens in the aberrant Bougainvilliidae genus *Nemopsis* (Nagao, 1964).

The main characteristic of this hydroid is its peculiar kind of asexual reproduction, producing new polyps by transverse fission along a linear axis instead of by lateral gemmulation. Cases of asexual reproduction by transverse fission are already known in some species of hydroids. Among thecate hydroids, free hydranths are produced only by *Zelounies estrambordi* Gravier-Bonnet, 1992 through a peculiar kind of autotomy, in which the polyp goes through a process of characteristic transformations before detachment from the original colony (Gravier-Bonnet, 1992). Among athecate hydroids, the first observation of transverse fission is that of "decapitation" in *Moerisia lyonsi* Boulenger, 1908, whose hydrants leave a "basal bulb" adhering to the substrate after the detachment of their apical portions (Ritchie, 1915). Other examples of "decapitation" are found in the free hydranths of *Euphysa* (Brinckmann-Voss, 1967), and in those of *Ectopleura larynx* (Ellis and Solander, 1786) (cf. Tardent, 1963, 1965; Mackie, 1966; Rungger, 1969, all as *Tubularia larynx*).

In our hydroids, however, transversal fission is preceded by the formation of a peculiar chain of three polyps. In the animal kingdom, asexual reproduction by means of a chain of elements produced along a linear axis is named strobilation and is present in several taxa such as: siphonophores, scyphozoans, polychaetes and tunicates. Although these groups do not show evident phylogenetic relationships, all the elements of the chain have the same polarity. Our hydroid, to the contrary, exhibits inversion in the oro-aboral axis in its chain. Another dissimilarity to strobilation in other animal taxa is the fact that, generally, the chains are composed of an undefined number of elements while in our hydroid the number of polyps, under normal conditions, is always three. Nevertheless, under conditions of stress, the chain never fragments and the number of polyps increases up to 14 (Hand and Jones, 1957).

In higher metazoans, the spatiotemporal development of morphological structures is regulated by homeobox genes. These genes are particularly involved in the repetition of metameric parts along an oro-aboral axis (Lawrence, 1992). Homeoboxes have also been observed in lower metazoans as such cnidarians (Schierwater *et al*., 1991) and sponges (e.g. Seimiya *et al*., 1997) but their meaning has been obscure until now. We hypothesise that the characteristic chain, with an inversion of polarity among its element as described here, may be controlled by these genetic structures.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

New observations and corrections concerning the trio of invasive hydromedusae *Maeotias marginata* (=*M. inexpectata*), *Blackfordia virginica*, and *Moerisia* sp. in the San Francisco Estuary*

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SUMMARY: New observations of *Maeotias*, *Blackfordia,* and *Moerisia* in low salinity waters of the San Francisco Bay estuary allow better understanding of the life cycles and natural history of these three genera of invading hydrozoans. *Maeotias inexpectata* Ostroumoff, 1896 is found to be a junior synonym of *Maeotias marginata* (Modeer, 1791). Moreover, *M. inexspectata* Ostroumoff, 1896b is an incorrect subsequent spelling of *M. inexpectata* Ostroumoff, 1896a. The clear presence of marginal statocysts in the medusa of this species places it back in the family Olindiidae of the Limnomedusae. Polyps previously attributed to *Maeotias* in San Francisco Bay are now known to belong to a *Moerisia* sp., whose medusa has also recently been found in the estuary system. Solitary *Moerisia* polyps have been found in the field amongst the general fouling fauna on floating docks in the Napa River. Small simple primary polyps of *M. marginata* were obtained in the laboratory. Polyps of *Blackfordia virginica* have been found in abundance in the field covering the valves of nonindigenous barnacles in the Napa River and laboratory-cultured colonies are pictured here along with their newly-released and juvenile medusae.

Key words: *Maeotias inexpectata, Maeotias marginata, Blackfordia virginica, Moerisia* sp., Olindiidae, Limnomedusae, Leptomedusae, San Francisco Bay, Cnidaria.

INTRODUCTION

Further observations in low salinity tributaries to San Francisco Bay of hydromedusae and their polyps in the genera *Maeotias, Blackfordia,* and *Moerisia* have revealed a number of errors in the Mills and Sommer (1995) paper, which first described the presence of two of these invasive species in California. We take this opportunity to clarify a number of important details concerning the nomenclature and biology of *Maeotias marginata (=inexspectata)*, *Blackfordia virginica* and *Moerisia* sp. and to add a few new observations.

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RESULTS AND DISCUSSION

Maeotias marginata (Modeer, 1791)

We have recently determined, thanks to a question by Ron Ates of Zaandam in the Netherlands, that *Maeotias inexspectata* Ostroumoff, 1896 is actually a junior synonym of *Maeotias marginata* (Modeer, 1791). The intricate early history of the synonomies of this distinct, yet confusing species were enumerated by Hummelinck (1938b, 1941). Kramp (1961), missing its significance, synonymized the highly distinct *M. marginata* with *Craspedacusta sowerbii* Lankester, 1880. Examination of the figures of Baster (1765), republished by Hummelinck (1938b, 1941) with accompanying detailed description, as well as two specimens in poor condition (COEL. 2046) from the Zoological Museum, University of Amsterdam, collected in there in 1889, leaves little doubt that there is only one species of *Maeotias*, which should therefore henceforward be called *Maeotias marginata* (Modeer, 1791). The specimens of Baster, subsequently described by Modeer as *Medusa marginata*, were up to 40 mm in diameter in life (Hummelinck, 1941). Most of our Petaluma River specimens were less than 35 mm in diameter, but a few exceptional animals reaching 50 mm in diameter have been collected in nearby Suisun Slough (see below), another tributary to San Francisco Bay. In all aspects they agree with the description detailed by Hummelinck (1941) for *M. marginata*.

With regard to the correct spelling of the junior synonym *M. inexpectata*, it has been pointed out that Ostroumoff described this species twice in 1896, and although *inexspectata* (Ostroumoff, 1896b) is better Latin, this spelling of the name is predated by three months by a description using the spelling *inexpectata* (Ostroumoff, 1896a), which therefore takes precedence as the original and correct spelling of this species name.

Statocysts were not observed around the bell margin of *Maeotias marginata* (as *M. inexspectata*) by Mills and Sommer (1995). Further observation of living specimens in August 1995 and on subsequent occasions showed unquestionably that *Maeotias* does indeed have numerous marginal statocysts (see also Rees and Gershwin, 2000). Such statocysts are clearly described and illustrated by Ostroumoff (1896b), as well as by Borcea (1928), Hummelinck (1941) and Denayer (1973), so any questions about their existence can be laid to rest.

The presence of marginal statocysts undermines placement of *Maeotias* within the Anthomedusae by Mills and Sommer (1995). At this time, until molecular genetic phylogenies are able to clearly sort out some of these relationships, it seems best to restore *Maeotias* to the family Olindiidae in the Limnomedusae.

In addition to the locations summarized by Mills and Sommer (1995) for *Maeotias marginata* in the Black Sea, Sea of Azov, Loire River estuary, Chesapeake Bay, South Edisto River estuary in South Carolina, and the San Francisco Estuary system in California, *M. marginata* has also been collected at least twice in the Netherlands. It was collected during the summer of 1762 in freshwater environments of the estuarine Spaarne River near Haarlem [making that collection one of the earliest known marine introductions (J.T. Carlton, personal communication)] and during July 1889 in the brackish Plantage Muidergracht canal, Amsterdam (Hummelinck, 1938b, 1941). Both of these locations occasionally received salt water from the Zuiderzee at that time (but do not now). The occurrence of live *M. marginata* in freshwater (Baster, cited by Hummelinck 1938a, 1938b, 1941) is not out of agreement with laboratory observations (C.E.M., unpublished) that San Francisco Bay specimens can survive in pond water [0 psu (practical salinity units)] for five days in good condition, and died only on the eleventh day in entirely fresh water. *Maeotias marginata* has most recently been collected in August 1999 at several locations in the Moonsund (Väinameri) Sea area of the Baltic Sea in western Estonia in salinity near 6.5 psu (R. Vainola, personal communication; Vainola and Oulasvirta, 1999).

Within the San Francisco Estuary, *Maeotias marginata* medusae have recently been found in additional low-salinity locations in north San Francisco Bay, including the Napa River and Suisun Slough (Rees and Gershwin, 2000). Field notes from California Fish and Game and U.S. Fish and Wildlife Service collections in the upper San Francisco Estuary near the confluence of the San Joaquin and Sacramento Rivers (personal communication to J.T.R.) indicate that *M. marginata* has been present in very low salinity portions of the San Francisco Bay estuary at least since 1959, moving the date of first observation back by 33 years if true, although no preserved specimens are available for verification. *M. marginata* has not yet been found elsewhere on the Pacific coast of North America.

Additional field and laboratory work revealed another surprise about *Maeotias* in California. Although Mills and Sommer (1995) reported only male medusae in the Petaluma River, a few female *M. marginata* (among numerous males) were found in the Napa River in September 1998. Eggs produced by these females were fertilized in the laboratory and developed into tiny, simple, primary polyps without tentacles (see Rees and Gershwin, 2000), very different than those polyps figured as *Maeotias* by Mills and Sommer (1995) (see below under *Moerisia*), but not out of character with other known limnopolyps such as *Craspedacusta*. Growth of these primary polyps did not occur in laboratory culture and they subsequently disintegrated without further development.

Blackfordia virginica Mayer, 1910

The first *Blackfordia virginica* specimens (medusae) were collected in the Napa River and the Petaluma River in the San Francisco Estuary, in 1970 and 1974 respectively, and were deposited in the California Academy of Science (Mills and Sommer, 1995). *B. virginica* polyps (Fig. 1a, b) were first discovered in the field in September 1997, densely covering living nonindigenous barnacles, *Balanus improvisus*, collected near the water line on floating docks in the Napa River at the public boat launch in John F. Kennedy Park (although a fragment of a *B. virginica* hydroid colony was tentatively identified and figured by Mills and Sommer (1995) from the gut of a *Maeotias marginata* medusa collected in 1993). Salinity in the Napa River was 19 psu and temperature about 16°C at the time of collection. The polyps were growing both on the outer valves and on opercular valves of the barnacles. Individual polyps and gonophores (each containing a single medusa) arise directly from creeping stolons. Both are very small, not exceeding 0.5 mm in height. The polyps have webbing between the tentacles. Positive identification of *B. virginica* was accomplished by raising medusae produced by these polyps to maturity in the laboratory. A newly-released medusa and older juvenile medusa are shown in Figure 1c and 1d.

Other than the San Francisco Bay estuary, the only other American west coast location known for *Blackfordia virginica* is Coos Bay, Oregon, where medusae were collected in both July 1998 (one specimen) and July 1999 (thousands seen) by James Carlton and identified by C.E.M. No polyps were located.

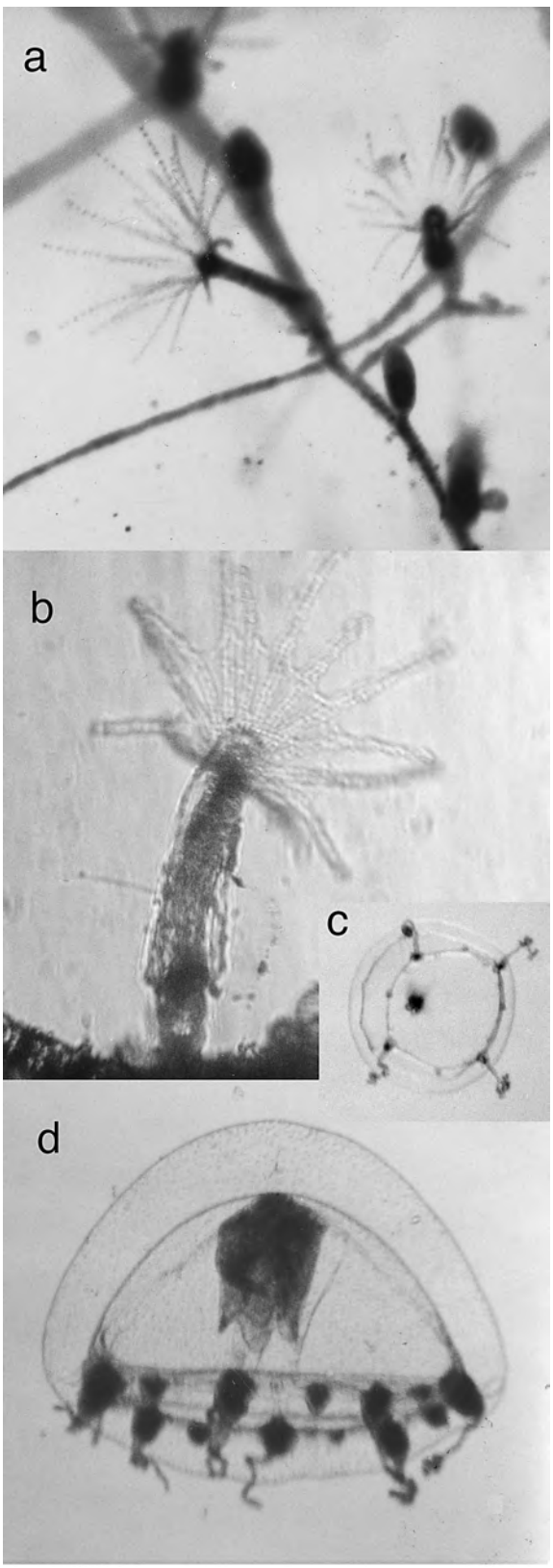

FIG. 1. – *Blackfordia virginica*. (a) polyp colony growing on glass slide, (b) single polyp showing webbing between tentacles, 0.5 mm tall measured from substrate to tip of tentacles, (c) newly-released medusa with four perradial marginal tentacles and four developing interradial tentacle bulbs, 0.8 mm bell height and diameter. (d) 16-tentacle juvenile medusa, bell 1.3 mm high and 1.6 mm diameter.

Moerisia sp.

Solitary polyps similar to those described as *Maeotias* by Mills and Sommer (1995) from the Petaluma River (collected in 1993, the first record of this hydroid in the San Francisco Estuary) have now also been found in the field in the San Francisco Estuary in the Napa River amongst the float-fouling community. These polyps have been identified through laboratory rearing as those of *Moerisia* [whose description they match, as noted by Mills and Sommer (1995)] rather than *Maeotias* (see Rees and Gershwin, 2000). Furthermore, the newly-released medusae of *Moerisia* have ocelli, which are not present in *Maeotias*, and only 4 tentacles rather than the 24 tentacles on newly-released *Maeotias marginata* (see Rees and Gershwin, 2000). Adult *Moerisia* sp. medusae have subsequently been collected in the same Suisun Slough site in the San Francisco Estuary where *M. marginata* was also present (Rees and Gershwin, 2000).

The San Francisco Bay system is now at least the third region in which all three genera of invasive hydromedusae, *Maeotias*, *Blackfordia* and *Moerisia* have become established. All are considered to be native to the Black Sea region (Calder and Burrell, 1969) and have also all been reported in the Chesapeake Bay (Calder and Burrell, 1969). The three genera are tolerant of very low salinities, but there may be other variables that cause them to move together. *Maeotias* and *Blackfordia* appeared together with the Atlantic American and European hydromedusa *Nemopsis bachei* in the Loire estuary (Denayer, 1973). [*Nemopsis bachei* has also been collected in the Zuiderzee (Kramp, 1961), not far from the region where *Maeotias* was found there, and is common in the Chesapeake Bay (Calder, 1971)]. A fourth "Sarmatic" hydroid (thought native to the Black and/or Caspian Seas), *Cordylophora caspia* (Pallas, 1771) which does not produce medusae, is also found in very-low salinity sites in the San Francisco estuary (Cohen and Carlton, 1995). *C. caspia* is apparently the most invasive of the group, and is known to be established in a large number of very-low salinity sites worldwide.

ACKNOWLEDGEMENTS

Several people contributed to the corrections and observations reported here. Most recently, Ron Ates inquired whether Modeer's *Medusa marginata* might be the same as *Maeotias inexspectata*. The 1997 collections of the polyps of *Blackfordia virginica* and *Moerisia* sp. were made during the Fourth San Francisco Bay Expedition, a rapid assessment inventory for nonindigenous marine species organized by Andrew Cohen. The 1998 collection of the female *M. marginata* and subsequent development of primary polyps occurred during the Fourth Workshop of the Hydrozoan Society at the Bodega Marine Laboratory, University of California. Thanks to Jim Carlton for sharing his collections of *Blackfordia virginica* from Oregon and to Shin Kubota for help in collecting in the Napa River. Thanks to Lisa Gershwin, who followed up the tip for *Maeotias* in Suisun Slough and for noting the precedence of *inexpectata* over *inexspectata*. Thanks to Dale Calder, who was the first to see the likelihood that our large solitary polyps were *Moerisia* rather than *Maeotias*, and for providing helpful comments on this manuscript. Thanks to Rob van Soest for supplying Dutch material from 1889 for study, and to Risto Vainola, who apprised us of the discovery of *Maeotias* in the Baltic Sea in 1999. J.T.R. wishes to acknowledge aid from the Interagency Ecological Program and the California Department of Water Resources (DWR agreement #B-81818) to California State University, Hayward and the CSUH Bay/Delta Shore Institute. We are sorry to preside over the demise of yet another imaginative species name with this paper.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Cnidarian "Parasites" on *Solmissus incisa*, a Narcomedusa*

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SUMMARY: A narcomedusa, *Solmissus incisa,* was collected off central California in the Monterey Submarine Canyon at 230 m in October 1996. The medusa was viewed and collected from the RV *Point Lobos* using the remotely operated vehicle (ROV) *Ventana*. Advantages of such *in situ* observation include the ability to recognize parasites (which appear as small, opaque circles) on the bell of the specimen. In the laboratory, the circular objects were found to be "parasitic," cnidarian-like juveniles developing within the epidermis, stages that moved freely, extending and retracting their tentacles. It is not known whether these juveniles are true parasites – *i.e*. juveniles of another species drawing nutrition from the host medusa, or whether they are offspring being brooded. *Solmissus* is one of the most numerous genera of medusae in Monterey Bay, and this is the first report of parasites on members of that genus in the California Current system.

Key words: Hydromedusae, Narcomedusae, *Solmissus*, parasites, brooding, midwater medusa, California Current, submersible collection

INTRODUCTION

Narcomedusae are a major group of open ocean cnidarians that are found in deep water. *Solmissus incisa* is a mesopelagic or bathypelagic narcomedusa living below 400 m (Larson, *et al.,* 1991). Narcomedusae have separated their tie from the benthos with no well-known associated stage other than parasitic juveniles. Parasitic associations can be very complex in the Phylum Cnidaria and some taxa brood their young in specialized pouches within the adult epidermis. Narcomedusae are dioecious and some larvae develop directly while others go through planula-actinula-medusa stages, but the ecology of some stages is complicated by parasitism by the actinula (Hyman, 1940). The egg may develop in the mesoglea or gastric pouches of the parent, sometimes with the aid of a nurse cell. Development may then proceed directly in the parent from egg to actinula; actinulae bud off other actinulae that later transform into medusae. Or planulae may leave the parent entirely and associate with other hydromedusae as a parasite, attaching to the manubrium or the subumbrella (Hyman, 1940). Very little is known about the complicated parasitic and/or brooding characteristics of deep-sea medusae. *In situ* observations of live organisms are recent and information about swimming behavior and associations of narcomedusae with other pelagic organisms has been very difficult to obtain from preserved specimens.

Narcomedusae generally are difficult to sample, both because they are delicate and they live in deep water. Sampling with submersibles and by SCUBA is imperative to successfully collect these gelatinous

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organisms without significant damage to their fragile bodies (Mackie and Mackie, 1963; Mills and Goy, 1988; Larson, *et al.,* 1989, 1991). Narcomedusae are called 'holoplanktonic', *i.e.* always floating freely; however, the developmental cycle may include parasitism of early life history stages on adult medusae, in stomach pouches or in the coelomic cavity (Bouillon, 1987). When parasitizing adult medusae, larvae remain associated with planktonic organisms so, in a wide sense, they are holoplanktonic. Our knowledge of narcomedusae life histories is limited, as fewer than ten species have a known life cycle. Development in narcomedusae is poorly understood because the parasite and host body forms are complex and most are still not identified (Bouillon, 1987).

Not only are narcomedusae parasitic on other hydromedusae but also, many pelagic cnidarians commonly have associations with juvenile organisms in separate, non-related taxa. For example, arthropod crustacean, *Cancer gracilis,* juveniles and megalopas are adapted to associate with or 'hitchhike' on the large scyphomedusa *Chrysaora fuscescens* in Monterey Bay and drop off the bell when they are transported close to shore (Graham, 1994). Observations of parasitic hyperiid amphipods living a benthic-like existence on medusae are also numerous. Hyperiid amphipods are hypothesized to be the descendents of benthic crustaceans which have developed an association with a pelagic substratum, gelatinous zooplankton (Laval, 1980). Hyperiids can be difficult to study because they leave their host when they are collected with nets (Laval, 1980). Fortunately, such associations can be documented using ROV collection, which usually does not cause hyperiids to flee. The complex associations of internal and external parasitic larvae, from the same or separate taxa, must be studied *in situ* and with gently collected, fresh specimens because with brooding and parasitism, the association can be fragile and require delicate handling of specimens.

MATERIALS AND METHODS

The collection was made from the 33.5 m Research Vessel *Point Lobos* based in Moss Landing, California and its remotely operated vehicle (ROV) *Ventana*, both owned and operated by the Monterey Bay Aquarium Research Institute (MBARI). MBARI has been collecting and surveying Monterey Bay with this submersible since 1989. The *Ventana* is an ISE Hysub 40-1850 powered by a 40hp electro/hydraulic power pack and equipped with low-impact collection devices, including detritus and suction samplers (Robison, 1993). ROV pilots on the ship maneuver the *Ventana* while video images and oceanographic data are relayed in real time to the scientists on the ship through optical fibers in the umbilical cable. A Sony BetaCam BVM30 is used on the ship to videotape each dive in its entirety, allowing subsequent viewing and analysis onshore. The study site was located over the axis of the Monterey Submarine Canyon at 36° 42' N, 122° 02' W where the water column depth is 1600 m. The submarine canyon begins not far from the Moss Landing Harbor mouth and cuts through the continental shelf, allowing the study of mesopelagic species within 5 km of shore in central Monterey Bay.

RESULTS

A single specimen (specimen 1) of *Solmissus incisa* was collected on 23 October 1996 at 230 m depth in Monterey Bay, California. As the vehicle approached the swimming medusa, the velum and the oral surface were directed upward. The tentacles were rigidly outstretched below the bell with distal ends curved outwards (Fig. 1a). The velum pulsed

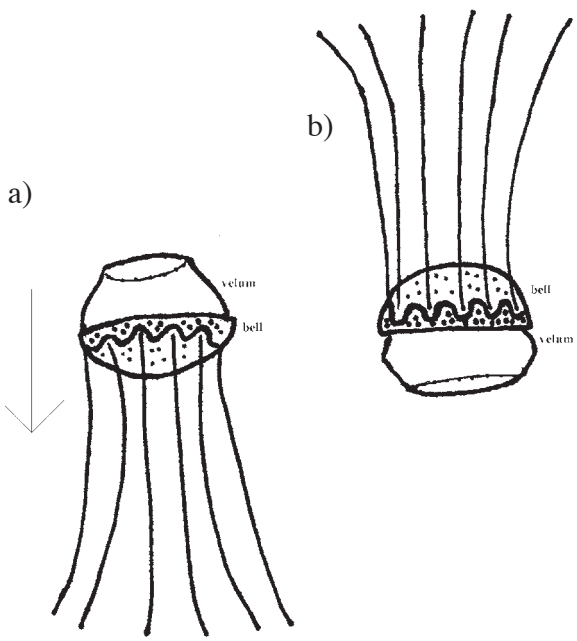

FIG. 1. – a) *S. incisa* swimming. The arrow shows the downward direction of movement. The velum is pulsing above the bell; b) Position when *S. incisa* stopps pulsing and rotates in the water column so the velum is below the bell.

consistently, propelling the medusa down, yet when the vehicle paused to videotape, the medusa stopped pulsing, rotated slowly around until its oral surface was downward, essentially suspended 'upside down' from the original position in which it was viewed (Fig. 1b). The medusa remained in this position for one minute and then turned over and began pulsing again. Video images were recorded of this specimen *in situ* for three minutes.

With the ROV video camera, it was possible to see a hyperiid amphipod associated with the surface of the bell. At first, a number of similarly sized, circular objects were seen concentrated on the bell. On closer observation, I saw two distinct types of objects, both appeared to be inside the organism, though their exact location was difficult to discern. Randomly dispersed, clear bubbles were spread along the entire surface of the bell, later found to be oil droplets. Distributed across three lappets were separate, concentrated opaque spheres, later found to be "parasites." This medusa was collected gently by the ROV in the suction sampler for closer examination at the onshore laboratory.

FIG. 2. – Cluster of parasites in different developmental stages on the rectangular lappets of *S. incisa* collected in Monterey Bay. Tentacles and a mouth are visible on more developed individuals. Oil droplets are visible as clear bubbles on the upper lappet.

The specimen (1) of *Solmissus incisa* with opaque, spherical bodies was collected in close proximity to three other specimens of *Solmissus incisa*. The first of these was seen at 223 m pulsing regularly with rectangular lappets and an amphipod on its surface, the second and the third were smaller specimens at 228 m and 264 m with no obvious amphipods. As the vehicle descended, at least six more *Solmissus incisa* were observed. I did not see any of the clear bubbles or opaque spheres on the bells so none of these medusae were collected. From the video transmission, I did not see eggs or reproductive products on any of these medusae, even though eggs can normally be seen with the ROV camera.

In the laboratory, the specimen (1) of *S. incisa* was found to have a 50 mm bell diameter and twenty tentacles with purplish hue. It appeared to be full-grown and possibly of reproductive size. The tentacles were approximately 60-70 mm long and 8 mm apart. There were twenty stomach pouches with an oval outline, longer than wide. The marginal lappets were rectangular in shape, each with an average of three statocysts. The mouth was a small, simple opening in the center of the bell. The specimen and associated parasites were photographed in the laboratory with a Nikon camera attached to a Zeiss dissecting scope at 1x power.

The opaque spheres or "parasites" were concentrated on one side of the bell (Fig. 2) and the epidermal, hyperiid amphipod was on the oral side of the medusa. There were approximately 45 individual parasites in different development stages and they ranged in size from 0.2-1.0 mm in diameter, not including small tentacles that were extended away from the body (Fig. 3). They each had 2-6 tentacles and smaller protrusions around the circumference that looked like developing tentacles and each had a small mouth opening in the center. No statocysts were visible. No food particles were seen in the developing parasites but some pigment gave them a distinguishable visual texture. The parasites did not look like food items for the adult medusa, as they were not being digested nor were they encapsulated in mucus. The parasites resembled juvenile cnidarians in body form, having tentacles, a small mouth and a rounded bell (Fig. 4).

With a dissecting scope, it was possible to see that the opaque spheres were constantly moving. The bodies could extend and contract tentacles, moving past one another to a different position. They reached out with a tentacle, touched another

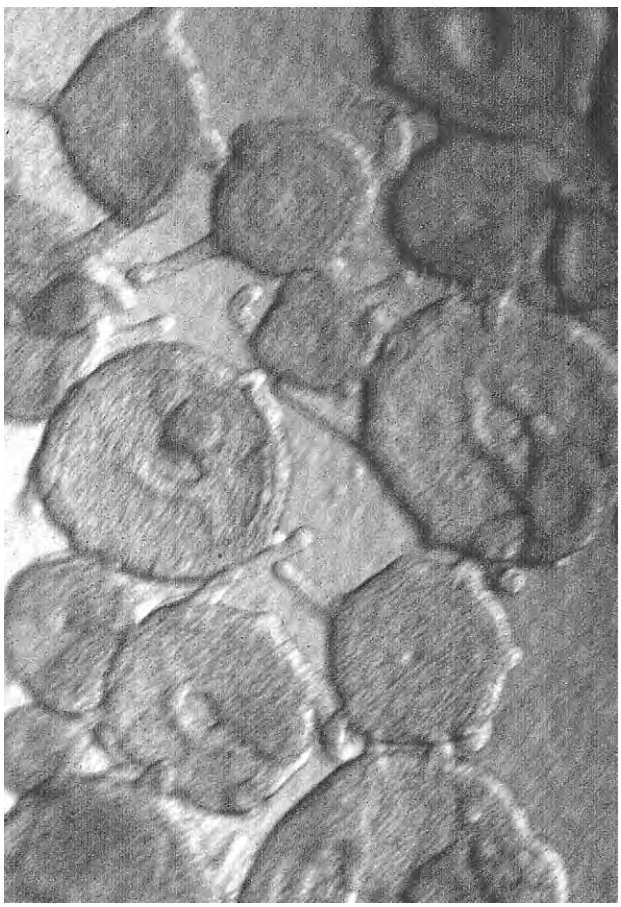

FIG. 3. – Numerous parasites (0.5-1.0 mm diameter) on *S. incisa* showing different developmental stages.

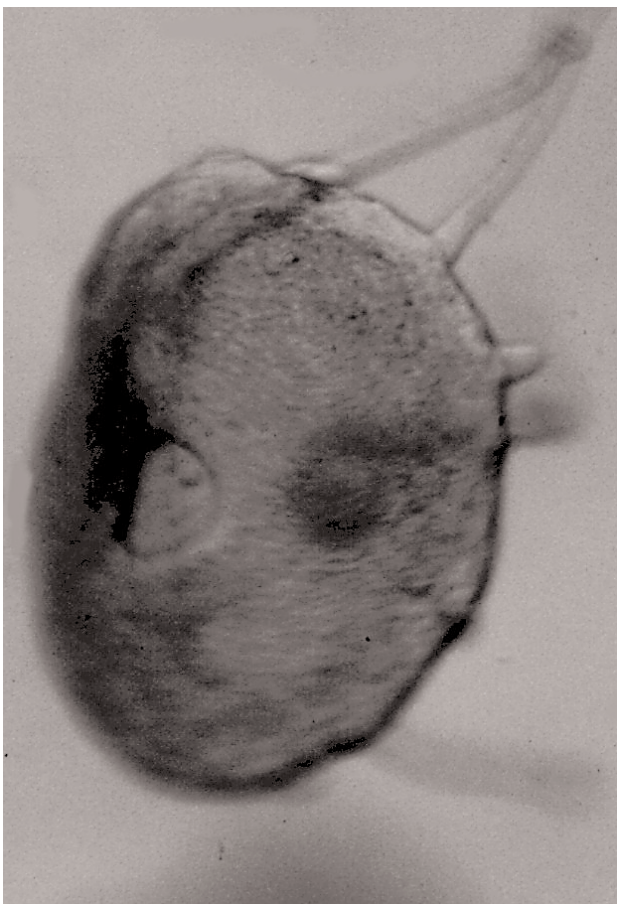

FIG. 4. – A single parasite (1.0 mm diameter) with tentacles of different lengths. The mouth opening is visible in the center of the body.

individual and contracted the tentacle. They seemed to move freely inside the medusa and were not attached to each other or the medusa. They glided using their tentacles to pull them along inside the medusa. Using forceps to peel back the thin, transparent, epidermal tissue layer on the oral side of the medusa, I removed one fairly well developed specimen from the outer edge of the bell near the stomach cavity. It was comparatively large and had six tentacles. This individual maintained its form for several days off the host body and developed further but not into an identifiable species.

DISCUSSION

Life histories and development

Narcomedusae have lost their connection to inorganic, benthic substrates and, unlike most of their benthic-dependent hydrozoan relatives, the entire narcomedusan life cycle occurs in the pelagic realm. However, there is another way to maintain an association with a substrate: utilize another living organism during a larval stage of the life cycle. The life cycles of several parasitic narcomedusae were described by Bouillon (1987), including revisions of previous accounts of their embryonic and larval development. He noted that narcomedusae consistently maintain close associations with other organisms, *e.g.*, other narcomedusae, anthomedusae, leptomedusae, trachymedusae and scyphomedusae in the mesopelagic zone. The life cycle of narcomedusae is complicated and the most remarkable parasitism is shown by some of the developing stages, which can be parasitic on other planktonic medusae (Russell, 1953). Two genera of narcomedusae produce an amoeboid cell stage that can be either self-parasitizing or which can inhabit the mesoglea of a trachymedusa. The amoeboid cells are associated with a nurse cell and develop into tentacled larvae, which may develop directly into free-swimming medusae or bud off similar larvae which

develop into medusae (Russell, 1953). The parasitic stage may also be a pelagic hydroid that can bud new medusae or form an asexually produced piece of tissue, the stolon (Mills, 1991).

Another example of complex life histories in narcomedusa is polyembryony, or embryonic budding when one sexually produced embryo buds into many genetically identical buds that are then brooded by the adult (Craig *et al.,* 1997). It is difficult to identify the diverse stages of narcomedusa parasites or brooded young, even after they are liberated from host medusae. Although they are no longer tied to the benthos, narcomedusae may still alternate between substrate-adapted morphologies, associated with other organisms, and pelagic stages, which are free living.

Brooding/parasitism

It is difficult to recognize the diverse stages of narcomedusae parasites, as they are small, not well developed and enclosed in tissue and therefore, are undoubtedly significantly underreported. Standard observation and collection techniques for deep-sea medusae can damage adult specimens and parasites beyond recognition (Larson, *et al.,* 1991). In the Mediterranean, the behavior of *Solmissus albescens* and *Solmissus marshalli* (Mills and Goy, 1988) was examined *in situ* and no parasites on the bell were reported. Parasites were not observed in studies of representatives of eight narcomedusan genera in the NW Atlantic, Arctic and Antarctic (Larson, *et al.,* 1989) or on medusae in northwestern Atlantic midwater (Larson *et al.*, 1991), when examined *in situ*. However, many small parasitic narcomedusae were reported developing in the stomach pouches of two *S. albescens* specimens in a manned submersible study (Mills, *et al.*, 1996) but these specimens were not examined further.

Medusae parasitic on narcomedusae have been reported in the literature, but little is known about their development. Hyman (1940) briefly noted larval and juvenile medusae in the stomach cavity of *Solmissus* found in Puget Sound but reported nothing more about them. Bouillon's (1987) list of narcomedusae parasites and hosts does not include *S. incisa* as a host. Larval and juvenile *Cunina*, a narcomedusa, have been found in the stomach cavities of other narcomedusae hosts, including *Solmundella*, *Pegantha* and *Cunina* (Bouillon, 1987). Perfectly formed young medusae were found in a bowl that adult *S. marshalli* had been kept in for three days. The young medusae were 3 mm wide but their taxonomic identity was not determined (Mackie and Mackie, 1963). Developing larvae have been reported in an adult specimen of *S. marshalli* collected in Puget Sound (Mackie and Mackie, 1963). In most studies, juveniles on medusae were more commonly considered parasites than brooded young, but more medusae must be collected *in situ* and reared in the laboratory to determine the nature of these associations and to exclude the possibility that the "parasites" are brooded offspring of the host or parent.

Advantages to brooding and "parasitism" include protecting and enhancing the survival of young by providing a safe, internal habitat for development. In other cnidarian taxa, brooding is the major function around which specialized reproductive structures have arisen (Campbell, 1974). For example, some scyphozoan embryos are brooded in special pouches in the folds of the mouth lobes, where they develop into ciliated planula larvae (Pearse *et al.*, 1987). Individual hydromedusae are dioecious and eggs may be freely spawned or brooded, retained for fertilization and development to larval stages (Mills and Strathman, 1987). In some jellyfish, brooding may occur internally within the gastric cavity or externally, following a free-swimming stage, with planulae brooded on a parent of a different species (Campbell, 1974). In many species, brooding consists of no more than harboring free- floating larvae. Internal brood chambers exist in platyctenid ctenophores yet there is no evidence that they receive nourishment from the parent. The brooding embryos may be in the same stage of development or several different stages simultaneously and have been observed "escaping" through the adults' epidermis (Pianka, 1974).

***S. incisa* specimen collected in Monterey Bay**

Brooding in cnidarians is commonly reported (Hyman, 1940; Campbell, 1974; Pearse *et al.*, 1987), especially for scyphozoans and anthozoans; however, for narcomedusae, an association with young life history stages is commonly reported as "parasitism" (Bouillon, 1987). From the diversity of sizes and shapes I saw on this specimen, I believe these "parasites" were successfully developing on their host. In the specimen from Monterey Bay, each parasite was independent, moving separately, not attached to the medusa. I cannot exclude the possibility that the "parasitic" juveniles developing in *S. incisa* were possibly juveniles brooding within the

adult/parent tissue. They were protected under a thin epidermis and could possibly have drawn nutrition from the adult through gastric secretions or by intercepting digesting amoeboid cells. Some were primary larvae, actinula-like with a mouth and at least two tentacles and when developed, the young medusa should leave the host, possibly through the mouth as they are developing within the gastric cavity.

I was able to view the living, healthy specimen with an ROV, which allowed me to examine behavior and physical characteristics not obvious unless specimens are viewed directly underwater. Close ROV observation gave me the opportunity to observe the size and distribution of parasites on the bell and collect the medusa without damaging its tissue. The parasites looked very similar to the oil droplets when viewed *in situ* and therefore could be easily misidentified. In the laboratory, I was able to distinguish these parasites from prey-derived oil droplets that indicate the medusa had fed recently (Larson *et al.,* 1991). The parasites resembled very early stages (54 hour embryos) of a hydrozoan with direct development (Freeman, 1983). Furthermore, the internal, cnidarian-like parasites are so small, that after a specimen is preserved and the color and/or texture is lost, the parasites may even then be too difficult to see or distinguish from oil droplets or pigment spots in the laboratory. Thus, making many observations of live, undamaged specimens aids in recognizing the parasitic association. It is much more effective to see such delicate associations with *in situ* viewing because not all medusae have parasites. Specimens with suspected parasites require gentle collection and further lab examination to verify the presence of juvenile cnidarian-stages versus oil droplets or external hyperiid parasites.

The swimming behavior of the *Solmissus incisa* reported here might favor contact with parasites and increase the encounter rate. The swimming speed and direction of *S. incisa* was affected only slightly by the presence of the ROV. *Solmissus marshalli* has been reported to be a weak swimmer with only the outer part of the umbrella and the velum contracting (Mackie and Mackie, 1963). *Solmissus albescens* was observed in the western Mediterranean with a submersible and was almost always actively swimming, regardless of the time of day. The tentacle positions during locomotion in *S. albescens* (Mills and Goy, 1988) differs slightly from those in the *S. incisa* specimen in Monterey Bay, although these two species are very nearly related (Russell, 1953). Postures for *S. albescens* include holding the tentacles up above the bell with tentacle ends slightly recurved or tentacles held straight down below the bell (Mills and Goy, 1988). The swimming behavior of *S. incisa* is unique and similar behavior has not been reported for other narcomedusae or *Solmissus* species: *S. incisa* holds its tentacles rigidly outstretched, arching below the bell while the velum is opening and closing above, propelling the medusa down in the water column. Continuous pulsing of the velum brings a backwash of water against the underside of the bell, allowing a greater volume of water to flush the oral side of the medusa thus possibly increasing contact with free floating "parasitic" organisms. This posture may make it easier for parasites to be pushed or sucked onto the surface of the bell and then into the stomach cavity, where they may remain during development.

This sighting of cnidarian-like parasites in association with *S. incisa* is rare. In Monterey Bay, where *S. incisa* is common, numerous specimens have been collected over the years and yet none have been reported to have parasites. If these parasites are developing narcomedusae, this modification of the holoplanktonic life cycle facilitates larval development in a protected environment, in an adult medusa of the same or different genus. Larvae able to parasitize adult medusae and develop in a semi-protected environment within the epidermis should continue to develop until being liberated as medusae. Other hydrozoans, including hydroids and siphonophores, accomplish this substrate association within self-colonies; in narcomedusae, brooding or parasitism provides an association with a safe, organic substrate and ensures release in a suitable habitat.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

A pandeid hydrozoan, *Amphinema* sp., new and probably introduced to central California: life history, morphology, distribution, and systematics*

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SUMMARY: A pandeid hydrozoan new to California, *Amphinema sp.*, was collected in 1998 as a hydroid living on the non-indigenous bryozoan, *Watersipora "subtorquata"*, attached to floats in Bodega Harbor 80 km north of San Francisco Bay. The hydroid was cultured in the laboratory and medusae it released were raised to maturity. No species name could be assigned because although the hydroid colony structure and morphology of the polyp most closely resemble descriptions of *Amphinema rugosum*, the immature and adult medusae best resemble *A. dinema*. These two described species are known from widely-spaced locations worldwide including Europe (British Isles and the Mediterranean), New England, the Caribbean, east Africa, India, Japan and China, implying that they may transport easily between sites by man's activities. Such wide-spread distributions of both species, coupled with the notable absence of *Amphinema* sp. from Bodega Harbor during a number of previous field surveys in the 1970's, strongly intimates that *Amphinema* sp. has been introduced from elsewhere into Bodega Harbor during the past 25 years. Two additional species of *Amphinema* medusae present on the west coast of North America are discussed.

Key words: *Amphinema rugosum, Amphinema dinema*

INTRODUCTION

The hydrozoan fauna of bays and harbors of the central California coast has been altered through non-indigenous species introductions over the past 50 years or more (Hand and Gwilliam, 1951; Mills and Sommer, 1995; Rees and Gershwin, 2000) and the rate of successful establishment of all non-indigenous species in San Francisco Bay appears to be accelerating in recent decades (Cohen and Carlton, 1998). The present paper describes what is believed to be a recently-introduced species of *Amphinema*, family Pandeidae, in Bodega Harbor, California, a shallow, sandy harbor located about 80 km north of San Francisco. This is the first formal description of a hydrozoan in the genus *Amphinema* from California, and the first of its type [either *A. dinema* (Péron and Lesueur, 1809) or *A. rugosum* (Mayer, 1900)] to be reported from anywhere on the Pacific coast of the Western Hemisphere.

One pandeid hydroid colony was collected by Ms. Cinzia Gravili on September 25, 1998, from the floating dock at the Coast Guard Station on the south shore of Bodega Harbor, California (during the Fourth Hydrozoan Workshop sponsored by the Hydrozoan Society and held at Bodega Marine Lab-

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oratory September 19–October 3, 1998). The colony was growing on the non-native, bright orange, encrusting bryozoan, *Watersporia* "*subtorquata* (d'Orbigny, 1852)" (see Cohen and Carlton, 1995). The initial generic identification of the hydroid as *Amphinema* was made on the basis of its morphology and a peculiar but specific "flexing" behavior of the polyp, in which disturbed polyps bend over nearly 180° towards the substrate (F. Boero, personal communication). Intriguingly, the *Amphinema* hydroid colony was bright orange-red and nearly invisible on casual inspection of the similarly-colored bryozoan. Without knowledge of the morphology of the adult medusa, a specific identification within the genus *Amphinema* was not possible, so I undertook to culture the hydroid as well as the medusae it was releasing. Medusa buds were present on the field colony, and upon isolation, aeration, and consistent daily feeding, newly-released medusae were reared to sexual maturity in the laboratory.

Taken together, *Amphinema dinema* and *A. rugosum* have a cosmopolitan distribution spanning several different biogeographic provinces. The medusae of *A. dinema* have been reported from northern and western Europe, the Mediterranean, the eastern Atlantic including Rhode Island, the Dry Tortugas, and Brazil, western Africa, and South Asia (Kramp, 1961). *A. rugosum* has been reported from the western Pacific (Japan) by Uchida (1927) as *Stomotoca rugosa* (Kubota, 1998), and in Europe from the British Isles and Italy (Rees and Russell, 1937; Russell, 1953; F. Boero, personal communication). The medusa of *A. rugosum* has also been reported from New England and the Caribbean (Kramp, 1961) and China (Chow and Huang, 1958).

MATERIALS AND METHODS

To encourage release of medusae, the bryozoan colony on which the hydroid colony was growing was tied onto a glass microscope slide using thread, and the slide placed in a 250 ml plastic rearing cup filled with sea water. The culture was aerated, and the polyps fed daily with *Artemia* nauplii and copepods collected from the sea with a plankton net. Both the hydroid polyps and bryozoan zooids fed avidly on the *Artemia* and copepods. The culture water was changed every other day, and temperature was maintained at about 18° C in the laboratory. Under these conditions both the hydroid and the bryozoan colonies flourished, and many medusae were released. Newly-released medusae were isolated in separate rearing cups and fed in a manner similar to the polyps. Medusae were maintained in the laboratory for up to 8 weeks, during which they grew and ultimately developed mature male gonads. The colony was maintained in the laboratory for about 6 months, and eventually spread onto the glass slide, where it thrived without the presence of the original host bryozoan, which had long-since perished.

Reference photographs were taken and a photolibrary maintained of all life history stages, including the colony, individual polyps, medusa buds, newly-released and adult medusae, and nematocysts. Photographs were taken with a Nikon 35 mm camera mounted on an American Optical binocular microscope. Nematocysts were examined under high power (500x and 1000x) and photographed. Nematocyst measurements given here are of undischarged capsules.

RESULTS

Polyp and Colony (Fig. 1)

The color of the polyps and stolons in the field-procured colony was a deep orange-red. The polyps arose from stolons growing on the surface of the bryozoan colony among the zooids. The hydroid colony was primarily reptant, with occasional upright branches (Fig. 1a). The uprights possessed at most 2 polyps, and protruded 2-3 mm above the surface of the bryozoan. Medusa buds, mounted on short pedicels, developed directly from the stolons as well as on the upright branches. Polyps had 8 to10 tentacles in two cycles, the top cycle being held upright, nearly parallel to the body of the hydranth, and the bottom cycle held perpendicular to the hydranth wall (Fig. 1a, b). The polyps were mounted on well-defined hydrothecae, at the base of which, adjacent to the stolon, were 2-5 annulations, (Fig. 1c, d). Both field and laboratory colonies possessed more than 100 polyps.

Medusae (Fig. 2)

Newly-released medusae (Fig. 2a, b) were slightly less than 1.0 mm in height and diameter. The jelly was thin and there was no apical projection. There were two opposite tentacles, each with a broad flat tentacle bulb, and two opposed rudimentary marginal swellings, four broad radial canals, and a total of 1–4

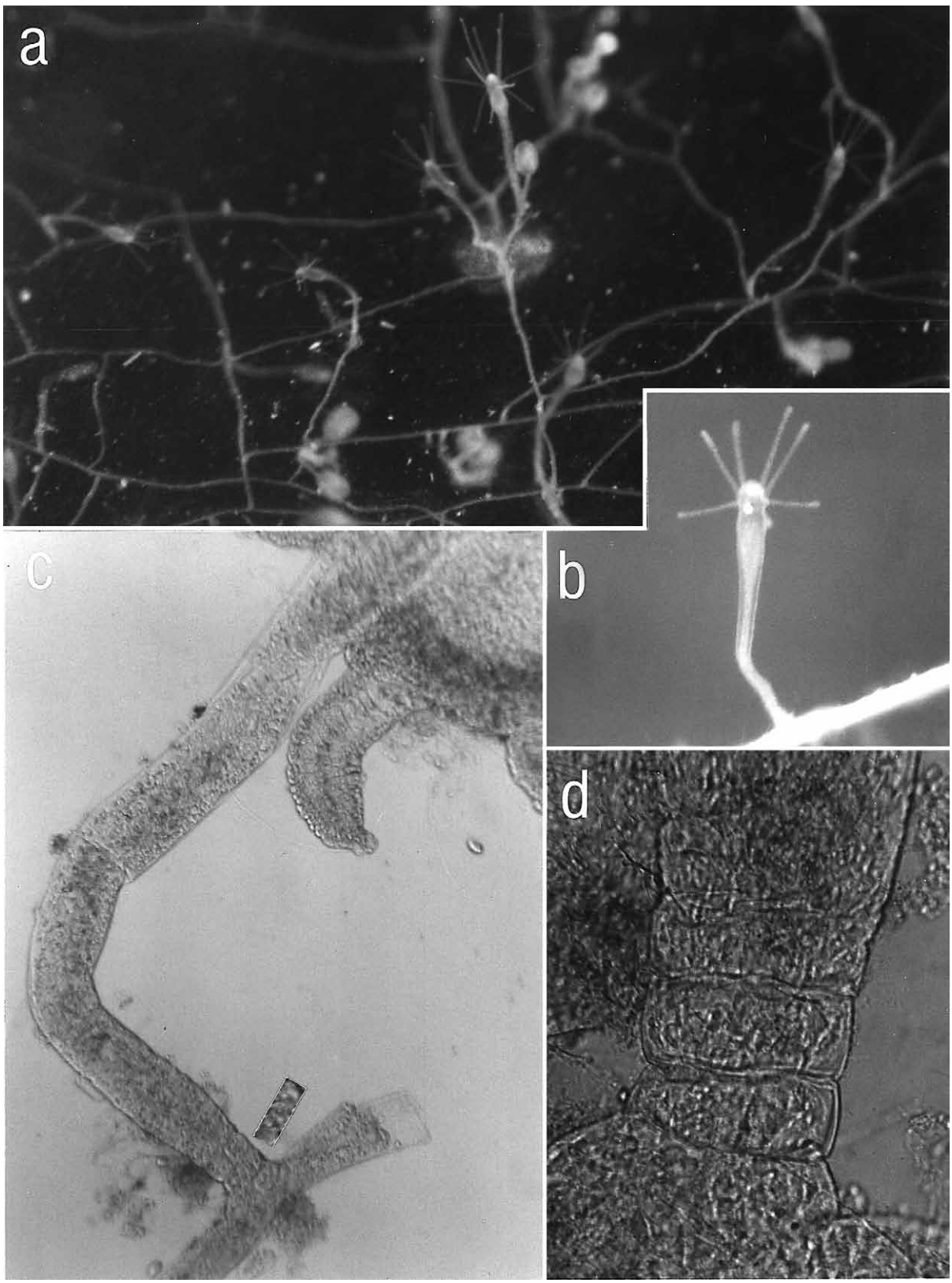

FIG. 1. – *Amphinema* sp. from Bodega Harbor, laboratory-reared specimens. (a) Hydroid colony, showing two polyps and a developing gonophore on an upright branch. (b) Single polyp, showing the two cycles of tentacles, held upward and outward from the body of the hydranth; polyp height, about 1 mm. (c) Photomicrograph of the lower part of a hydranth, showing (from bottom to top) stolon, annulations of the perisarc, perisarc proper, the distinct lip of the perisarc, and the body of the hydranth (one tentacle of the polyp is evident in the photograph). Note the single banana-shaped microbasic eurytele nematocyst on the hydranth; the same nematocysts are scattered over the tentacles. (d) Enlargement of (c), showing annulations of the perisarc at the base of the polyp.

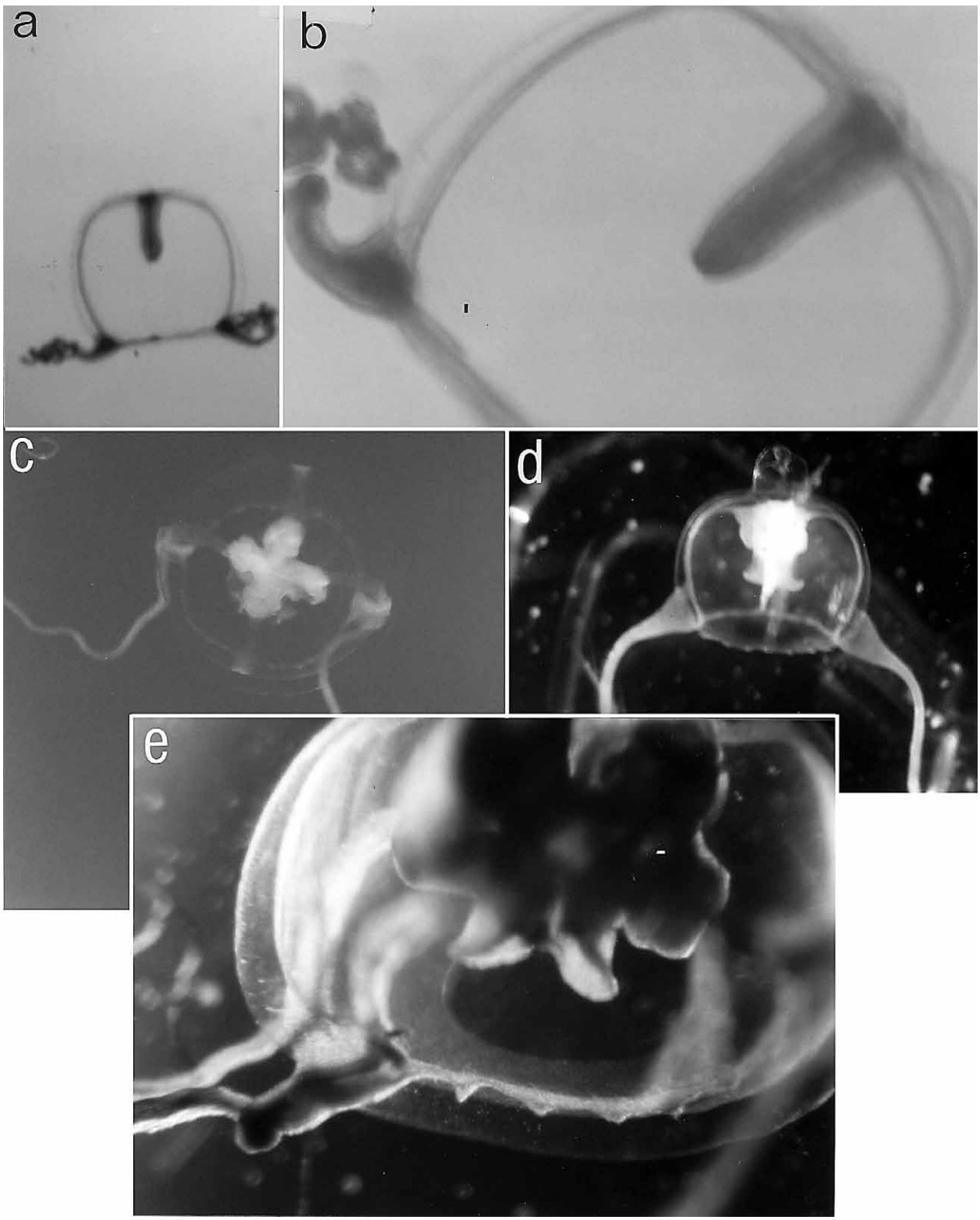

FIG. 2. – *Amphinema* sp. from Bodega Harbor, laboratory-reared specimens. (a and b) Two views of the newly-released medusa, about 0.9 mm wide by 0.7 mm in width. Note the manubrium, which reaches about half-way into the subumbrellar cavity, the two tentacles with large flattened tentacle bulbs, the perradial marginal wart on the rim of the umbrella, and absence of an apical projection. (c) Six week-old medusa, aboral view, 2 mm bell height, 3 mm bell width. Note the cruciform-shaped gonad as seen from above, showing irregularities and convolutions of the gonad structure. Note also the broad radial canals and two extended marginal tentacles. (d) Four week old medusa, side view, 1.5 mm bell height, 1.7 mm bell width. Note the apical projection, very thin jelly of the bell, two prominent tentacle bulbs with tentacles, cruciform manubrium with developing gonad above, and the numerous marginal warts around the base of the bell. (e) Seven-week old senescent medusa, 2.5 mm bell height, 3 mm bell width. The cruciform lips, smooth gonads, and marginal warts are clearly visible. An "abnormal" third tentacle has developed on a previously atentaculate marginal wart.

small interradial warts along the bell margin. The manubrium was simple and extended about 1/2 way into the subumbrellar cavity. The exumbrella was sprinkled with nematocysts. When not extended, the tentacles were held coiled up against the bell (Fig. 2a)

Mature medusae 4–5 weeks old attained a bell height and diameter of about 2.5 mm. The two tentacle bulbs had enlarged and become laterally compressed, extending upwards onto the bell along each corresponding radial canal (Fig. 2d). The extended tentacles were very long, reaching 5 cm or more in length when medusae swam up into the water column. The tentacle bulbs were rose-red in color, which also extended somewhat down the length of each tentacle; the four radial canals were the same rose-red. Three to four marginal warts were present in each interradial sector of the bell margin - none of these warts produced tentacular processes of any kind. The manubrium had four well-developed cruciform lips with a bright greenish tinge. Gonads had formed on the upper half of the manubrium, and consisted of four interradial, somewhat-convoluted areas extending, when viewed from the top, in a cruciform shape out adjacent to the radial canals (Fig. 2c). No oblique, inwardly-pointing, folds on the surface of the gonads, characteristic of many species of *Amphinema,* including *A. rugosum,* were ever observed. No eggs were seen, and all mature medusae observed were assumed to be males; the gonads were whitish and opaque.

The oldest medusae reared, about eight weeks old, attained a bell diameter of about 3.5 mm. Senescence had apparently begun to set in by this stage, and one specimen developed a third tentacle from a previously-atentaculate marginal wart (Fig. 2e); the same tentacle bulb also had a finger-like growth directing inward towards the velum. Russell (1953) noted similar developmental abnormalities in *A. rugosum,* as have Boero *et al*. (1997) in senescent specimens of the pandeid *Codonorchis octaedrus*, which developed multiple manubria and bifurcated tentacles. Whether these senescent morphologies occur in field populations, or are an artifact of laboratory culture, is not known.

Newly-released *Amphinema* sp. medusae were phototactic and swam towards a light source; similar behavior was noted by Boero *et al*. (1997) by the pandeid *Codonorchis octaedrus*. In culture, *Amphinema* sp. medusae usually rested on the bottom of the container and caught brine shrimp nauplii or copepods passively with the lips of the manubrium; the tentacles were never seen to actively capture or ensnare any provided crustacean food item in the laboratory.

Cnidom (Fig. 3)

The Bodega Harbor *Amphinema* sp. had two types of nematocysts. Both types were found in the tentacles of the polyp: banana-shaped microbasic euryteles (Fig. 3a, b) were 8–9 x 2–2.5 mm, and very small, almost-round capsules, probably desmonemes, were 2–2.5 mm diameter (Fig. 3c). Only one type of nematocyst, microbasic euryteles 6.5–8.0 x 2.5 mm, were found in the tentacles of the adult medusa (Fig. 3d). The types and sizes of nematocysts seen in the Bodega Harbor animals were virtually identical to those described for both *A. dinema* and *A. rugosum* by Russell (1938).

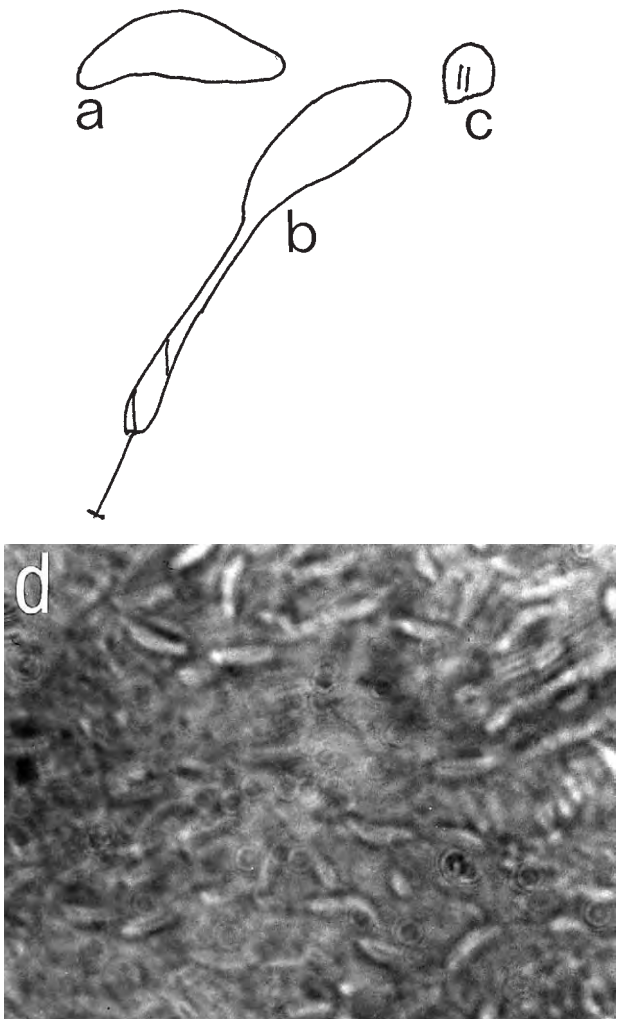

FIG. 3. – Nematocysts of *Amphinema* sp. Sketches of (a) unexploded and (b) exploded euryteles from the tentacles of an adult medusa. (c) Second type of nematocyst, presumably unfired desmoneme, in the tentacles of the polyp. (d) Photomicrograph of a tentacle squash preparation from an adult medusa showing unfired banana-shaped euryteles; the eurytele capsules are all about 10 μm in length.

DISCUSSION

Differences between *Amphinema dinema* and *A. rugosum* from the British Isles were described in detail by W. J. Rees and Russell (1937) and Russell (1953), for both field and laboratory-reared specimens. Similarities were sufficient to cause Rittenhouse to attempt to cross the two species, but he was unsuccessful (Russell, 1953).

The hydroid of *A. dinema* from Britain, may or may not have annulations at the base of the hydrocaulus and has a membranous, poorly-defined hydrocaulus rim. Medusa buds are borne only on the stolons. The newly-released medusa had no apical projection and the adult medusa has marginal protuberances of the bell that are "mere thickenings of the edge of the umbrella" (W.J. Rees and Russell, 1937, p. 61), also called marginal warts. Mature gonads are "simple adradial plates," lacking lateral folds.

The British *A. rugosum* hydroid has an annulated base of the hydrocaulus and the distal rim of the hydrocaulus ends abruptly; the hydranth is not seen at all retractile. The newly-released medusa has an apical projection, and the adult medusa has prominent marginal tentaculae along the umbrellar rim. The gonads of the adult medusa have inwardly-pointing adradial folds.

Amphinema sp. from Bodega Harbor shares taxonomic characteristics of both *A. dinema* and *A. rugosum* (see Table 1). The polyp of the Bodega Harbor *Amphinema* possesses an annulated hydrocaulus, and like *A. rugosum*, bears medusa buds on both the reptant stolon and the branched uprights. The Bodega Harbor *Amphinema* medusa, however, matches the description for *A. dinema*, in its absence of an apical projection in the newly released medusa, lack of oblique folds on the mature gonads, and presence of marginal warts around the margin of the umbrella, rather than the more elongate tentaculae of *A. rugosum*.

Due to the above inconsistencies in aligning characters in the life cycle of the Bodega Harbor *Amphinema* with either *A. dinema* or *A. rugosa,* it is concluded that a species designation is not possible at this time, although the possibility is certainly indicated that *A. dinema* and *A. rugosum* might be one variable species rather than two separate species. Overlapping specific characters are unfortunately common in hydrozoan systematics. In a group with a limited range of morphologies with which to separate species, this tendency towards character overlap renders identification of many hydrozoan species dubious. Innovative and integrated approaches are needed in hydrozoan systematics, including a "molecular taxonomy" in which species level markers are identified. It is hoped that such markers can be used in association with standard taxonomic characters to more clearly define species in difficult hydrozoan genera such as *Amphinema*. Without new innovative approaches, we continue to be stymied by situations exemplified by the Bodega Harbor *Amphinema*, hobbled by our inability to make field identifications and engaged in fruitless debates over morphological minutiae, which only a specialist who has worked with a particular genus or group of species over a long period of time can interpret.

Both *A. dinema* and *A rugosum* are apparently widely distributed globally, but neither has been reported from any other location on the Pacific coast of North or South America. It is probably significant

TABLE 1. – Comparison between *Amphinema dinema, A. rugosum*, and the *Amphinema* sp. from Bodega Harbor, California. Definitive morphological differences between *A. dinema* and *A. rugosum* are summarized from W.J. Rees and Russell (1937) and Russell (1953).

	Amphinema dinema, (Péron and Lesueur, 1809)	***Amphinema rugosum,*** (Mayer, 1900)	***Amphinema* sp.** Bodega Harbor
Hydroid:			
Base of hydrocaulus	With or without annulations	Annulated	Annulated
Upper end of hydrocaulus	Membranous and delicate	Not membranous	Well-defined, not membranous
Placement of medusa buds	On stolons	On stolons and uprights	On stolons and uprights
Color	Orange-red	Orange-red	Orange-red
Newly released medusa:			
Apical projection	Absent	Present	Absent
Color of stomach	Reddish orange, tinged green	Ochre yellow	Brick-colored; no green
Adult medusa:			
Umbrella rim	Marginal warts	Marginal tentaculae	Marginal warts
Gonads	Oblique folds absent	Oblique folds present	Oblique folds absent
Color	Tentacle bulbs vivid purple-violet; stomach bright green	Tentacle bulbs and stomach orange to brownish-yellow	Tentacle bulbs light brownish red; stomach yellowish brown with bright green lips

that neither species has previously been reported from either central California, which has been sampled repeatedly by hydroid specialists over the past 100 years (see Fraser, 1937; Rees, 1975), or the Puget Sound/Strait of Georgia region in Washington State and British Columbia, despite relatively intensive sampling there (Foerster, 1923; Arai and Brinckmann-Voss, 1980; Mills, 1981; Mackie, 1985; Wrobel and Mills, 1998). The entire float-fouling fauna in Bodega Harbor has shifted in the past 20 years to a highly disturbed community, dominated by non-indigenous species, not so different from that of San Francisco Bay (C.E. Mills, personal communication). Neither the polyps nor medusae of any *Amphinema* species were collected in Bodega Harbor during intensive surveys of the area 1971–1974 (Rees, 1975), and again in 1980. The host *Watersipora* bryozoan, like many of the other non-indigenous invertebrates now present in Bodega Harbor, was also not present in the 1970s. The only pandeid polyps in the Bodega Harbor region during the 1971-1974 period were thought to be *Leuckartiara octona*, collected on several occasions on the shells of living gastropods, *Olivella biplicata*; *L. octona* medusae were also collected in the plankton during that period (J.T. Rees, unpublished).

There have been two other records of medusae in the genus *Amphinema* from the North Pacific. Arai and Brinckmann-Voss (1983) described a new species, *A. platyhedos*, from British Columbia, collected from depths exceeding 350 m. Wrobel and Mills (1998) show a different *Amphinema* medusa from surface waters in Monterey and Santa Barbara (incorrectly labelled as *A. platyhedos*, but clearly a different species, C.E. Mills, personal communication). *A. platyhedos* can be distinguished from the Bodega Harbor *Amphinema* sp. by its long marginal tentaculae and the smooth (unfolded) structure of its gonads. The *A. "platyhedos"* pictured by Wrobel and Mills (1995) is almost certainly a third west coast species. Its gonads show much distinctive lateral folding, a characteristic absent from *A. platyhedos*, and it has numerous marginal warts; it also does not correspond well to the Bodega Harbor specimens described here. A more complete comparison of the medusae of nine species belonging to the genus *Amphinema* is given by Bouillon *et al*. (2000).

The polyp of the Bodega Harbor *Amphinema* sp. seems to be a facultative, rather than obligate, commensal as evidenced by the ability of the colony to grow and thrive under laboratory conditions without the presence of its bryozoan host. Many pandeid polyps are symbiotic, including *Octotiara russelli*, also a bryozoan symbiont (Boero and Bouillon, 1989), and *Merga tergestina*, found on sea urchins and polychaetes (Vannucci, 1960). At least one pandeid genus, *Hydrichthys*, is parasitic on fish (Larson, 1982; Boero *et al*., 1991). A systematic review of symbioses within the Pandeidae would be most enlightening, and might provide insight into the mechanisms by which hydrozoan polyps have evolved from facultative to more obligate forms of symbiosis.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Photosynthetic planulae and planktonic hydroids: contrasting strategies of propagule survival*

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SUMMARY: Settlement delays can be important to prevent propagule waste when proper settling substrates are not immediately available. Under laboratory conditions, the planulae of *Clytia viridicans* underwent two alternative developmental patterns. Some settled on the bottom, forming a hydranth-gonotheca complex that produced up to four medusae and later either degenerated or gave rise to a hydroid colony. Other planulae settled right below the air-water interface, forming floating colonies that eventually fell to the bottom and settled. *Halecium nanum* released planulae with a rich population of symbiotic zooxanthellae that survived into a rearing jar for three months. After a long period of apparent quiescence (possibly fuelled by photosynthetic activities of zooxanthellae) the planulae produced new colonies. Both photosynthetic planulae and settlement at the interface air-water allow a delay in the passage from a planktonic to a fully functional benthic life.

Key words: Hydrozoa, life cycle, planula, floating hydroid, Zooxanthellae, dispersal

INTRODUCTION

Supply-side ecology (e. g., Gaines and Roughgarden, 1985) stressed the importance of organism substitution for the persistence of marine benthic communities. Individuals die and are replaced, so that the future of a community depends on the success of larval settlement. In this framework, larval ecology (usually a plankton-based affair) becomes crucial in benthic ecology and life cycles acquire a central position in marine ecology (Boero *et al*., 1996; Marcus and Boero, 1998).

The efficacy of dispersal of benthic organisms is linked to propagule vagility, with the possibility of delaying settlement until proper substrates become available. As remarked by Vance (1973), the planktonic larvae of benthic invertebrates can be either lecithotrophic (with their own reserves) or planktotrophic (feeding on external food sources), this affecting their possibilities of dispersal and survival. Scheltema (1966, 1988) described long-lived larvae of benthic invertebrates able to cross oceans: teleplanic larvae. A severe constraint to such wide-range dispersal is the impossibility, for non-feeding larvae like those of most hydroidomedusae, to have the necessary reserve to lead a long larval life before settlement (see Cornelius, 1992).

Boero and Bouillon (1993) and Boero *et al*. (1997) reviewed the variety of hydroidomedusan cycles, describing a vast array of modifications of the classical polyp-medusa alternation: in many cases, either the medusa or the polyp can be suppressed. The primary larva in the hydroidomedusan life cycle is the planula, a stage with generally low vagility which, in some cases, even remains connected to the mother colony by mucous threads

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before settlement (Wasserthal and Wasserthal, 1973; Hughes, 1977). There are no records of feeding planulae and their settlement should occur soon after release, this limiting dispersal for species deprived of other planktonic stages, i. e. medusae. Boero and Bouillon (1993), however, remarked that the presence of a medusa in the life cycle is not correlated to wide distribution, so that other ways of dispersal are available to these animals (see also Cornelius, 1992).

In this paper we report on the life cycle features of two species, relating their developmental features to their strategies of dispersal while leading a larval or post-larval planktonic life.

MATERIAL AND METHODS

Colonies of *Clytia viridicans* and *Halecium nanum*, growing on algae of the genus *Cystoseira*, were collected by SCUBA diving from the rocky shore of the Ionian Sea (Porto Cesareo, Italy) during April-July (*C. viridicans*) and October (*H. nanum*) 1997. Fertile colonies were removed from the supporting algae and maintained in glass tanks with filtered seawater (FSW, 0.45 μm). Both temperature and photoperiod were controlled so to match field conditions. The medusae released in the laboratory were fed with *Artemia* nauplii and reared until maturity in FSW. Males and females were kept together so to have fertilisation.

The planulae, either released from the gonotheca (*H. nanum*) or produced in the water after fertilisation (*C. viridicans*), were maintained in glass tanks with FSW (0.22 μm). The presence of zooxanthellae was investigated *in vivo* by a light microscope with fluorescence apparatus.

RESULTS

Clytia viridicans (Leuckart, 1856)

Metschnikoff (1886a) described a medusa that he retained as identical with those described by Leuckart in 1856 as *Phialidium viridicans*. Later, Metschnikoff (1886b) reported and figured primary hydranths of *C. viridicans* with a gonotheca arising from a basal plate. Both hydroid and medusan features agree with the present material. Russell (1953) considered the possibility that *Clytia flavidula* and *C. viridicans* were referable to a single species. Later, Cornelius (1982) referred *P. viridicans* to *C. hemisphaerica* because of their similarity. Finally, Calder (1991) described the hydrotheca of *C. hemisphaerica* with "U-shaped pleat extending inwards towards hydrothecal cavity"; he also reported an extensive synonymy list for *C. hemisphaerica*, including in also *P. viridicans* Leuckart, 1856.
The following is the first description of the life cycle of *C. viridicans*.

Hydroid (Fig. 1a, b)

Colony stolonal, growing on algae; pedicel short, annulated at base and below hydrotheca; hydrotheca conical, about 0.6 mm high, with 7-9 cusps, projecting inwards and with outwards perisarc projections in the bays between nearby cusps; hydranths about 0.5 mm high with a peduncled hypostome and 14-18 amphicoronate tentacles, the ones oriented downwards laying on the perisarc projections between adjacent cusps, the ones oriented upwards being sustained by cusps; gonothecae on hydrorhiza, either corrugated or smooth, about 0.8 mm high, containing a row of up to four developing green medusae.

Newly released medusa (Fig. 1c)

Umbrella hemispherical, about 0.5 mm high, bright green, with four radial canals, four perradial tentacled bulbs, four small interradial bulbs sometimes present; manubrium tubular, with four lips, reaching half of subumbrellar cavity; velum wide, with a small opening; eight statocysts along the circular canal, with one statolyth each. A row of nematocysts often present on exumbrella, parallel to umbrellar margin.

Medusa development (Fig. 1d)

Gonad rudiments developed on radial canals two days after release, along with other four tentacles on interradial marginal bulbs and other statocysts between adjacent tentacles. Marginal bulbs, tentacles and statocysts are gradually added and umbrella tends to flatten until, 30-45 days after release, medusae reach maturity.

Mature medusa (Fig. 1 e)

Umbrella flattened, about 6 mm wide and 1.5-2 mm high, with 14/16 tentacles; manubrium, gonads and tentacular bulbs bright green, exumbrella trans-

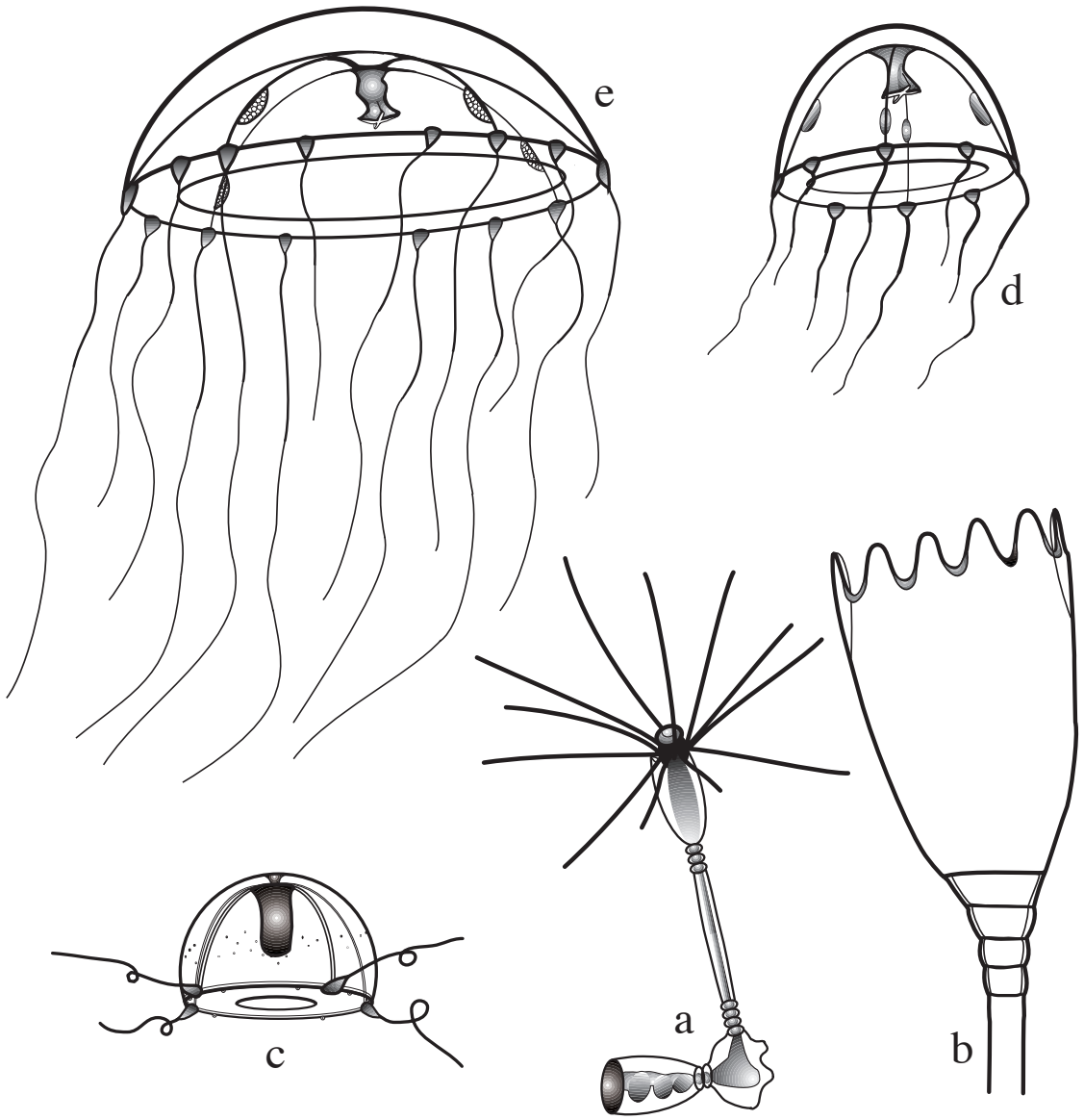

FIG. 1. – *Clytia viridicans*. a: primary hydranth with gonotheca; b: hydrotheca; c: newly released medusa; d: young medusa; e: mature medusa.

parent; manubrium short, on a short peduncle, mouth with four corrugated lips; gonads on distal part of radial canals, not reaching umbrellar margin.

Planula production (Fig. 2)

Spawning took place at night; the eggs, about 200 μm in diameter, were immediately fertilised, and the embryos reached a gastrula stage within 10 hours, to differentiate into free-swimming hollow planulae within 20 hours. Settlement took place within 48 hours and 6-10 days after zygote formation.

Settlement on the bottom (Fig. 2)

Most planulae settled on the glass of the rearing jars, forming a wide basal disc from which a primary polyp arose within 12 hours followed, after 24 hours, by a gonotheca. Two days later, 3 or 4 medusae were released. After medusa liberation, some polyps degenerated, but most developed a hydrorhiza and new polyps, producing large colonies that started to develop gonothecae and to release medusae. Several generations were easily obtained under laboratory conditions. No differences were observed in development and growth

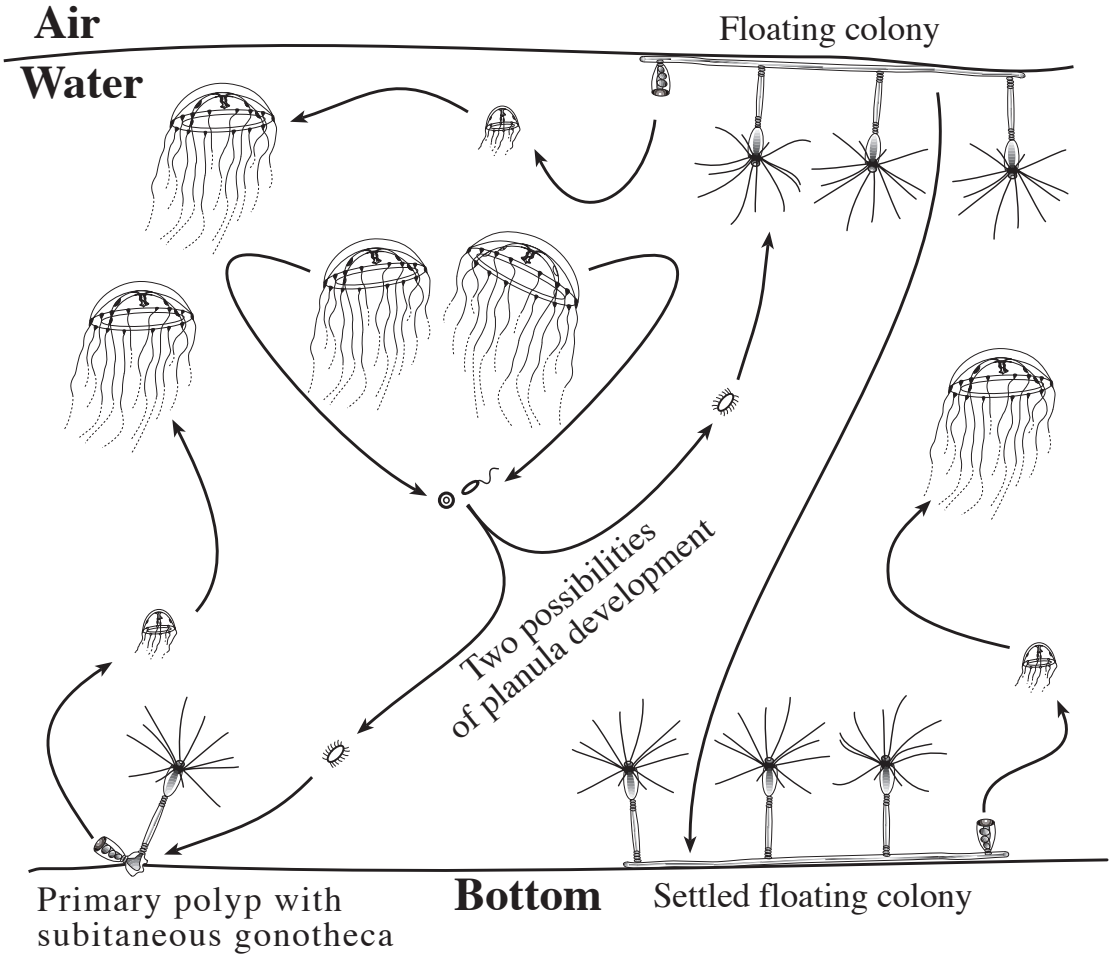

FIG. 2. – *Clytia viridicans*, alternative life cycle pathways involving planula settlement either on the bottom or on the air-water interface.

pattern between medusae from natural colonies and medusae from laboratory-reared ones.

Settlement on air-water interface (Fig. 2)

Some planulae settled on the underside of the surface film of water and gave rise to floating colonies that developed for weeks in that position. Accidental break off of the interface induced colony sinking to the bottom. Sunken colonies settled on the bottom of the rearing jars, becoming indistinguishable from those that immediately developed there.

Remarks

The development of gonothecae from the base of primary hydranths confirms Metschnikoff's observations. Such developmental pattern was never reported for other *Clytia* species, but recalls what Bouillon *et al*. (1991) described for *Laodicea indica*. The bright green colour of the medusa, furthermore, is clearly suggested by the specific name *viridicans*, from the Latin *viridis:* green. The adult medusa is similar to *Clytia hemisphaerica* but the green colour of gonads, manubrium and marginal bulbs is a diagnostic character in living material. The shape of the hydrothecal cusps, with the perisarc projections between adjacent cusps, is a diagnostic character for the hydroid.

The origin of floating colonies directly from a planula is rare for usually benthic hydroids; Billard (1917) reported on three free-living species, two of which perhaps represent fragments of detached colonies, while the third species, *Campanularia pelagica* Van Breeman can be derived from an unsettled planula or from a planula settled on a sand grain (see also Cornelius 1995). Cornelius (1982) considered *Campanularia pelagica* as identical with *Clytia hemisphaerica***.**

Halecium nanum Alder, 1859

Together with *H. conicum*, *H. pusillum* and *H. tenellum*, *Halecium nanum* is one of the small *Halecium* of the Mediterranean. Motz-Kossowska (1911) provided a good description of this species which, in spite of being rather common, has been rarely treated in hydroid literature.

Planula production

The colonies of *Halecium nanum*, as well as the planula, are packed with zooxanthellae. The solid planulae, with a ciliate epidermis, moved slowly by ciliary propulsion. They also crawled on the bottom with peristaltic movements, continuously changing from an elongated to a spherical shape.
The majority of the about 100 planulae released in the laboratory died within one week, but two survived and went through the following states:
- 0-30 days: they continuously crawled on the jar bottom;
- 31-70 days: they stopped, and became nearly spherical, as if they were encysted;
- 71-75 days: they became active again, acquiring a pear shape with a pike from which, after three days, a polyp was born;
- 76-100 days: a colony with five hydranths was built.

Remarks

The colonies produced by the two planulae had zooxanthellae in their tissues and regressed to hydrorhiza within a week after settlement; they were not kept further to see if they were able to regenerate after a period of rest.

DISCUSSION

Planula dispersal is generally limited because planulae are lecitothrophic: in most hydrozoan species, in fact, planula settlement occurs within few hours or days (Sommer, 1992), so their contribution to dispersal should be little. Planula types reflect the vagility of species with and without medusae: those produced by medusae are usually hollow and able to swim, so to reach the bottom from the water column, whereas those produced by hydroids are usually solid, do not swim much and just crawl on the bottom (see Bouillon, 1994). These different properties should lead to higher endemicity in species without medusae and wider distribution in species with medusa. This is not what Boero and Bouillon (1993) found in the distribution of Mediterranean species, hypothesising a dispersive role also for the hydroid. The specialised asexual propagules of *Halecium pusillum* are a paradigm of hydroid dispersal (Huvé, 1955), even though many species simply disperse by colony fragments.

Medusae are obvious dispersal agents, but they can become ripe while distant from proper settling substrates for the planulae, so that sexual reproduction might result in a failure. Such inconvenient can be overcome by prolonging the life of medusae by fission, by multiple gonad ripening, or by production of gonothecae and fertile polyps on the medusa, but in *Clytia viridicans* this risk is faced with the possibility for the planula to produce hydroid colonies attached to the air-water interface that can become secondarily benthic.

Cornelius (1992) discussed the possibility for hydroids to settle on floating organisms and raft on them so to become widely dispersed. The efficacy of this way of dispersal has not been tested experimentally but, however, is probably very high for the hydroids that settle on algae, such as the ones we investigated. That of *Clytia viridicans*, with planulae producing floating hydroids, is a particular case of rafting, because the hydroid itself is the raft, contrary to what happens for the species settling on other organisms, labelled as rafters. Floating hydroids are usually originated by fragmentation of benthic colonies whereas, for *C. viridicans*, the rafting colonies derive from planulae. It might be questionable wether this planula behaviour can really occur in the field. The study of many hydrozoan species, to our knowledge, never recorded such planula behaviour whereas, in *C. viridicans*, it occurs quite often. Particularly calm conditions are common in the Mediterranean during summer months, so allowing planula settlement on the interface. Once the colony is established, then, it can even become detached from the surface and be transported by the currents.

As described for dozens of species by Boero and Fresi (1986), many hydroids deprived of medusa paradoxically, after sexual reproduction, “disappear” for several months instead of becoming more abundant. The zooxanthellate planulae of *Halecium nanum*, remaining viable for at least three months, are one of the keys of this lapse between planula production and appearance of new colonies. For

species deprived of zooxanthellae, another possibility might be that planulae settle, become wrapped by perisarc and remain dormant until the return of favourable conditions.

These planula behaviours were observed in the laboratory and we do not know their importance in the field. The contribution of planulae to species dispersal, however, might be greater than usually thought and it is possible that further types of planula development will be found in the future, showing still unexpected aspects of the renewed developmental plasticity of hydrozoans.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Life in tidepools: distribution and abundance of two crawling hydromedusae, *Staurocladia oahuensis* and *S. bilateralis*, on a rocky intertidal shore in Kominato, central Japan*

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SUMMARY: Two crawling medusae, *Staurocladia oahuensis* (Edmondson, 1930) and *S. bilateralis* (Edmondson, 1930) were found to be abundant in intertidal rock pools in Kominato from late summer until early winter. The two species were found to rarely share the same individual alga, and sometimes showed exclusive occupancy of pools at higher intertidal levels. The abundance of the two species of medusae fluctuated widely over time with both species showing similar population structures during their period of occurrence. The asexual reproduction of the medusae was considered to be a cause of the distributional pattern and the fluctuation in abundance. An experiment was conducted to evaluate the rate of asexual reproduction under different conditions. At 12°C neither species performed asexual reproduction, while at 17°C and higher temperatures both species reproduced asexually at a high rate. The number of each population was found to nearly double in about a week. The coexistence of the two species of medusae in tidepools is discussed in relation to the habitat characteristics. *S. oahuensis* and *S. bilateralis* were not known previously from Japan; this constitutes a new record of both species from Japanese waters. We also found both species in several other warm water locations in Japan.

Key words: crawling medusae, new record, asexual reproduction, tidepools, distribution and abundance, coexistence.

INTRODUCTION

Crawling medusae are small benthic medusae that live on algae and seagrasses. Most of them are known to reproduce asexually either by budding or fission including schizogony (Bouillon, 1978); all are hydrozoans. They are primarily non-swimmers and move by crawling or creeping on the substrate by means of walking tentacles, although swimming behavior has also been observed in a few species (Browne, 1910; Brinckmann, 1964). Because of this they are confined to coastal waters, and are sometimes common along shorelines (Gilchrist, 1919; Edmondson, 1930; Millard, 1975). However, with an exception of the European species, *Eleutheria dichotoma* Quatrefages, 1842, they have been rather neglected animals, and little is known about their life in natural habitats.

We found that two species of crawling medusae were common in Kominato and they often occurred in abundance in intertidal rock pools from late summer to early winter. The two species were identified as *Staurocladia oahuensis* (Edmondson, 1930) and *S. bilateralis* (Edmond-

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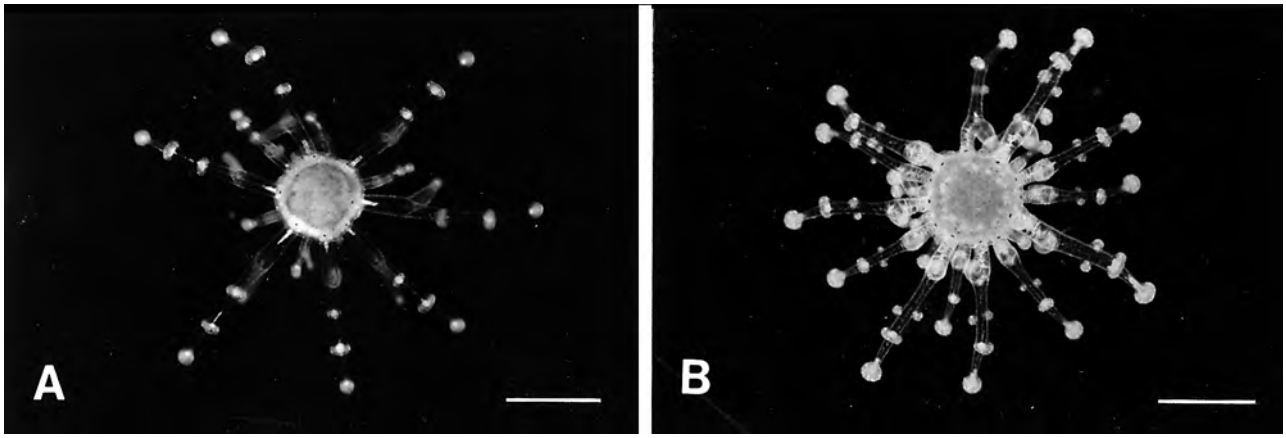

FIG. 1. – A: *Staurocladia oahuensis*. B: *Staurocladia bilateralis*. Scale bars, 0.5 mm.

son, 1930), based on extensive morphological comparisons. This is the first record of both species from Japanese waters. There has been only one other published report on each species since they were described from Hawaii in 1930. *S. oahuensis* is also known from Chile (Kramp, 1952) and *S. bilateralis* is also reported from the Seychelles (Bouillon, 1978). These species are thus still poorly known.

The abundance of the two medusae allowed us to redescribe both species based on many specimens, and to investigate some aspects of their biology. As they were often abundant in tidepools, we focused on their life history in relation to the habitat. Tidepools are considered to be unique habitats in marine environments, and a number of ecological studies have been made on the community structure and organization of their inhabitants (see Metaxas and Scheibling, 1993, for references). Crawling medusae are distinctive, with supposedly high population growth rates by asexual reproduction and limited dispersal ability. It is of special interest to see how such organisms are distributed in tidepools and how their abundance fluctuates over time. In this study we describe patterns of distribution and abundance of the two crawling medusae in the habitat, and discuss it in light of their biology.

MATERIALS AND METHODS

Identification of the species of *Staurocladia*

The characters described and illustrated by Edmondson (1930) were used to differentiate *Staurocladia oahuensis* and *S. bilateralis*. In *S. oahuensis*, the upper or dorsal branch of each tentacle is provided with two aboral clusters of nematocysts in addition to the capitate terminal cluster (Fig. 1A), whereas *S. bilateralis* bears one aboral and two lateral clusters besides the terminal cluster (Fig. 1B). The bell diameter and number of radial canals, which seem to be helpful for identification of some other species of *Staurocladia* (Kramp, 1961; Bouillon, 1978), cannot discriminate these two species, because they are similar in size (about 0.5 mm in bell diameter on average), and the number of radial canals varies from about five to more than 15 among individuals in both species. The number of tentacles is also a variable character for both species. Some specimens are provided with only about five fully-grown and five newly-developing tentacles whereas others have more than 20 fully-grown and several young tentacles. The bell of both species is provided with a number of red eye-spots along the margin on its aboral surface and has a marginal nematocyst ring on its oral side. The velum is very thin and broad, reaching the manubrium.

Study site

Samples for the field investigations and the experiment of this study were made on a rocky intertidal shore in Kominato, Boso Peninsula (35°07'N, 140°11'E). It is in a small bay (about 1.5 km wide and 1.5 km deep) at about 0.5 km from the entrance. The bay opens directly to the Pacific Ocean, facing south with most of the coast quite exposed to ocean surges. The tidal amplitude in Kominato is about 150 cm (mean high water spring tide, mean tide level and mean low water spring tide are respectively 153 cm, 94 cm and 0 cm above sea level), and the surface seawater temperature ranges annually from about 10°C to 25°C.

Field investigations

In order to see how abundance of the two medusae fluctuated over time, density of the medusae on *Sargassum thunbergii* (Mertens) and *Ulva conglobata* Kjellman, which are among the most common algae in the intertidal zone in Kominato, was investigated from the end of August, 1995, until late February, 1996, at about one-month intervals. The two algae were haphazardly collected from three adjacent tidepools (T-2, T-3 and T-4 in Table 1) and separately put in 500 ml plastic containers. Formalin was added to the two containers (to a concentration of about 5%) to fix the medusae. Each container was emptied into a plastic bowl where the collected algae were shaken vigorously to detach crawling medusae; the algae were then removed to measure wet weight. Crawling medusae were sorted out from the residue, identified under a Nikon stereomicroscope (model SMZ-U), and the number of specimens was counted for each *Staurocladia* species. Bell diameter of the specimens was measured with an ocular micrometer calibrated to 0.033 mm to see how population structure of the two medusan populations changed during their seasonal occurrence. As some medusae were oblong in bell shape, length of the bell was measured at two perpendicular axes, and the mean of the two values was regarded as a representative diameter.

The distribution between tidepools of the two species was investigated twice, on November 3 to 4, and November 30 to December 1, 1998. Small pieces of algae were picked up haphazardly from eight tidepools (T-1 to T-8 in Table 1), and a "Lower" site, which consisted of six shallow pools at the lower mid intertidal fringe. The tidepools T-1 to T-8 are scattered almost along a line in the order with a distance of about one to several meters between one another. The height of the pools above sea level is shown in Table 1. Most of the algae taken for both investigations were *Sargassum* spp. (87.5% and 80.0% of the first and second investigation respectively), as they were the most common algae in the study area, but a number of *Ulva conglobata* (11.7% and 16.3%) and a few other algae (0.8% and 3.7%) were also examined where found. The weights of algal pieces were 2-34 g for *Sargassum* spp., 1-6 g for *Ulva* and 4-7 g for other species. Each algal piece was put in a separate plastic bag and brought back to the laboratory, where each was carefully searched for living crawling medusae. Medusae collected from the pieces were identified under the stereomicroscope, and the number of each species was counted.

Culture experiment

Medusae of moderate to large size were collected on November 5 and 6, 1998, kept at 15°C without food for a day or two, and were then separated into groups of ten specimens which were put in the same plastic container (6 cm in diameter and 3 cm in depth) to be reared together during a nine-day experiment. Sixteen and 12 groups were obtained for *Staurocladia oahuensis* and *S. bilateralis* respectively. Eight groups of *S. oahuensis* and four of *S. bilateralis* were given no food throughout the experiment, while the other eight sets of both species were fed newly hatched *Artemia* sp. nauplii in saturation every other day. The starved groups and those given food were both evenly divided into four subgroups, each of which were cultured at four different temperatures, 27°C, 22°C, 17°C and 12°C. Thus eight experimental groups, shown in Table 2, were set up for each species

TABLE 1. – The number of *Staurocladia oahuensis* and *S. bilateralis* collected from different tidepools on November 3 to 4, 1998 (1) and on November 30 to December 1, 1998 (2). "The number of algal pieces found with medusae / the number of pieces examined" at each pool is also shown as "inhabited". Height is in cm above sea level (mean low water at spring tide).

Pool	Height (cm)	1			2		
		inhabited	*S. oahuensis*	*S. bilateralis*	inhabited	*S. oahuensis*	*S. bilateralis*
T-1	95	4/10	86	0	3/20	1	2
T-2	118	3/20	5	0	0/20	0	0
T-3	117	15/20	0	35	15/20	0	61
T-4	99	2/10	0	2	2/10	6	1
T-5	79	5/10	3	12	7/10	41	6
T-6	80	5/15	6	10	12/20	33	17
T-7	114	0/5	0	0	0/5	0	0
T-8	120	0/10	0	0	1/10	2	0
"lower"	ca. 60	6/20	3	8	11/20	88	26
Total		40/120	103	67	51/135	171	108

TABLE 2. – The bell diameter in mm (mean±SD) of *Staurocladia oahuensis* and *S. bilateralis* before and after experiment under different conditions. The numeral of the group shows the temperature (°C) at which the experimental group was cultured, with "s" or "f" standing for "starved" or "fed" respectively. The difference of the size before and after the experiment is given as positive (+) or negative (-) growth. All differences shown were found to be significant ($P<0.05$ for all) based on the t-test (t-test with Welch's correction was applied for 22f of *S. oahuensis*, and for 27f, 22f, 17f of *S. bilateralis*).

Species	Group	Before	After	Growth
S. oahuensis	27s	0.559±0.062 (20)	0.321±0.059 (27)	-
	27f	0.570±0.063 (20)	no data	
	22s	0.587±0.067 (20)	0.349±0.048 (35)	-
	22f	0.563±0.044 (20)	0.402±0.128 (48)	-
	17s	0.587±0.047 (20)	0.426±0.058 (31)	-
	17f	0.582±0.071 (20)	0.451±0.091 (40)	-
	12s	0.573±0.051 (20)	0.499±0.069 (20)	-
	12f	0.589±0.057 (20)	0.473±0.152 (14)	-
S. bilateralis	27s	0.531±0.048 (10)	0.291±0.041 (14)	-
	27f	0.538±0.052 (20)	0.451±0.098 (13)	-
	22s	0.538±0.070 (10)	0.347±0.065 (9)	-
	22f	0.549±0.053 (20)	0.606±0.153 (37)	+
	17s	0.535±0.065 (10)	0.306±0.065 (9)	-
	17f	0.560±0.068 (20)	0.631±0.131 (39)	+
	12s	0.528±0.067 (10)	0.434±0.032 (10)	-
	12f	0.563±0.060 (20)	0.489±0.094 (20)	-

by nutritional condition and temperature. During the experiment the number of individuals in each vessel was counted every day. After counting the number, individual medusae were transferred by a pipette to a new vessel with fresh filtered seawater of adjusted temperature. Bell diameters of all specimens in each experimental group were measured at the beginning and end of the experiment. The same method as described above for the investigation of the size distribution of field populations was applied for the bell diameter measurements.

RESULTS

Temporal fluctuation of abundance

Abundance of the two species fluctuated considerably during the six months of the field study (Fig. 2). *Staurocladia bilateralis* was very abundant in late August, 1995, when nearly 500 medusae were collected from about 40g of *Ulva conglobata*, and 150 medusae from about 100 g of *Sargassum thunbergii*. In early October, however, this rich population was virtually gone and no specimens of *S. bilateralis* were collected. In November the population showed some recovery, and it grew further from then into December. On the other hand, *S. oahuensis* was similarly rare in August and October. It became

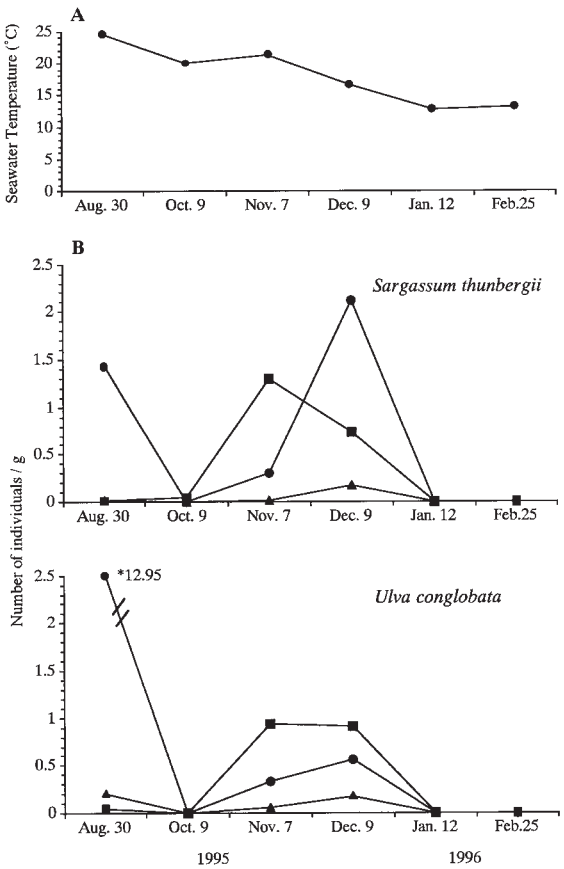

FIG. 2. – A: Seawater temperature in Kominato during the investigation. B: The density of *Staurocladia oahuensis* (n), *S. bilateralis* (l) and unidentified medusae (s) on *Sargassum thunbergii* (upper) and *Ulva conglobata* (lower).

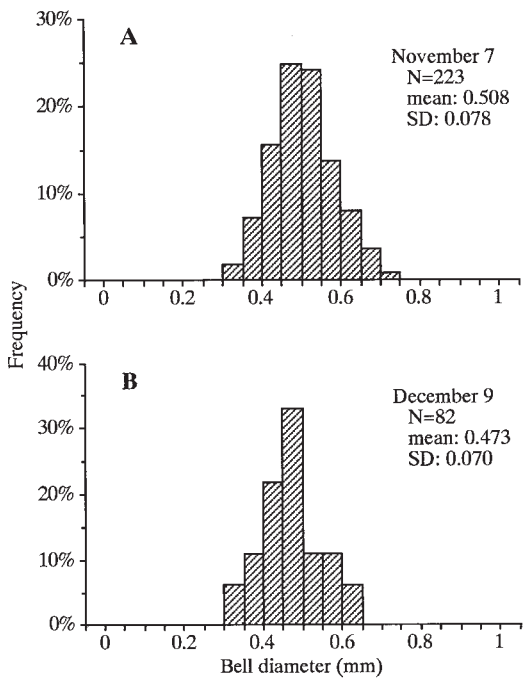

FIG. 3. – Size distribution of *Staurocladia oahuensis* on November 7, 1995 (A) and on December 9, 1995 (B).

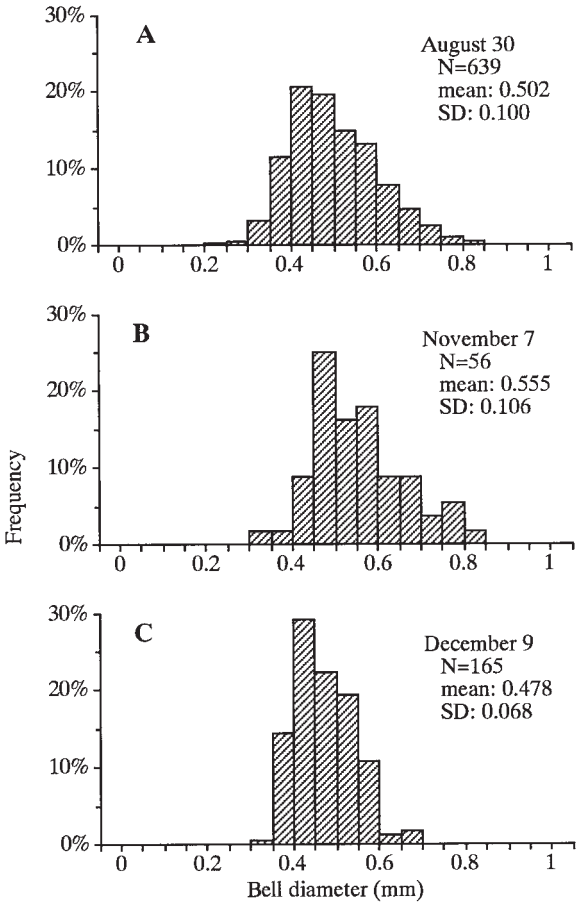

FIG. 4. – Size distribution of *Staurocladia bilateralis* on August 30, 1995 (A), on November 7, 1995 (B) and on December 9, 1995 (C).

quite abundant by early November, and was still common in early December. However, by mid-January both species had totally disappeared and the populations had not started to recover yet by late February.

Population structure

Size distribution of the tidepool populations was similarily unimodal for both species with the mode at 0.4 to 0.6 mm in bell diameter, and did not change much during the investigation (Figs. 3 and 4). The mean ± SD of the bell diameter of *S. oahuensis* in August and October was 0.507±0.206 mm, and 0.501±0.037 mm, respectively. A slight difference was found in the average size of the *S. oahuensis* population between November and December (t-test, $P<0.001$). The bell diameter of *S. bilateralis* was larger in November than in August (t-test, $P<0.001$), and then slightly decreased from November to December (t-test with Welch's correction, $P<0.001$).

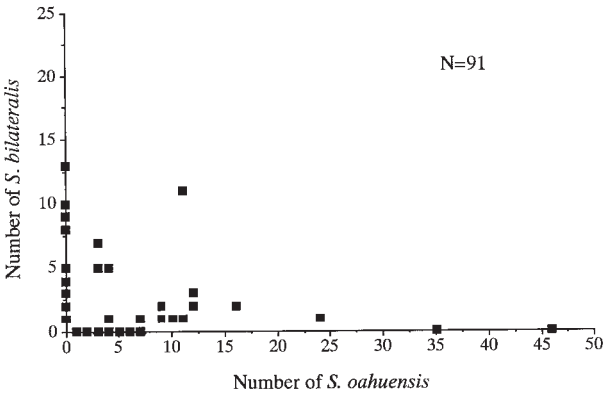

FIG. 5. – Relative abundance of *Staurocladia bilateralis* (vertical) to *S. oahuensis* (horizontal) on each algal piece.

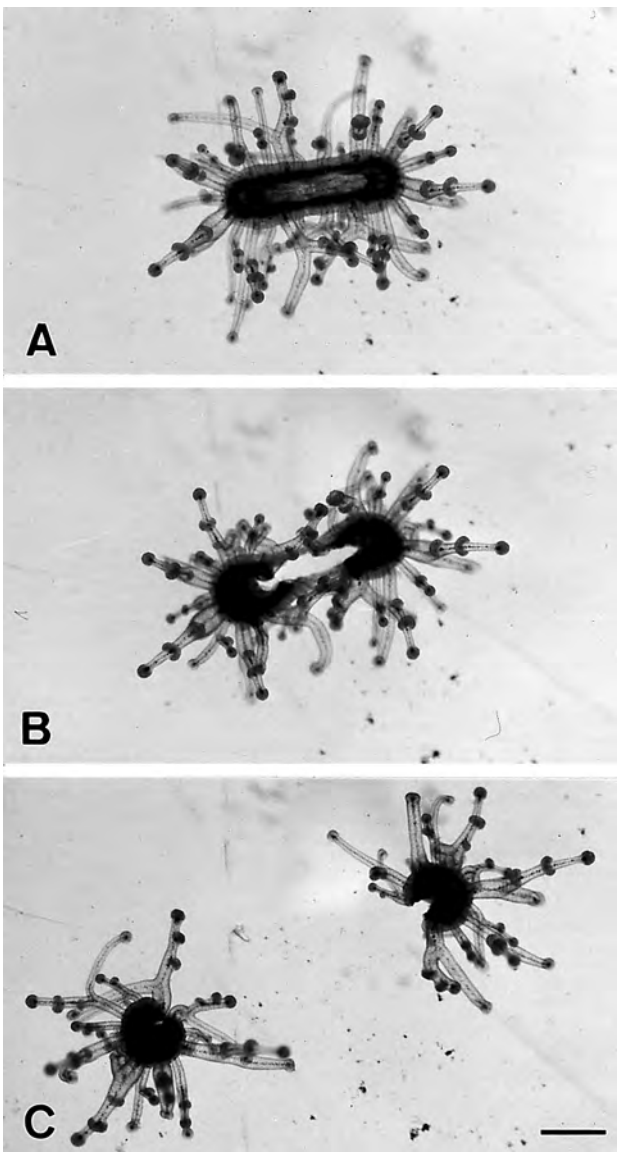

FIG. 6. – Three stages (A, B, C in series of time) of asexual reproduction, illustrated by *Staurocladia oahuensis*. Scale bar, 0.5 mm.

Distribution of two medusae among tidepools and algae

Six out of the eight upper tidepools were found to be harboring crawling medusae on both occasions, as were the "Lower" pools (Table 1). The two species were found together in lower pools such as T-5, T-6 and those grouped together as "Lower", while higher pools were often found to be occupied by a single species alone.

More than half of the investigated algal pieces were found to be devoid of medusae (Table 1), and the majority of the algae inhabited by medusae was occupied by only a single species (Fig. 5) in both investigations. The number of the algal pieces with two species in a mixture was only 2 and 13 in the first and second investigations, respectively. No correlation was detected between the abundances of the two species of medusae (r=-0.103, P=0. 329)

Asexual reproduction

Both medusae reproduced asexually by fission. A medusa elongated and the bell became very thin, especially in the central part (Fig. 6A). In the course of this bell elongation, a hole appeared at the center, and it gradually enlarged leaving only narrow marginal parts to be barely connected (Fig. 6B). Finally the medusa pulled completely apart, dividing into two daughter medusae each with an indentation on one side (Fig. 6C). The indentation was filled in and the bell became rounded within one day.

Rate of asexual reproduction

The rate of asexual reproduction was affected by temperature and also by the nutritional state of medusae. Both species were able to perform asexual reproduction at 17°C and higher temperatures, but not at all at 12°C (Fig. 7). Reproduction rate, however, did not show a clear temperature-dependent increase at temperatures higher than 17°C in either species. When starved, medusae reproduced only for a few days in most cases, and reproduction virtually ceased thereafter in both species (Fig. 7, A and C). A decrease in numbers by death of some specimens occurred on several occasions, although death was a rather rare event during the nine days of starvation for both species.

On the other hand, when fed, all specimens of *S. oahuensis* and half of *S. bilateralis* died at 27°C (Fig. 7, B and D) within three days. *S. bilateralis*,

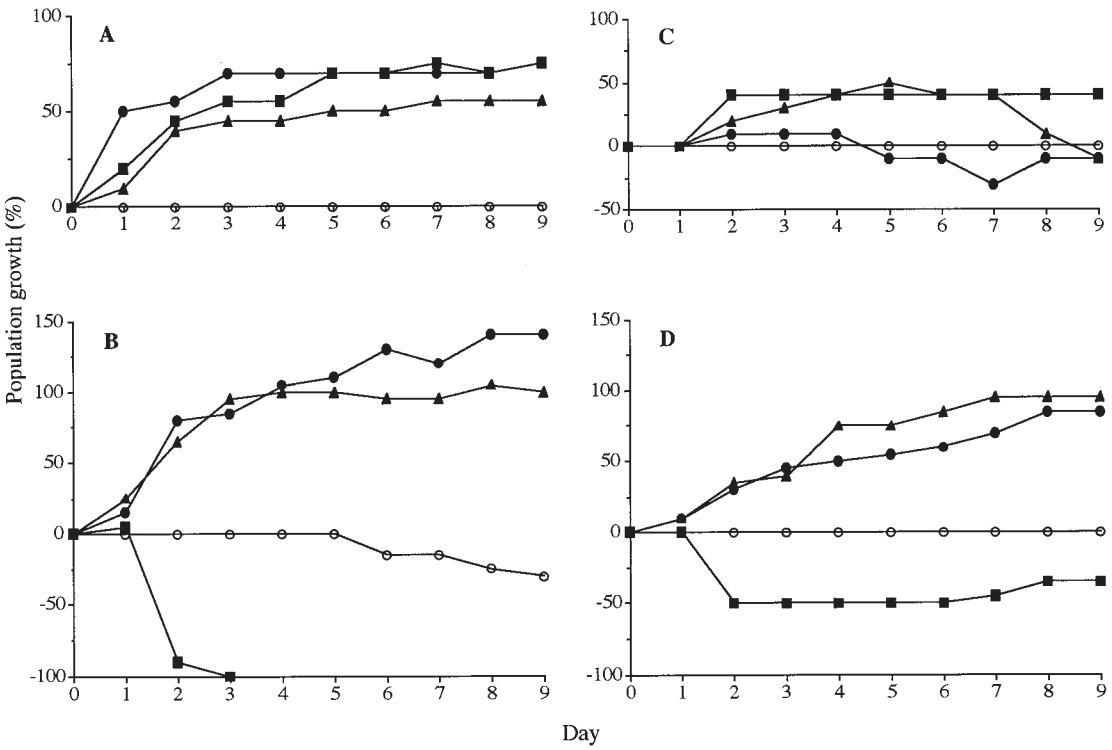

FIG. 7. – The rate of population growth under different experimental conditions. A: *Staurocladia oahuensis* under starvation. B: *S. oahuensis* given food. C: *S. bilateralis* under starvation. D: *S. bilateralis* given food. The experiment was done at four different temperatures, 27°C (n), 22°C (s), 17°C (l) and 12°C (m) for each group.

however, did not decrease further in number, and the survivors showed some population growth afterward at the same temperature. At the lower temperatures *S. bilateralis* given food did not die at all, whereas some of *S. oahuensis* died at lower temperatures as well. Both species continuously reproduced asexually when given food at 22°C and 17°C, where in nine days the experimental populations grew on average by 90% in *S. bilateralis* and by 120% in *S. oahuensis*.

At the beginning of the experiment, the size distribution of individuals was not different among all experimental groups both in *S. oahuensis* (ANOVA, P=0.584) and *S. bilateralis* (ANOVA, P=0.647). After nine days both species showed significant size decrease at all temperatures when starved (Table 2). Even when given food every other day, *S. oahuensis* medusae significantly decreased in size at all temperatures, and *S. bilateralis* decreased in size at 27°C and 12°C. At 22°C and 17°C, however, *S. bilateralis* grew significantly during the nine days of experiment. The bell diameter of medusae did not differ between starved and fed groups in *S. oahuensis* at any temperature, while it was significantly larger in groups given food than in starved medusae in *S. bilateralis* at all temperatures (t-test with Welch's correction for all, $P<0.001$ for 27°C to 17°C and $P<0.05$ for 12°C).

DISCUSSION

Being the smallest members of the genus, *Staurocladia oahuensis* and *S. bilateralis* would be easily overlooked in the field, and might be mistaken for a juvenile form of some larger species even when found. We discovered some *S. bilateralis* specimens in a collection of *S. vallentini* deposited in the Showa Memorial Institute, National Science Museum, Tsukuba. The *S. bilateralis* specimens were collected in Misaki, Sagami Bay, in 1936. We visited the place on July 9, 1998, and confirmed the occurrence of *S. bilateralis*, and there we also found *S. oahuensis*. A medusa assignable to *S. oahuensis* has been found in a filtration tank in a fish farm in Hota, Tokyo Bay (Oikawa, 1995), and we have collected *S. bilateralis* at Yobuko, Kyushu, too. *S. oahuensis* and *S. bilateralis* seem to be widely distributed in warm waters in Japan.

We have also looked for polyps of *S. oahuensis* and *S. bilateralis* in the field, but with no success. Polyps of the genus *Staurocladia*, where known, have oral capitate tentacles and some filiform tentacles in the middle, are up to a few millimeters in height, and form stolonal colonies (Gilchrist, 1919; Lengerich, 1923; Prévot, 1959; Kakinuma, 1963; Brinckmann, 1964; Hirohito, 1988; Schuchert, 1996). It should be very difficult to find them in the field. We have also not been successful in obtaining planulae of the two species to raise them to polyps in the laboratory. Thus polyps of the two species are still unknown. B. Schierwater similarly did not locate any polyps of *Eleutheria dichotoma* during field collections of that species of crawling medusa in the Mediterranean Sea (personal communication, Bodega Bay, CA, 1998).

Staurocladia oahuensis and *S. bilateralis* in Kominato were observed to reproduce asexually by fission as described by Edmondson (1930) for Hawaiian specimens. In Hawaiian specimens, however, division into two daughter medusae was initiated by a constriction on the central part of the mother medusa (Edmondson, 1930). There may be slight differences in the details of asexual reproduction between localities, but Edmondson (1930) seems to have inappropriately used the word "budding" for the mode of asexual reproduction. This is probably why budding has been known as the mode of asexual reproduction for *S. bilateralis* (Bouillon, 1978).

A high rate of population growth by asexual reproduction in both species was demonstrated by the experiment; the rate is comparable to that of *Eleutheria dichotoma* by budding (Schierwater and Hauenschild, 1990). Low temperature proved to be a critical inhibitory factor for asexual reproduction for both species of *Staurocladia* (Fig. 7), and probably plays an important role in the mid-winter disappearance of the two species (Fig. 1). The lowest tides in Kominato occur at night during winter so that medusae living in tidepools may often be subjected to lower air temperatures than the ambient sea water low temperatures of 12°C.

Low temperature not only inhibits asexual reproduction but also seems to have a bad effect on the medusae themselves. At 12°C several medusae of *S. oahuensis* died during the last few days of the experiment (Fig. 7). *S. bilateralis* specimens decreased in size even when fed at 12°C, despite no dividing event, whereas at 17°C and 22°C specimens grew while performing fission (Table 2). Harada (1954) reported that medusae of *Staurocladia acuminata* (Edmondson, 1930) became inactive with tentacles shrinking at about 10°C. Such low temperatures

may produce great stress in species also capable of living in the tropical waters in Hawaii. The decrease of bell size in the field population in December (Figs. 3 and 4) may also be attributable to the effect of low temperature on these crawling medusae.

High temperature may also provide great stress for both species, as suggested by the mass mortality of both medusae at 27°C when they were fed. The seawater temperature in Kominato often reaches this value in shallow water from summer to early autumn, when temperatures can attain even higher values in tidepools that are well separated from the sea for long daytime periods under high radiation. Crawling medusae living in such pools may often experience temperatures higher than 27°C. In fact we have found many medusae in tidepool water as warm as 35°C. This implies that both species of medusae can tolerate temperatures higher than 27°C; death in the experiment may have been caused by deterioration of the culture water in the small containers under an oversupply of food.

As mentioned above, the mid-winter disappearance of the two medusae seems to be a seasonal event, although the brief disappearance in early October, 1995, cannot be similarly explained. It is impossible to specify its cause by this study, but one possibility is a big typhoon (940 hPa) that hit Kominato on September 17 of that year. The coast of Kominato is very susceptible to high waves generated by typhoons. Typhoons are often accompanied by heavy rain, as the September 1995 typhoon was. Wave action and precipitation are among the main disturbances for shoreline animals, and they may cause large temporary damage to populations of crawling medusae in Kominato.

Tidepools are subjected to various disturbances, and both species of crawling *Staurocladia* medusae often occur in abundance in such pools. The crawling medusae are also found on algae growing subtidally, but not in such abundance as in tidepools. These medusae are both small in size, and can achieve rapid population growth by asexual reproduction. These features show that the medusae are suited to living in tidepools (Emson, 1985). Furthermore, pool-dwelling might provide an advantage to the medusae. Hadrys *et al*. (1990) suggested an increase in the risk of the crawling medusa *Eleutheria dichotoma* becoming detached by water currents during feeding. Crawling medusae are able to firmly adhere to the substrate with their tentacles, but when walking and feeding, some tentacles leave the substrate and they become less resistant to water currents. When well separated from the sea, tidepools provide much less turbulent water where the crawling medusae can feed and probably perform fission more easily. Higher population growth may thus be achieved in tidepools than in the subtidal zone.

S. oahuensis and *S. bilateralis* occur sympatrically in Kominato, but looking at the distribution on smaller scales, the two medusae are rarely close neighbors (Fig. 5). This is especially true at higher intertidal levels where tidepools are often occupied by a single species of *Staurocladia* (Table 2). Because of a longer time of separation from the sea, these upper pools probably receive fewer immigrants, which may facilitate such exclusive occupancy. In contrast, crawling medusae should meet more frequent dispersal events at lower intertidal levels, where well-mixed populations of the two medusae are formed. But even in lower pools, the two species do not share the same algal blade very often.

As there is no negative correlation between the numbers of the two species living on a same algal piece, competitive interaction is unlikely to be the determinant of this spatial segregation. Even when they are directly touching, two individuals of different species or of the same species showed no aggressive behavior, and they remained fairly immobile even in crowded containers in the laboratory. The possibility of interference between the two medusae seems very low. As the two species both prey mainly upon harpacticoid copepods (Y.M. Hirano, unpublished data) they may compete over food on some crowded algae. However, the population density of medusae seems to be not very high on most algal pieces, and a number of algae are left uncolonized. Exploitative competition between the two species of crawling medusae is also unlikely in such environment.

Many forces of disturbance in tidepools might prevent either species from constantly competing with the other, and would permit *Staurocladia oahuensis* and *S. bilateralis* to live together even without significant niche differentiation. In fact, the two species are similar in ecological requirements such as habitat algae, seasonal occurrence and prey, although they may differ in life history strategies. The experiment suggested that *S. oahuensis* may reproduce at a higher rate than *S. bilateralis* with the bell diameter decreasing even when given food. In contrast, *S. bilateralis* increased in size while performing fission at a moderate rate, and seemed to

have higher tolerance to temperature extremes. These differences may play some role in further facilitating the coexistence of the two crawling medusae. Tidepools experience wide fluctuations of physical variables such as temperature and dissolved oxgen (Huggett and Griffiths, 1986). In such habitats *S. oahuensis* may predominate over *S. bilateralis*, thanks to its high rate of reproduction, under favorable conditions, while *S. bilateralis* may be more competitive because of its robustness under less favorable conditions.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

The hydroid and medusa of *Sarsia bella* sp. nov. (Hydrozoa, Anthoathecatae, Corynidae), with a correction of the "life cycle" of *Polyorchis penicillatus* (Eschscholtz)*

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SUMMARY: A new hydrozoan, *Sarsia bella* sp. nov. is described in both its hydroid and medusa stage from north of Puget Sound, Washington in the San Juan Islands, USA and off the southernmost tip of Vancouver Island, Canada. The medusa is distinguished from other *Sarsia* species by 16 exumbrellar nematocyst patches and in being more transparent or "glass like" when living than any other known species of the genus. The exumbrellar nematocyst patches become indistinct in mature specimens and in those crowded in culture, with single nematocysts increasingly spaced out. The hydroid, both field-collected and raised in culture from its medusa, forms small, upright stolonal colonies not more than 1.5 mm high. The hydranths bear an oral whorl of four to five capitate tentacles, and immediately below a second whorl of slightly shorter capitate tentacles. In thriving colonies there is occasionally a whorl of small filiform tentacles on the lower part of the hydranth. Medusa buds develop in the middle of hydranth below the capitate tentacles and above the reduced filiform tentacles, if present. Young medusae are liberated with the typical 16 exumbrellar nematocyst patches. The hydroid of this species was originally mistaken for the hydroid of *Polyorchis penicillatus*. Brinckmann-Voss (1977) reported a small corynid hydroid living on the margin of rock scallop shells. Medusae liberated from this hydroid were at that time believed to be those of *Polyorchis penicillatus* (Eschscholtz) present in the plankton. Immature medusae of these two species appear strikingly similar, especially with regard to their exumbrellar nematocyst patches, four tentacles and abaxial ocelli. Since then however, this connection has been proven wrong, because an identical hydroid was raised from the medusae of the new species *Sarsia bella*. Second generation medusae raised in the laboratory were carefully compared with medusae liberated from field collected hydroids (thought to have been *Polyorchis penicillatus*), and these were found to be identical with meduse of *Sarsia bella*. Young medusae of *P. penicillatus* from the plankton can be clearly distinguished from *S. bella* medusae by the number of their exumbrellar nematocyst patches. Both *P. penicillatus* and *Sarsia bella* have eight adradial rows of exumbrellar nematocyst patches when young, however each row in *P. penicillatus* consists of at least three vertically alligned patches whereas each row never has more than two patches in *S. bella*. In both species the patches consist of microbasic p-mastigophores, but capsules in the case of *P. penicillatus* are larger than those in *S. bella*. Later stages of the two species are easily distinguished using other morphological characters with only four tentacles in *S. bella* and more than four in *P. penicillatus*. No hydroid of the genus *Polyorchis* has been described to date.

Key words: Leptolida; Anthoathecatae; Corynidae, *Sarsia*; Polyorchidae, *Polyorchis*.

INTRODUCTION

The taxonomy of the genus *Sarsia,* especially from the North East Pacific, has been problematic for a long time (Arai and Brinckmann-Voss, 1980; Mills, 1982; Brinckmann-Voss, 1985). Only those species of *Sarsia* having medusae with a long manubrium, treated by Miller (1982) as belonging to the "tubulosa complex" will be considered here. The medusa stage of this species complex - common in the eastern section

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of the Juan de Fuca Strait and the San Juan Islands (Fig. 1), occurs in different forms either considered "species," "subspecies" or "morphotypes" (Miller, 1982) in the same habitat. Miller's paper dealt with the medusa stage and first cleavage of their embryos only. Brinckmann-Voss (1985) followed the development of some of Miller's "types" to the hydroid and next medusa generations comparing them with additional field-collected hydroids. Some of Miller's results were confirmed, while certain morphotypes were definitely assigned to valid species. However, one of Miller's morphotypes, designated by him as the "L" type on account of the large eggs in the females, proved to be a separate, yet undescribed, species. Based on both young and adult specimens, or its hydroid raised from the medusa in the laboratory, and on observation of field-collected hydroid material, this species is described here as *Sarsia bella* sp. nov. The formerly mistaken connection (Brinckmann-Voss, 1977) of the hydroid of this new species with *Polyorchis penicillatus* will be discussed and corrected.

Sarsia viridis (see Brinckmann-Voss, 1980) will not be discussed in this paper. Although it is sympatric with *Sarsia bella* and the other species of the genus listed in Table 1, it can be easily distinguished from them morphologically by its small size and persistant green colour. In addition, *Sarsia viridis* is very rare and more information, especially about the hydroid, is needed.

MATERIAL AND METHODS

Sarsia bella medusae were collected regularly from floats in Friday Harbor, Washington, Becher Bay, and occasionally in the harbour of Sooke, British Columbia (Fig. 1). Females and males were placed in pairs in small custard cups to spawn. Filtered sea water was used, being at least three days old to avoid contamination with sperm from other *Sarsia* medusae in the field. As an additional control, female medusae alone and their eggs were observed. Embryos developed from the mating pairs were left in the same container for observation of settlement and development of the hydroids. Primary hydranths were raised to colonies as described in Brinckmann-Voss (1985). As the hydranths of this species have a tendency to regress if not aerated, leaving only the hydrorhiza, small stones with barnacles from the intertidal were added to the colonies. This stirring by natural feeding action of the barnacle cirri was preferable to simple aeration

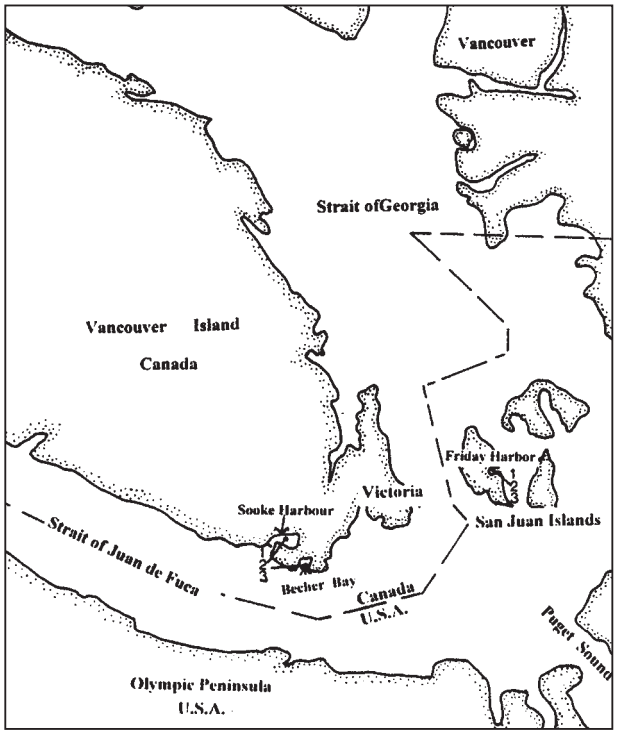

FIG. 1. – Main collection sites of the various *Sarsia* species being sympatric with *Sarsia bella* sp. nov. 1: *Sarsia bella* sp. nov.; 2: *Sarsia apicula* (Murbach and Shearer, 1902); 3: *Sarsia princeps* (Haeckel, 1879).

with air stones because nauplii released from the barnacles acted also as perfect-sized prey for the small *Sarsia bella* hydranths. The hydroid cultures were kept at 5-10°C in an unheated room during the winter months, and outside or in a refrigerator during the summer (the research region of southern Vancouver Island region has cool summers with night temperatures rarely above 15ºC.)

Field collected hydroids were collected from the outer and inner margin of rock scallop shells, *Hinnites multirugosus* (Gale) (syn. *Hinnites giganteus* Grey) from Departure Bay, British Columbia.

TAXONOMIC ACCOUNT

Sarsia bella sp. nov.
(Figs. 2-6)

Type material: Holotype ROMIZ B3124, adult male medusa, 9 May, 1995, Becher Bay, off Vancouver Island, B.C. Canada, surface. Paratypes: ROMIZ B3125, male and female adult field collected medusae, 10 May,1995; Becher Bay, off Vancouver Island, B.C. Canada; surface ; with their hydroids and second generation medusae cultured. Paratype: RBCM 999-381-1; immature and mature medusae; 9 May, 1995; Becher Bay, off Vancouver Island, B.C.,Canada; surface.
RBCM: Royal British Columbia Museum, Victoria, British Columbia, Canada
ROM: Royal Ontario Museum, Toronto, Ontario, Canada

Etymology: The new species was named *Sarsia bella* in reference to the delicate glass-like bell of the medusa.

Diagnosis: Medusae with 2 vertically alligned nematocyst patches in each of eight exumbrellar adradii; clearly visible in young specimens; individual cnidocysts more widely spaced and diminishing in numbers when medusae are crowded in culture or reaching maturity (compare Fig. 2 with Fig. 5). Gonads in distal half of manubrium only; female medusae with larger eggs than other *Sarsia* (Miller 1982, Table 1); Reddish (never blue) manubrium and marginal bulbs. Hydroids *Sarsia*- like, upright short stolonal colonies; hydranths emerging directly from hydrorhiza without distinct hydrocaulus; with feeble perisarc around base of hydranths; hydranths small, maximum length 1.5 mm (measured from hydrorhiza); with oral whorl of 4-5 tentacles, and second whorl with shorter tentacles just beneath the oral one; with or without minute filiform tentacles.

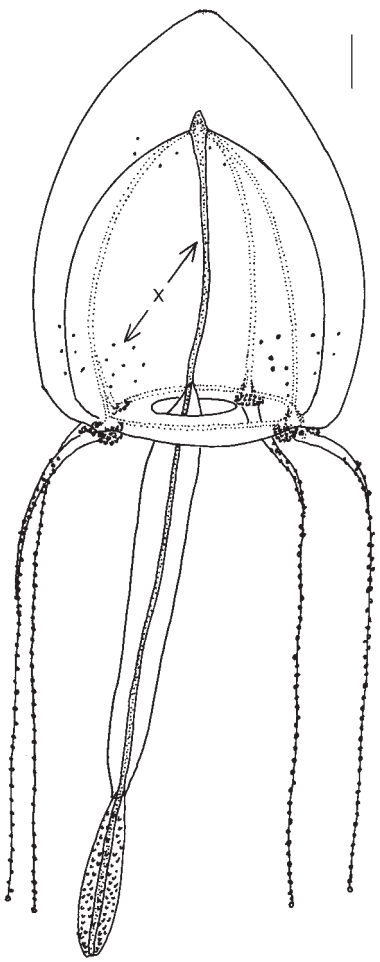

FIG. 2. – *Sarsia bella* ; adult male medusa Becher Bay; 0 m; x: specific characters: gonad free part of manubrium and exumbrellar cnidocyst patches. Scale = 1.0 mm

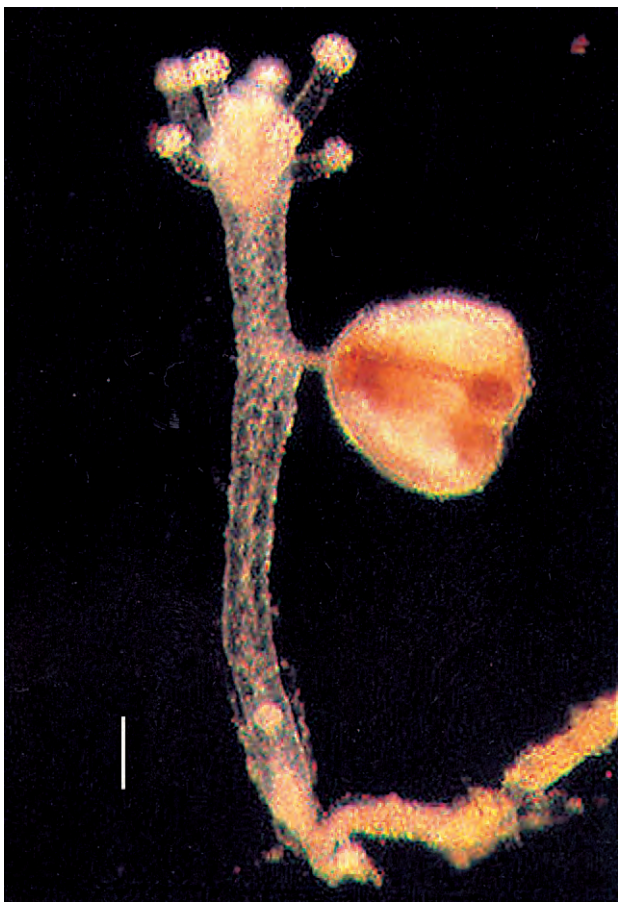

FIG. 3. – *Sarsia bella,* hydroid with medusa bud raised from medusae in the laboratory; tentacles slightly contracted. Scale = 0.1 mm.

Description of medusa (Fig. 2): Living adult medusa with rounded to conical bell reaching a maximum 9 mm high, and 7.5 mm wide; with exumbrella thicker apically than laterally; with short, conical apical canal; with exumbrellar cnidocyst patches faintly visible or absent; with individual cnidocysts more widely separated than in young specimens; manubrium nearly three times as long as exumbrella, with gonads encircling distal part of manubrium except stomach leaving about proximal half of manubrium gonad free. Four marginal bulbs with abaxial ocelli , but without abaxial spurs; tentacles with cnidocyst clusters scattered proximally, becoming more moniliform distally.

Hydroid raised from medusa (Fig. 3): stolonal short colonies not more than 1.5 mm high with hydranths rising directly from a creeping net-like hydrorhiza without clear separation of hydranths and hydrocaulus (terminology used after Millard

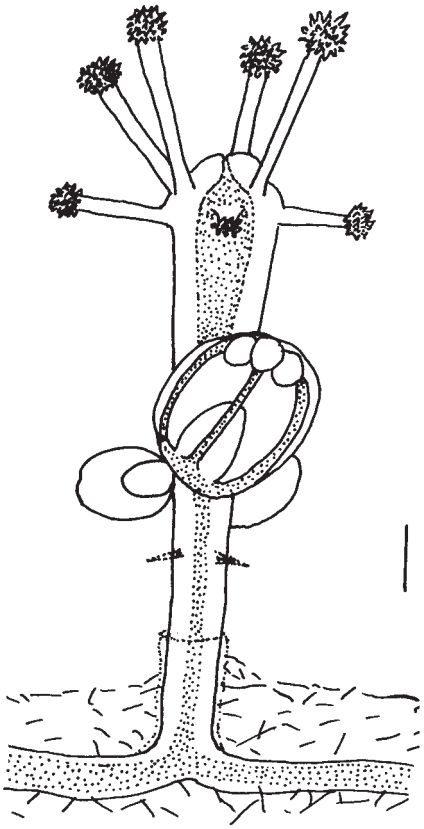

FIG. 4. – *Sarsia bella,* after sketch from living hydroid from field-collected rock scallop. Small filiform tentacles below medusa buds only occasionally present. Scale = 0.1mm

1975, Cornelius 1996); with an oral whorl of 4-5 short capitate tentacles, each not more than 0.4 mm long; with tentacles of second whorl half the length of the oral ones; maximum thickness of oral tentacle bulb 56 μm, 40 μm in lower tentacles; usually only 10 endodermal cells in oral tentacles; up to five medusa buds in middle of hydranths; occasionally (Fig. 4) four small, reduced filiform tentacles in area below medusa buds, these present in thriving and relaxed colonies only; usually absent as in Figure 3.

Hydroids from field-collected material (Fig. 4) on the shell margin of rock scallops appear identical to hydroids raised from the medusae in the laboratory, except for the hydrorhiza and proximal part of hydranth being imbedded in an incrusting sponge which often covers part of the margins of rock scallops.

Medusae liberated from their hydroids are 1 mm high and 1 mm wide. Exumbrella with 16 patches of nematocysts - two per each of eight adradii (Fig. 5); each patch consisting of 6-11 densely packed microbasic p-mastigophores. During growth of the exumbrella, the nematocysts become more scattered or widely separated and often disappear as the medusae mature; the upper patches tend to disappear before the lower ones. During development, the manubrium getting longer than the subumbrella and gonads develop. Initially when the manubrium has not reached its full length, gonads seem to be thicker distally than proximally, so that the manubrium appears spindle-shaped in juvenile specimens; but in mature medusae, the gonads are limited to the distal part of the manubrium, leaving its proximal part gonad-free. In alcohol-preserved specimens, mature medusae measure between 5.8/5.0 mm and 8.0/7.6 mm in height/diameter. Diameter of eggs is 129 μm (see Miller 1982 for *Sarsia* "L"). In the present study the egg diameter is slightly less, 110-120 μm, but still considerably larger than those of the sympatric species *Sarsia apicula* (Murbach and Shearer 1902) (as *Sarsia* "S" in Miller, 1982; see Discussion).

The ciliated planula settles on the bottom of glass dishes and primary hydranths were observed 10 to

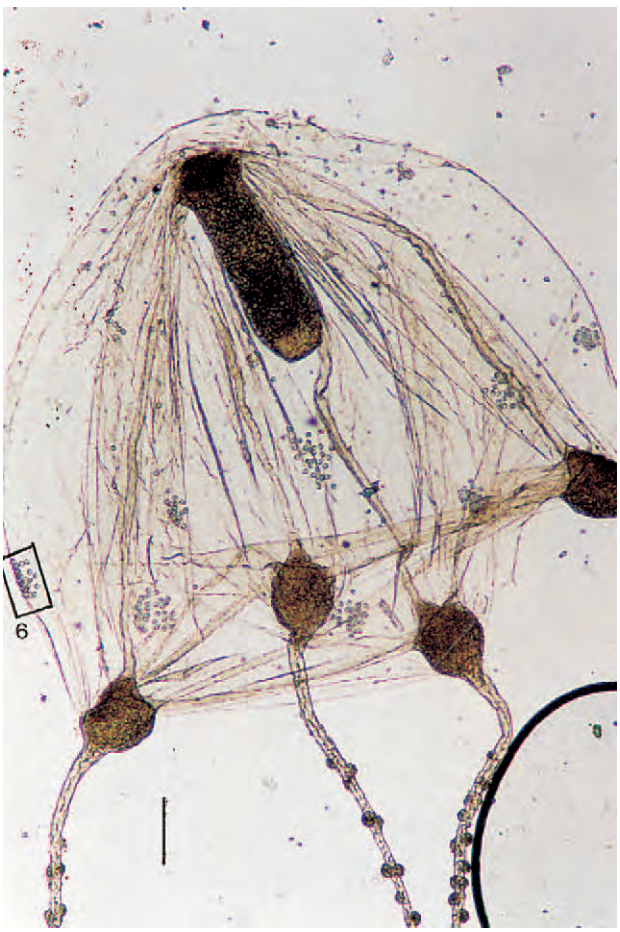

FIG. 5. – *Sarsia bella,* one day old medusa liberated from hydroid. The box at the left indicates nematocyst cluster shown enlarged in Figure 6a. Scale = 0.09mm.

FIG. 6. – *Sarsia bella;* a. enlargement of box insert from Figure 5: magnified: part of bell of young medusa with group of microbasic p mastigophores photographed at kink of exumbrella, unexploded ; b. hydroid: large isorhiza with 2 adjacent stenoteles. Scale a,b = 10 μm.

14 days after spawning. In contrast to this new species, the planulae of *Sarsia apicula* develop into primary hydranths after only 48 hours.

Medusa buds develop between March and May in cultures kept at about 8-12°C. The field-collected hydroids off the British Columbia coast were found with medusa buds in the beginning of March at a sea-water temperature of 9°C.

Nematocysts (all measurements are in μm): *Sarsia bella* medusae: stenoteles undischarged 9-13×7-9; desmonemes undischarged 7-9×4-5; microbasic p-mastigophores11-12.5×8-10; Hydroid: stenoteles undischarged 12-18×7-12; homotrichous isorhizas 14-15×5-7.

Remarks: although stenoteles in other species of *Sarsia* typically appear to be in two size groups (Brinckmann-Voss, 1985, 1989; Calder, 1988; Kubota and Takashima, 1992; Schuchert, 1996) these two size ranges are less distinct in *Sarsia bella*.

Distribution: the medusa stage of *Sarsia bella* has been found occasionally in Sooke, frequently in Becher Bay and Friday Harbor (Fig. 1). The hydroid was collected in Departure Bay off Nanaimo, B.C. Although intensive collecting was done in Departure Bay, *Sarsia bella* medusae were not found there.

DISCUSSION

Generic distinctions within the Corynidae have been under discussion for a number of years. Petersen (1990), with the help of cladistic methods, improved earlier concepts by trying to arrange the family into three genera (*Sarsia, Coryne* and *Dipurena)* according to different characters of hydroid and medusae. Although *Sarsia bella* fits Petersen's definition of the genus *Sarsia,* some of the characters used by him seem to be unreliable as a generic distinction: these include shape of of marginal bulbs of the medusae and position of medusa buds on the hydroids (author's personal observation; Kubota and Takashima 1992). Additional characters such as morphology of the tentacles in the hydroid, should also be considered to define the three genera.

Sarsia bella is one of the two common "sibling"species of the genus *Sarsia* which occur in certain bays off southern Vancouver Island and Friday Harbor. Miller (1982) considered these belonging to a *Sarsia tubulosa* complex, but recent hybridization experiments between Friday Harbor and Becher Bay specimens and subsequent raising of the primary hydranths of both forms (Brinckmann-Voss, work in progress) reveal that Miller's Friday Harbor "S" type is actually *Sarsia apicula* (Murbach and Shearer, 1902) and not a morphotype of *Sarsia tubulosa* (M. Sars, 1835) as suggested in Figure 4 of his paper (Miller, 1982, p.161). The species *Sarsia tubulosa* (M. Sars, 1835) is present in Sooke Harbour, but much rarer or absent in Friday Harbor, where Miller did his work; Miller's "L" type is, as he suggested, a separate species described above as the new species *Sarsia bella*. Although Miller reported up to 37.7% successful early cleavage stages in his hybridization experiments of *Sarsia bella* sp.nov. and *S. apicula* (Murbach and Shearer,1902) (S and L morphotype in Miller, 1982), recent hybridization

TABLE 1. – Different *Sarsia* species sympatric with *Sarsia bella* spec. nov. in three locations off the south coast of Vancouver Island, B.C. Canada and San Juan Islands,Wash. USA.

Species	Distinctive Characters		Seasonal Distribution	Location
	Medusa	Hydroid		
Sarsia bella sp. nov. (*Sarsia* "L" in Miller, 1982)	16 exumbrellar dense cnidocyst patches in immature specimens, diminishing in adults; gonads in distal half of manubrium only; egg diameter more than 100μm; sympatric with *S.apicula*, but no hybridization *	small, less than 1.5 mm, no distinct hydrocaulus. 2 whorls of capitate tentacles; 2nd whorl smaller than 1st; endodermal cells in oral tentacles not more than 10	Medusa: early May to mid- June; Hydroid: with medusa buds in March	Medusa: Friday Harbor Labs floats abundant; Becher Bay abundant; Sooke, rare. Hydroid: on margin of live rock scallop shells.
Sarsia apicula (Murbach and Shearer, 1902) (*Sarsia* "S" in Miller, 1982)	scattered exumbrellar cnidocysts in immature specimens, none in adults; gonads entire length of manubrium, leaving only most proximal part free; egg diameter less than 100μm	large, 2 mm or longer; distinct hydrocaulus of various lengths; 3 whorls of capitate tentacles; more than 15 endodermal cells in oral tentacles	Medusa: early May to mid-July;	Medusa: Friday Harbor off floats; Becher Bay; Sooke; common in all three locations. Hydroid: intertidal
Sarsia princeps (Haeckel, 1879) (*Sarsia* "P" in Miller, 1982)	exumbrellar cnidocysts in 8 loose exumbrellar patches plus scattered exumbrellar cnidocysts in liberated medusa; none in adult; gonads entire length of manubrium, leaving only most proximal part free; egg diameter less than 100 μm; more pointed exumbrella than any other *Sarsia*	hydroid slender, two capitate tentacle whorls; hydrocaulus clearly separated from hydranth	Medusa: May	Medusa: Friday Harbor off floats; Becher Bay; Sooke; not abundant in all three locations. Hydroid on live swimming scallop shells.
Sarsia tubulosa (M. Sars, 1835) and *Sarsia tubulosa*, small blue variety (*Sarsia* "B" in Miller, 1982)	this species and its varieties are either spatially or temporally separated from the new species *Sarsia bella* (Brinckmann-Voss 1985, and work in progress)			See Discussion

*In this paper hybridization means development from heterozygotic matings not just to first cleavage stages but to the primary hydranths, and subsequent formation of colonies.

experiments (Brinckmann-Voss,A. work in progress) between the two species - or heterotypic matings of the two morphotypes as phrased by Miller (1982, p. 163) - did not result in any primary hydranths. Instead embryos from homotypic matings developed into primary hydranths about 90% of the time (Brinckmann-Voss, A. work in progress). Furthermore, *Sarsia bella* can be morphologically distinguished from a small blue *Sarsia*, " B" type in Miller (1982), collected in Parks Bay, Shaw Island (San Juan Islands), and considered by him belonging to the same "tubulosa" morphotype. In addition to their morphological distinction in the medusa and hydroid stage, both species are spatially or temporally separated from each other. *Sarsia"* P" type (Miller, 1982), or *Sarsia princeps* (Haeckel, 1879), can be easily distinguished from *Sarsia bella* (Table 1) by the morphology of the medusa as well as the hydroid (Arai, and Brinckmann-Voss, 1980; Brinckmann-Voss, 1985).

The hydroid now identified as *Sarsia bella* which occurs on the rim of the shells of the rock scallop, *Hinnites giganteus,* was mistakenly reported as the hydroid of *Polyorchis penicillatus* Eschscholtz in an earlier paper (Brinckmann-Voss, 1977). The mistake happened because of the similarity between newly-liberated medusae from the hydroid living on rock scallops and the youngest stages of the medusa *P. penicillatus* collected separately from the plankton. Although both species look strikingly similar in their youngest medusa stage, they can be easily distinguished from each other: S*arsia bella* never has more than two vertically alligned nematocyst patches on each of the 8 adradii as shown earlier (Fig. 5

present paper and Brinckmann-Voss, 1977, Fig. 2 mistakenly as *P. penicillatus*), whereas the youngest *P. penicillatus* - known from plankton only - has three or more (Mills, 1976, Fig. 2.7; Brinckmann-Voss, 1977, Fig. 3), and as described for the similar *Polyorchis karafutoensis* (Nagao, 1970). Nematocysts on the exumbrellar patches of the young medusae are microbasic p-mastigophores in both species, but they are nearly one-third larger in *P. penicillatus* than in *S. bella* . The hydroid of *P. penicillatus* is still not known, nor is any hydroid of its family, the Polyorchidae.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Ecological characteristics of the Mljet Island seawater lakes (South Adriatic Sea) with special reference to their resident populations of medusae*

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SUMMARY: Ecological properties and distribution and abundance of medusae were studied over an 18-month period in the Mljet Island seawater lakes, south-east Croatia. Strong stratification during the summer differentiates these lakes from the oligotrophic South Adriatic ecosystem. The lakes are designated as a moderately eutrophicated ecosystem. Very small numbers of hydromedusae were noted, representing only the Anthomedusae and Leptomedusae. A new species of the genus *Tima* was found in considerable numbers of individuals. High abundance of the scyphomedusa *Aurelia* sp. was observed throughout the year. This species differs in terms of genetic divergence from *Aurelia aurita* found elsewhere in the Mediterranean and could be attributed to the boreal origin.

Key words: seawater lake, stratification, medusae, *Tima*, *Aurelia*, South Adriatic.

INTRODUCTION

The island of Mljet is an offshore south Adriatic island that extends in a NW-SE direction (Fig.1). The 8-10 km wide Mljet Canal separates it from the mainland. As the southernmost island in the Adriatic, Mljet is directly exposed to the incoming Ionian sea current and is influenced by it (Zore-Armanda *et al*., 1991; The POEM Group, 1992). Plankton community structure and density values in the south Adriatic correspond to the general values for oligotrophic eastern Mediterranean waters (Viličić, 1985, 1991; Benović and Lučić, 1996; Kršinić, 1998; Hure and Kršinić, 1998).

The seawater lakes "Veliko Jezero" and "Malo Jezero" are located on the western part of Mljet Island and are connected to the surrounding sea from the south. They are natural phenomena of karstic depressions that were filled by seawater about 4200 years B.P. (M. Juračić and V. Onofri, unpublished) and 7000 years B.P. (Seibold, 1958; Schmidt, 1993), respectively. The first scientific data on the marine fauna and hydrographic conditions of the Mljet lakes were published in 1935 (Ercegović, 1935). More intensive research was conducted in the period from 1951 to 1955 and from 1985 to 1986 (see: Vučetić, 1995; Benović and Onofri, 1995).

Previous scientific research in the Mljet lakes only partially included data on medusae and made no attempt to estimate their importance in structuring the plankton communities. When present in high numbers, the scyphomedusa *Aurelia aurita* has been shown to have a significant impact on the structure

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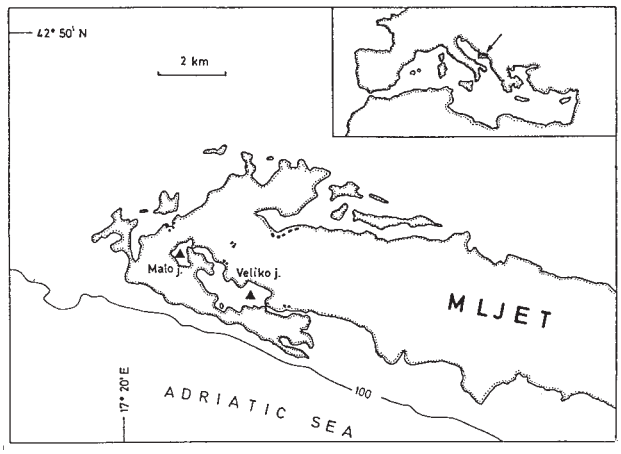

FIG. 1. – Map of the seawater Mljet lakes "Veliko jezero" (VJ) and "Malo jezero" (MJ). The black triangles indicates the deepest locations (46 m and 29 m, respectively), which in VJ is also the most frequent swarm site for *Aurelia* sp.

of coastal planktonic communities by its predation on zooplankton (Shushkina and Musayeva, 1983; Behrends and Schneider, 1995; Olesen, 1995; Omori *et al*., 1995; Lucas, 1996**;** Ishii and Bamstedt, 1998). The present paper describes the physical, chemical and plankton characteristics of the Mljet seawater lakes ecosystems and relates them to the large and persistent populations of *Aurelia* sp. and to other medusae present there, particularly an unusual large, unidentified species of *Tima*.

MATERIALS AND METHODS

The study reported here was carried out from June 1997 to January 1999 in the two seawater lakes of Mljet Island. The "Veliko Jezero" (VJ) has a surface area of 1.45 km^2 and maximum depth of 46 m while the "Malo Jezero" (MJ) has a surface area of 0.25 km^2 and maximum depth of 29 m. Narrow and shallow straits (from the outer sea to "VJ": 10 m wide, 2.5 m deep; from "VJ" to "MJ": 3 m wide, 0.5 m deep) connect the lakes with the outer southern Adriatic Sea (Fig. 1).

A multiprobe Hydrolab-Surveyor-3 was used for measuring temperature, salinity and oxygen. Samples of nutrients were collected by water bottles and determined by standard oceanographic methods (Strickland and Parsons, 1972; Grasshoff *et al*.1983). Zooplankton was collected by vertical tows with a 0.125 mm mesh Nansen plankton net and whole samples were analyzed under a stereomicroscope. All samples for plankton analyses were preserved in 2.5% neutralized formaldehyde. SCUBA diving was used during daylight and at night for the field observation and sampling of *Aurelia* sp. and *Tima* sp. Underwater recording was performed using a Camcorder Sony-DCR-VX 1000 E 3CCD DIGITAL with underwater lights and depth indicator. Video editing was performed using a PC Capture Board: Fast DV-Master with frame rates 25 f/s.

For the purpose of this paper, unpublished hydrographic and SCUBA diving data from the 26th to 28th of August 1996 were included in the analysis.

Nutrients data were subjected to the analysis of variance (ANOVA) and SNK multiple range tests.

RESULTS

Temperature ranges in MJ (Fig. 2) were from a minimum of 9.7°C in February 1998 to a maximum of 29.4°C in August 1997. In VJ, temperature (Fig. 3) ranged from the constant minimum of 11.0°C in the bottom layers to a maximum of 28.0°C at the surface in July 1998. During the winter months, isothermal spreadout of values was characteristic, while in summer months a very strong thermal stratification existed in the layers between 10-15 m (MJ) and 15-20 m (VJ). A very pronounced thermocline occurred in July (VJ) and September (MJ) when in only one meter, the temperature dropped 4°C and 6°C, respectively. Other months were characterized by transitive values.

Salinity ranges in MJ (Fig. 2) were between 36.5 psu in the surface layer in May 1998 and 38.2 psu 10m above the bottom in June 1997. However, most values were between 37.0 psu and 37.5 psu. In VJ (Fig. 3) salinity ranged between 36.3 psu and 39.0 psu, but most values are between 37.5 psu and 38.0 psu. High values throughout the entire water column were noted in summer months of 1997. A slight stratification with lower salinity values down to 20 m, and minimum of 36.3 psu at the surface were noted in 1998.

Dissolved oxygen saturation ranged from 4.3% (MJ) and 17% (VJ) in October 1997 near the bottom, to 122% (MJ) and 130% (VJ) in August 1998 in the thermocline layers (Figs. 2 and 3). Stratification occurred during summer and autumn months. In general, dissolved oxygen and most saturation values were between 80% and 110%. Though waters are well-saturated, an anoxic event in VJ was noticed briefly between 26 and 28 August 1996. During that event the strongest thermocline of 7.0°C was observed between 17 and 20 m. In the layer

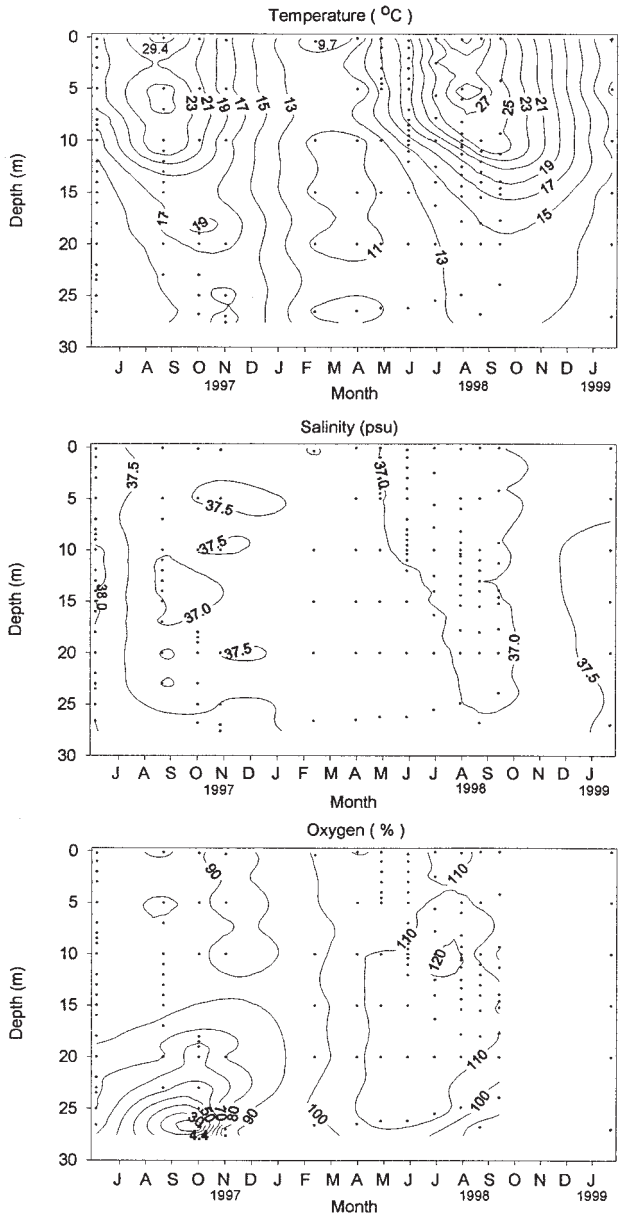

FIG. 2. – Distribution of temperature, salinity and dissolved oxygen in "Malo jezero" (MJ) from June 1997 to January 1999.

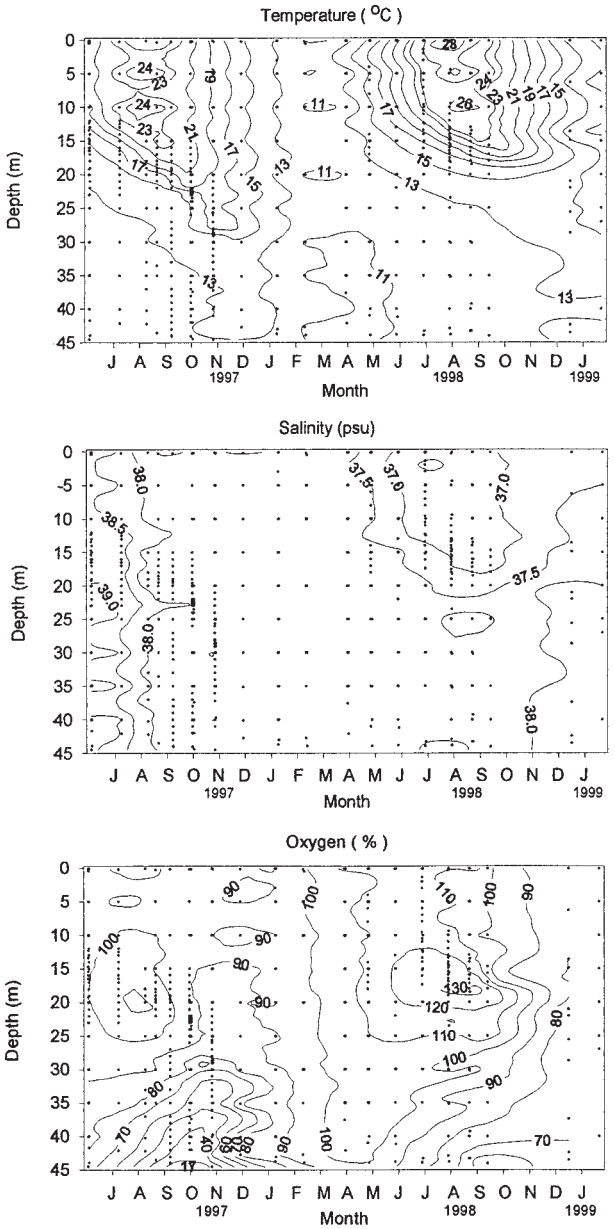

FIG. 3. – Distribution of temperature, salinity and dissolved oxygen in "Veliko jezero" (VJ) from June 1997 to January 1999.

extending from the surface to 17 m, temperature was from 26.0°C to 23.0°C, and from 20 m to the bottom it was 16.0°C to 12.5°C. Salinity values were relatively low, and ranged from 34.9 psu at the surface to 36.7 psu which occurred from 35 m to the bottom. Within the thermocline layer, slight variations of salinity were noticed. Oxygen saturation values from the surface to 17 m were between 91% and 95%, yet in the thermocline layer values slightly increased to the maximum of 101% at 18.5 m, and thereafter rapidly decreased to 0% in the layers from 39 m to the bottom.

Nutrient values (Fig. 4a) in VJ were different in 1997 and 1998 and, with the exceptions of reactive silicates, all values were higher in 1998. Statistical significance ($P < 0.01$) was noted only for differences in ammonia and reactive phosphorus. Ranges of the concentration values in 1997 and 1998 were as following: NO_3 (0.1-3.42; 0.01-4.54); NO_2 (0.01-0.33; 0.01-0.62); NH_4 (0.07-0.33; 0.21-1.2); N_{tot} (2.19-7.82; 0.68-32.13); PO_4 (0.01-0.15; 0-0.26); SiO_4 (0.61-37.99; 0.54-28.71). In the summer of 1997 and 1998, a pronounced nutricline was present between 20 and 25 m, while during the fall it was noted at depths greater than 30 m. Statistically significant differences ($P < 0.05$) for concentrations of

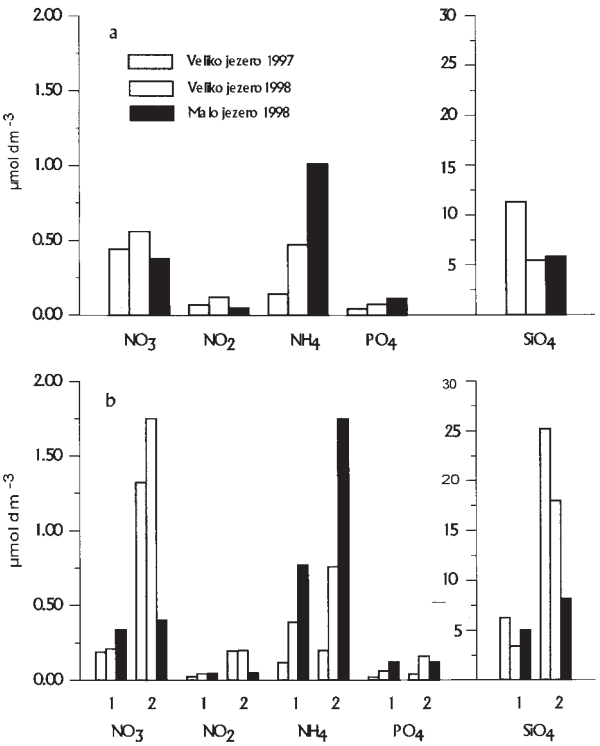

FIG. 4. – Nutrient concentrations during the investigated period in VJ and MJ. Values are expressed as water column mean in (a), and for different layers (1= above nutricline; 2= below nutricline) as mean of layers in (b).

nutrients within vertical gradients were noted both years. In layers above and below the nutricline, significant differences ($P < 0.05$) in concentrations of NH_4 and PO_4 were noted during periods of strong stratification (Fig. 4b). During the isothermal winter period concentrations of nutrients were homogenous in the entire water column.

In 1998 in MJ, the concentration values for NO_3 and NO_2 were notably lower than in VJ (Fig. 4). Concentration ranges were as follow: NO_3 (0.04-1.65), NO_2 (0.01-0.15), N-tot (0.64-7.21), SiO_4 (2.42-11.76). Concentrations of NH_4 (0.46-2.18) and reactive phosphorus (0.03-0.67) were significantly higher than in VJ ($P < 0.01$). Only the NH_4 concentration showed a vertical gradient below 20 m.

There were no differences in mean values of reactive SiO_4 for both lakes (Fig. 4), but their vertical distributions were different. In MJ the concentrations of SiO_4 were quite equal for the entire water column, but in VJ maximum values were noted in the near bottom layer (Fig. 4b).

A small number of species characterized the zooplankton in both lakes. In VJ most zooplankton density values were less than 5000 ind. m^{-3} with a maximum of 17 097 ind. m^{-3} in September and minimum of 580 ind. m^{-3} in November (Fig. 5). The total zoo-

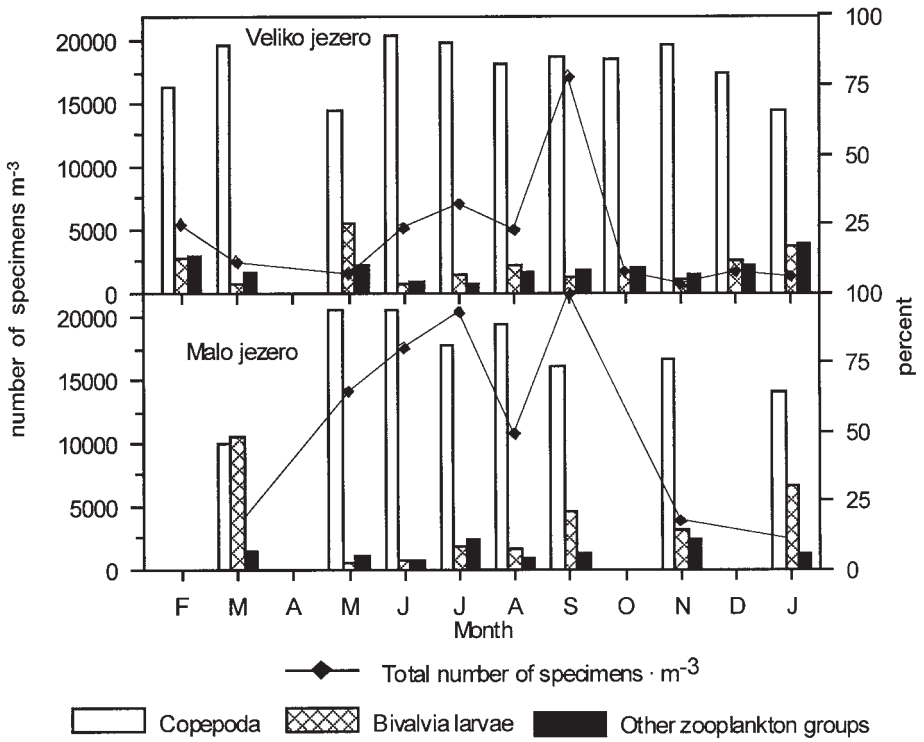

FIG. 5. – Total zooplankton (no. ind. m^{-3}; left-hand scale) and percentage occurrence (right-hand scale) from February 1998 to January 1999 in VJ and MJ.

plankton in MJ was high during spring and summer (Fig. 5). In this period, zooplankton density values ranged between 10 757 ind. m^{-3} and 21 864 ind. m^{-3}. A minimum of 2275 ind. m^{-3} was noted in January. Annual averages ratios of copepods to total zooplankton numbers were 82% (VJ) and 77% (MJ). Among 22 species of copepod in VJ and 12 species in MJ, the most numerous were copepodites of *Oithona nana* and *Paracalanus parvus*, followed by *Acartia clausi*, *Oithona similis*, *Isias clavipes*, and *Diaixis pygmaea*. In VJ *Calanus helgolandicus* was present in small numbers throughout the year. Among different copepod groups, cyclopoids had the highest density values (VJ: range 19%-86%, average 61.2%; MJ: range 48%-82%, average 67.1%). They were followed by calanoids (VJ: range 14%-62%, average 34.1%; MJ: range 18%-51%, average 32.5%). Poecilostomatoids were recorded only in VJ (range 0%-10%, average 2%) and harpacticoids were the minor group in both lakes (VJ: range 0%-4%, average 0.7; MJ: 0%-1%, average 0.4).

Medusae

Hydromedusae were present in both lakes in very small numbers of species and specimens, as indicated by plankton tows (Table 1). Their maximum occurrence was 181 ind. m^{-3} in September 1998 in VJ, and 81 ind. m^{-3} in May 1998 in MJ. Two of four species of Anthomedusae were collected in each lake and the same four species of Leptomedusae were present in both lakes, the most frequent and abundant of which was *Obelia* spp. (maximum: VJ: 140 ind. m^{-3} September; MJ: 61 ind. m^{-3} May).

A fifth Leptomedusae, *Tima* sp. (Fig. 6) was observed and collected by SCUBA divers in both lakes, but was never taken in the plankton samples. This distinctive, large species, with a nearly hemispherical umbrella, fairly thick jelly, well-developed velum, 60-84 mm bell diameter, and 55-75 mm bell height was present during summer and autumn, always below the thermocline. This species was more sporadic at VJ while a density of about 3 ind.m^{-3} (between 17 and 20 m) was noted at MJ. *Tima* sp. was not observed in the central parts of MJ where depth is greater than 20 m. At night, slowly moving individuals were found approximately 1-2 m above bottom and their exposure to diver's light caused rapid movements in opposite direction from the light. During daylight, *Tima* sp. moved slowly, touching the bottom with its tentacles and feeding on some particles that were collected. One captured mysid (e.g. *Mesopodopsys slabberi*) was recognized by a SCUBA diver on a tentacle that was pulled to the mouth.

The scyphozoan *Aurelia* sp. was present in both lakes throughout the year but its ephyrae were never collected in plankton samples. In VJ *Aurelia* sp. were always observed in swarms of very large numbers of individuals, with the exception of September 1997. *Aurelia* sp. density ranged from 10 ind. m^{-3} to

TABLE 1. – Abundance of hydromedusae collected by plankton net in the seawater lakes "Veliko Jezero" (VJ) and "Malo Jezero" (MJ), June 1997 - January 1999. In April (VJ) and February, April, October and December (MJ) plankton was not collected because of technical problems. (No.ind. m^{-3} : + = <1; r = 1-10; c = 11-50; cc = >50).

Species / Month	J	F	M	M	J	J	A	S	O	N	D
VJ											
ANTHOMEDUSAE											
Podocoryne minima			+		c	+	r	c	+	r	+
Podocoryne minuta					r	r					+
LEPTOMEDUSAE											
Obelia spp.	r	c	c	+	c	cc	cc	cc	cc	r	+
Clytia hemisphaerica					+		r	r	+		
Eirene viridula							+	+			
Eutima gracilis						r	+				
MJ											
ANTHOMEDUSAE											
Sarsia gemmifera			r								
Bougainvillia muscus					r						
Podocoryne minima		r	c		c	r	r	r			
Podocoryne minuta							c				
LEPTOMEDUSAE											
Obelia spp.	r	r	cc		c	c		+		c	
Clytia hemisphaerica								c			
Eirene viridula								+			
Eutima gracilis							+				

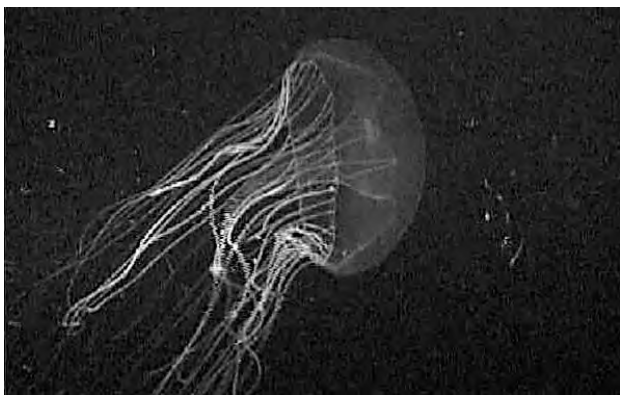

FIG. 6. – *Tima* sp. swimming horizontally near the bottom at 17 m in MJ. Photo by V.Onofri at 10.00 p.m., August 1998.

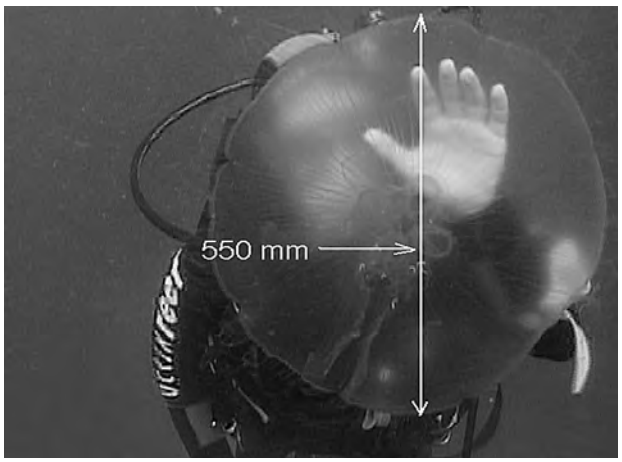

FIG. 7. – The largest *Aurelia* sp. specimen observed at 22 m in MJ. Photo by V. Onofri at 2.00 p.m., July 1998.

approximately more than 600 ind. m^{-3}. Diameter of the swarm was typically about 150 m. Horizontal movements of the entire swarm were observed as well as the active swimming of individuals toward the center of the swarm. Density of *Aurelia* sp. was always higher when the swarm was concentrated in either surface or deep layers. Within the swarm an especially high number of smaller aggregations of individuals were observed (e.g. from a small aggregation, 80 individuals were collected in a 20-liter container). Strong vertical migrations were noted. In summer months swarms were concentrated above the thermocline during the daylight, and below the thermocline at night. In winter months swarms were distributed unequally during the daylight, concentrating near the surface at dawn and sunset, migrating to deep layers after sunset and concentrating in deep layers through the night. Diameter of individuals was between 10 mm (December 1998) and 120 mm. During the anoxic event on 26-28 August 1996, the *Aurelia* sp. swarm, with specimens quiescent or only slowly moving, was observed just above the anoxic layer.

In MJ *Aurelia* sp. were distributed randomly. Though more individuals were always noted in the deeper layers (cca. from 17-25 m) and in the central part of the lake, neither vertical migrations nor swarms were observed. Individual numbers were from 1 to 10 ind. 100 m^{-3}, approximately. Diameters of these individuals were between 100 mm and the maximum of 550 mm (Fig. 7).

DISCUSSION AND CONCLUSIONS

Ecological characteristics of the Mljet Island seawater lakes are influenced by the surrounding terrestrial area and by restricted communication with the open sea through the naturally-formed shallow strait. Similar effects have been described in Ireland for Lough Hyne (Ballard and Myers, 1996). The long-time isolation of the Mljet lakes (Schmidt, 1993) has probably caused their specific environmental conditions and influenced the persistence of the primitive Tethys fauna that is known for some parts of Mediterranean Sea (Gili *et al*., 1998). It is well known that coastal environments are subjected to intensive changes of hydrographic conditions depending on local atmospheric and other coastal influences. The stratification is pronounced during summer months when a very strong thermocline divides the water columns and influences all hydrographic, chemical and biologic parameters (Buljan and Špan, 1976; Kršinić and Lučić, 1994; Benović and Onofri, 1995; Carić and Jasprica, 1998). Though general ecological conditions are similar in both lakes, some important differences should be noted. While in MJ the summer thermocline appears between 10 and 15 m, in VJ it is deeper, at 15-20 m. The influx of hyperhaline waters from the surrounding sea to the VJ that was noted in June-July 1997 has only slightly influenced the salinity values of MJ.

The consequences of the pronounced thermocline include constantly lower temperatures at depth (in layers 20-45 m) than in the surrounding sea at the same depth. This probably causes persistence of specific communities in deeper layers of both lakes (Kršinić and Lučić, 1994; Onofri and Marguš, 1995; Vučetić, 1995). Since in both lakes there are no upwellings or other forces to exchange waters from the bottom layers to the surrounding sea, we believe that the ecology in layers below the thermocline maintains primordial entities, beginning with an influx of waters from 4200 or 7000 years ago (M.

Juračić and V. Onofri, unpublished; Schmidt, 1993).

The nutrient water column profile in VJ generally shows lower values in upper layers (Carić and Jasprica, 1998). It could be supposed that the typical trophic relationship between nutrients and phytoplankton exists in upper zones of VJ. In deeper zones, and especially in near-bottom layers, higher nutrient concentrations might be related to excretions from the *Aurelia* swarms (Schneider, 1989) and other zooplankton aggregations (Kršinić and Lučić, 1994; Benović and Onofri, 1995), as well as to sinking of organic matter and its decomposition (Azam, *et al*., 1983). In MJ, nutrient concentrations were homogenous throughout the entire water column. However, a vertical gradient for NH_4 concentrations is shown below 20 m. It is important to note than during this survey these concentrations of NH_4 were notably higher than averages elsewhere in the coastal and open southern Adriatic (M. Carić, pers. comm.). These concentrations could be related to the large number of big *Aurelia* (Schneider, 1989) permanently present in the deeper layers of MJ.

Anoxic conditions occasionally occur in deep layers of closed marine ecosystems (Buljan, 1956; Buljan and Špan, 1976; Ballard and Myers, 1996). In VJ anoxic conditions were noted for the first time on 27 and 28. August 1996 when brief anoxia occurred in the layer below 35 m. It is possible that similar anoxic conditions appear regularly in VJ during periods of the strongest stratification, but because of short duration they can readily be missed. The formation of anoxic conditions is mostly related to bacterial activity and the decomposition of organic compounds (Azam *et al*., 1983), but presence of large numbers of *Aurelia* near the anoxic layer (Thuesen and Childress, 1994) could also be a substantial contributing factor (Hansson and Norrman, 1995). It is possible that marine snow also plays an important part in ecological processes of the lakes (Ott and Herndl, 1995). In VJ, in layers above the thermocline, concentrations of marine bacteria are in concentrations similar to those of highly productive coastal areas (S. Bobanović-Ćolić, pers. comm.). Especially high bacteria concentrations that are temporarily found in near bottom layers (i.e. in 1988: range of 0.35×10^6 ml^{-1} to 8.34×10^6 ml^{-1}, S. Bobanović-Ćolić, pers. comm.) are usually characteristic only for extremely eutrophicated areas (Azam *et al*., 1983; Ducklow, 1983). Decomposition processes induced by very high numbers of bacteria provide a probable reason for high concentrations of silicates in the VJ near-bottom layers, while large numbers of randomly distributed *Aurelia* in MJ probably caused higher concentrations of ammonia there (Schneider, 1989).

According to Viličić (1989) categorizing ecosystems on the basis of the phytoplankton population density and biomass, the Mljet lakes would be a "moderately eutrophicated ecosystem". Similar annual average phytoplankton population densities have been recorded in highly productive south Adriatic coastal zones (Jasprica, 1989). In the spring of 1986 an atypical seasonal phytoplankton succession was noted, similar to what is known to occasionally appear in the coastal Adriatic areas (Viličić *et al*., 1995; Jasprica and Carić, 1997), and in the Mediterranean (Carrada *et al*., 1980). Higher Chl *a* concentration in deeper zones of VJ could be explained by the high percentage of the nanophytoplankton fraction (Jasprica and Carić, 1997), sinking of particles (Ott and Herndl, 1995) and moderate metabolic processes of zooplankton due to the constantly low temperatures (Carić and Jasprica, 1995).

The zooplankton of similar ecosystems is typically characterized by domination by very few species that are occasionally present in very high densities (Raymont, 1983). Both of the Mljet lakes are inhabited by small numbers of zooplankton species and are dominated by calanoid and cyclopoid copepodites (*Paracalauns parvus* and *Oithona nana*). Copepods are permanently separated in the water column: copepodites and smaller species (i.e. *P. parvus*; *O. nana*) were always more abundant near the surface and above thermocline, and adults of larger species (i.e. *C. helgolandicus*) below the thermocline (Vučetić, 1995), aggregating in deeper layers (Kršinić and Lučić, 1994). As in the results of previous studies, one of the most abundant Adriatic-calanoids, *Pseudocalanus elongatus* (Vučetić, 1957; Kršinić and Lučić, 1994) was not present in our samples. In MJ the zooplankton densities were constantly higher than in VJ (except in September 1998), especially during spring and summer months. These results correspond to the distribution and abundance of *Aurelia* sp. medusae. In other ecosystems similar to the Mljet lakes, medusae are abundant and present with high numbers of zooplankton species (Ballard and Myers, 1996).

In the present study we noted very low numbers of hydromedusae, representing only the Anthomedusae and Leptomedusae. We found only seven species in VJ and nine species in MJ, with only *Obelia* spp. and *Tima* sp. being present over long periods. In surrounding areas of the southern Adri-

atic, representatives of all hydromedusan orders are present and the majority of specimens have been identified as the trachymedusae *Rhopalonema velatum, Aglaura hemistoma* and *Liriope tetraphylla* (Benović, 1976). None of these common species were collected in the present study in Mljet lakes, while Vučetić (1957) found only two of them more than 40 years before our study. Lučić and Bender-Pojatina (1995) found eight hydromedusan species in VJ, including a few individuals of *A. hemistoma* and *R. velatum*, shortly before our study.

For the first time in the Adriatic Sea since the studies of Neppi and Stiasny (1913), specimens from the genus *Tima* are reported. We designate these animals as *Tima* sp. because identification could not be precisely determined to the species level, but the genus has been confirmed to be *Tima* (S. Kubota, pers.comm.). The only *Tima* species previously known from the Mediterranean Sea is the rare *Tima luculana* (Mayer, 1910, Vannucci, 1966, Brinckmann-Voss, 1987, Gili *et al*., 1988). Our results differ from earlier records of *T. luculana* in the number of specimens, large size of individuals and frequency of appearance observed by the specialist SCUBA diver. Probably, this species has not been frequently collected elsewhere because of its very fragile body structure that results in disintegration of any individuals collected by standard plankton net sampling. The very restricted near-bottom areas of its distribution would increase the unlikeliness of collecting this species with standard techniques. The probable permanent presence and apparent abundance of *Tima* in the Mljet lakes may imply a relationship to the putative primitive Tethys fauna found elsewhere in deep pockets in the Meditrerranean (Gili *et al*., 1998). Future studies should determine whether the Tima sp. present in the Mljet lakes is *Tima luculana* or represents a new near-bottom species in an unusual habitat.

The presence of great numbers of *Aurelia* sp. medusae, usually identified as *A.aurita,* is a worldwide phenomena, distributed approximately between 70°N and 40°S (Kramp, 1961). In temperate zones, *Aurelia* medusae appear in masses during spring-summer, and reproduce from December onward (Lucas, 1996). On the contrary, swarms of *Aurelia* in VJ and randomly distributed big individuals in MJ were present throughout the year, but were never found in the surrounding open Adriatic (Benović and Bender, 1987). This may be related to the permanently low temperature values in the deeper layers of both lakes and thermal requirements for strobilation (Omori *et al*., 1995).

We identify our species as *Aurelia* sp. because recent studies do not confirm this species to be the same as *A. aurita*. Analyses by W. Schroth and B. Schierwater, Zoologisches Institut, Frankfurt am Main, Germany (pers.comm.) provide compelling molecular evidence (nuclear and mitochondrial DNA sequence analyses) that *Aurelia* sp. from Mljet lakes substantially differs in terms of genetic divergence from *Aurelia aurita* found elsewhere in the Mediterranean, and could be attributed to its boreal origin. In previous plankton studies of the Mljet lakes (Vučetić, 1957; Lučić and Bender-Pojatina, 1995) *Aurelia* was noted throughout the year, but only sporadically. Local fishermen from the island of Mljet regularly observe *Aurelia* swarms during low atmospheric pressure on cloudy and drizzling days at the surface of VJ. This phenomenon is known to occur in other shallow ecosystems and has been related to the particular light intensity (Yasuda, 1970). The numbers and sizes of *Aurelia* individuals in the Mljet lakes are greater than reported in other parts of the world (Yasuda, 1970, Olesen, *et al*., 1994, Lucas, 1996). Our specimens with the bell diameter of 550 mm from MJ (Fig. 7) were bigger than the maximum known diameter of 500 mm observed in the La Plata river mouth (H. Mianzan, Mar del Plata, Argentina, pers. comm.) We believe that the constantly decreasing value of zooplankton abundance, low number of hydromedusae, and disappearance of certain plankton copepod species (*Pseudocalanus elongatus*) is the consequence of the impact of *Aurelia* swarms and their selective feeding (Behrends and Schneider, 1995, Omori *et al*., 1995). Though *Tima* sp. is not present in masses, it also has an important impact because it inhabits different parts of the lakes than *Aurelia* does. The noticeably larger *Aurelia* individuals in MJ than in VJ are apparently in direct relationship with higher food availability (Mills, 1995; Ishii and Bamstedt, 1998). It is hypothesized that MJ contains only imported medusae from VJ, since the absence of ephyrae, and swarms and random distribution may be interpreted as indicating that *Aurelia* probably do not form scyphistomae in MJ, where the high sedimentation rate (Juračić *et al*., 1995) and occasional appearance of H_2S (Buljan, 1956) may prevent settlement of scyphistomae. The high nutrient concentrations, high bacterial and phytoplankton values, lower abundance of zooplankters and anoxia in VJ designate the Mljet lakes as a potentially highly vulnerable ecosystem. Our results indicate that the

Aurelia sp. medusae play a crucial role in maintenance of this sensitive boreal ecosystem. Because of the long isolation of the Mljet lakes, we believe that the same processes have determined ecosystem stability from early ages.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Gonangium development and medusoid of *Nemalecium lighti* (Hargitt, 1924) (Cnidaria: Hydrozoa, Haleciidae)*

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SUMMARY**:** Based on live specimens of *Nemalecium lighti* collected in the coasts of La Réunion (Indian Ocean) and São Sebastião (Southeastern coast of Brazil) and kept in the laboratory, we observed the release of short-lived medusoids. The gonangia pass through six developmental phases*: growing, ripening, migrating, stripping, liberating* and *spawning*. The medusoids are tall, lack tentacles, bulbs, circular and radial canals, and the sexual products are packed around the eccentric manubrium; they are provided with a velum and with a subumbrellar ectoderm rich in transverse striated muscle fibers. There are refringent and isotropic corpuscles within vacuolated and ciliated large cells located around the aperture of the medusoid, which possibly function as statoliths. The corpuscles are similar to those already described for the families Plumulariidae and Aglaopheniidae. The gametes are liberated shortly after the release of the medusoid from the gonotheca. The female medusoid spawned 40-62 ova; spermatozoa exhibited a semicircular nucleus, and planulae were formed *c.* twelve hours after fertilization. Colonies with medusoids of only one sex or with both male and female medusoids.

Key words: Cnidaria, Leptomedusae, hydroids, reproduction, life cycle, medusoid, gametes, hermaphroditism

INTRODUCTION

Nemalecium is a haleciid genus with a relatively wide distribution on the tropical regions of the world, being remarkable by having a pair of nematodactyls: short and thick fingerlike tentacles armed with large, pseudostenotele nematocysts (Bouillon, 1986; Bouillon *et al*., 1986; Calder, 1991; Migotto, 1996).

Bouillon (1986) thoroughly redescribed *Nemalecium lighti* (Hargitt, 1924) from Papua-New Guinea, including an account of the female gonophore. By histological sections he established the presence of a velum and of remains of a circular canal, and hypothesized that these gonophores could be released into the water and have a short free life. Calder (1991: 27) described the male gonotheca of specimens from Bermuda. Sexual reproduction by medusoids during the austral summer (November to April), together with data on feeding and on ecological preferences, has been described previously from Indian Ocean material (Gravier-Bonnet and Mioche, 1996).

Up to now the genus *Nemalecium* is monotypic. But some characters (see Discussion) differ slightly

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among specimens from São Sebastião, La Réunion and those described by Bouillon (1986) and Calder (1991), what could indicate that there are more than one species of *Nemalecium*. And in Papua New-Guinea, Boero suspected the presence of two species (pers. comm.). However, as the specific name *Nemalecium lighti* were already cited from São Sebastião (Migotto, 1996) and La Réunion (Gravier-Bonnet and Mioche, 1996), we decided not to name new species until thoroughly morphological comparisons are not undertaken.

Based on live specimens of *N. lighti* collected in the coasts of La Réunion island (SW Indian Ocean) and São Sebastião (southeastern coast of Brazil) that were kept in the laboratory, the development of male and female gonangia was followed until the release of mature, short-lived medusoids. The successive developmental phases of the gonangia and the released medusoids are herein described.

The similarities of the medusoid of *N. lighti* with those few already known from Plumulariida (for references and discussion on medusoids and swimming gonophores see Migotto, 1998) stress the importance of these structures for phylogenetic inferences for higher taxa.

MATERIALS AND METHODS

Colonies of *N. lighti* were collected on experimental plates and rocks of the shallow infralittoral of the São Sebastião Channel, São Paulo State, Brazil (see Migotto, 1996 for a brief description of the collecting area) and from crevices on the littoral reef platform of Cap Homard in La Réunion (see Gravier-Bonnet and Mioche, 1996 for a description of the collecting area).

Stems with gonothecae, from São Sebastião, were transferred to glass beakers (600 and 1000 ml) with filtered seawater, and kept in a constant temperature chamber at 24ºC, until the release of medusoids. Poorly ramified colonies from La Réunion, collected with fragments of the hard substratum, were kept at 23-25ºC in an aquarium with natural seawater (closed system, 6000 ml) and aeration, and the formation and growth of the gonangia were observed from the outset. The development of the gonangia was seen in material from La Réunion (phases 1-4) and the liberation of the medusoid in the material from São Sebastião (phases 5-6), but newly released medusoids and gametes were seen in materials from both locations.

Observations at La Réunion were undertaken using a stereomicroscope (Leica M420) and a light microscope (Leitz Diaplan). Photographs were taken with a mono CCD camera connected to a SVHS magnetoscope (Panasonic NV-FS100), a monitor (Sony Trinitron), a computer (Power Macintosh 8500), and a video printer (Sony UP-3000). Photographs have been treated with Adobe Photoshop and drawings by Adobe Illustrator. Experiments altering the day-night rhythm were carried out in order to record the release on videotape. The migrating and stripping phases of the medusoids occurred at very low speed and were only perceivable by time-lapse photography and by fast motion video recording.

Most of the specialized terms used here are clearly defined in the glossary proposed by Cornelius (1995) except the following two. The word "mantle", already used by Harris (1990) and Bouillon (1986; "manteau"), for the "ectoderm lining" of Millard (1975), is a double layer of ectoderm which covers the inner side of the perisarc and surrounds other coenosarcal structures: hydranths into hydrothecae, gonophores into gonothecae and coenosarc into hydrocladia and stems. "Plateau terminal" (apical plate), used by Bouillon (1986) in describing the gonophore of *N. lighti*, is as a large, hollow vesicle at the terminal part of the gonangium, being covered by the mantle.

RESULTS

Colonies collected without gonangia developed them in the laboratory within 8 to 10 days either on gonochoric or hermaphroditic colonies. At La Réunion island, the colonies collected were either only male or female, or both; only gonochoric colonies were seen in São Sebastião. The successive developmental phases observed are summarized below and illustrated by schematic drawings (Fig. 1) and photographs (Fig. 2).

From the small bud that appeared from the hydrorhiza and hydrocauli internodes (gonothecae grew only from hydrocauli internodes in the well ramificated colonies from Brazil), there was an initial *growing phase* (phase 1; Fig. 1 a-c). The gonangium gradually elongated, assuming its definitive shape and maximal size after 4 to 5 days. Concomitantly a large mass of cells, the apical plate, formed at the apex of the gonotheca. Both the apical plate and the gonophore below were covered by the mantle, which

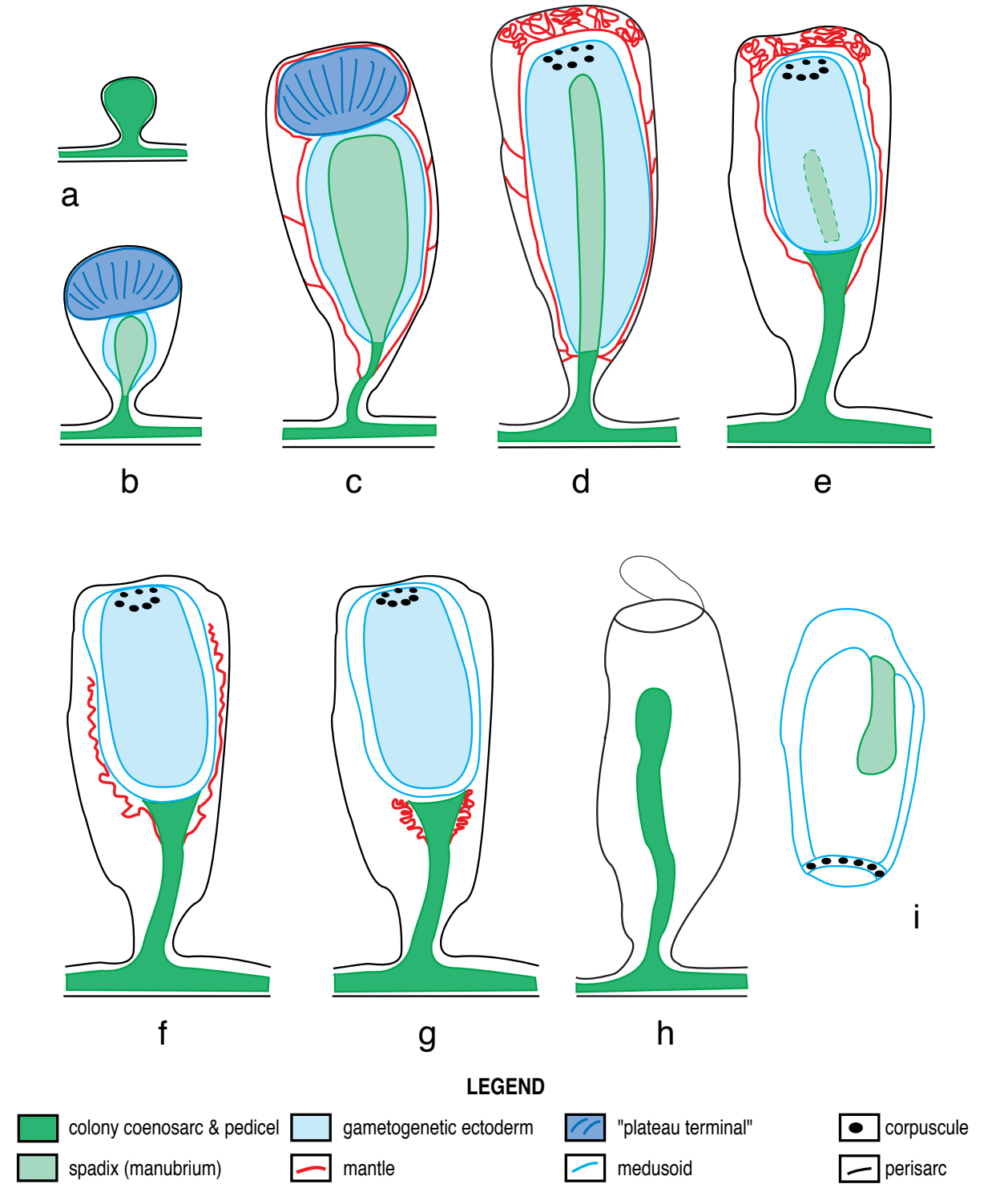

FIG. 1 – Development of the gonangium of *Nemalecium lighti* and medusoid release (schematic drawings). a-c, growing phase; d, ripening phase; e, migrating phase; f-g, stripping phase; h, empty gonotheca with operculum; i, spent male medusoid.

was discontinuously connected with the perisarc of the gonotheca and provided with many nematocysts. At this phase the manubrium was usually thicker than the layer of ectoderm that enclosed the gametes.

The second phase was the *ripening phase* (Fig.1d). The apical plate had completely disappeared, but the gonophore remained surrounded by the mantle where nematocysts, especially pseu-

dostenoteles, were in particular packed at the apex of the gonangium, below the top of the gonotheca. The manubrium became narrower than previously, and the ectoderm enveloping the gametes became wider. This ectoderm included not only the gametes but also nematocysts, glandular cells (like the ones at the base of the tentacles of the hydranth described by Bouillon, 1986) and peculiar needle shaped and crystalline elements (with a small angle of extinction under polarized light) that were distributed radially from the manubrium and could be excretive products. At this stage the presence of a ring of refringent corpuscles encircling the region to the future aperture of the bell was clear.

Observing live gonangia gave us almost no clue about the phases of the gametogenic processes. From the beginning of oogenesis, the oocytes – usually arranged in mosaic shape and with well visible darker nuclei - were opaque and white in color, being difficult to perceive any change inside the gonads. Conversely, during ripening, the male gonad underwent a change in color from translucent to white opaque, probably indicating the transformation of spermatids into spermatozoa. The duration of the ripening phase was 2 to 3 days.

During the third phase (Figs. 1e, 2a) – *migrating phase* – the gonophore, which previously almost completely filled up the gonotheca, became concentrated at the distal part, leaving the base empty. Simultaneously, the peduncle that linked the gonophore and the coenosarc of the colony elongated. This was a relatively slow process, taking about 6 hours.

During the fourth phase (Figs. 1f-g, 2b-d) – *stripping phase* – the gonophore became detached from the mantle. The event began at the distal end (oral) of the gonophore, where, as a first consequence, the circle of refringent corpuscles appeared clearly. Then, at low speed, the mantle withdrew towards the base until the gonophore remained uncovered inside the gonotheca. Concomitantly the oocyte nucleus became invisible and the shape of the oocytes changed from polygonal to round, probably indicating the beginning of their detachment from the manubrium. At this stage, the top of the gonotheca looked more convex under the pressure of the medusoid. This phase took half a day.

The medusoid was released from the top of the gonotheca, where there was an indistinct operculum that could either remain closed or open after medusoid release. The medusoids pulsated during and after release from the gonotheca. Spawning occurred during the process of release from the gonotheca or shortly after. Therefore, the fifth and sixth phases – *liberation* and *spawning phases* –, occurred simultaneous or near so, and were relatively short compared with the other phases.

Spent medusoids (Fig. 2e-g) lived up to two hours in the culturing dishes. A short time after spawning, the peduncle was still present within the empty gonotheca (Fig. 1h), being longer than before medusoid release. Occasionally some medusoids reached the outside water and spawned while still attached to the colony by the peduncle (Fig. 2e). The peduncle could have a role in pushing the medusoid out; at least the medusoid remained linked by the peduncle during the whole process of release from the gonotheca, detaching from it only when on the outside.

At La Réunion island, medusoid release took place about dawn. Experiences of leaving fertil colonies or isolated gonothecae under light during the night, in order to record the events on videotape, prevented the release from occurring. Conversely, colonies kept in darkness did not delay medusoid release.

Male and female medusoids were very similar, but the spent male gonophore (Figs. 1i, 2f-g) was more ovoid than the more spherical female gonophore (Fig. 2e). Both were 1050 to 1400 μm high and 420 to 550 μm in maximum diameter. They lacked mouth, tentacles and tentacle bulbs, radial and circular canals, ocelli and true statocysts. The bell had an aperture delimited by a velum (Fig. 2g). The remaining part of the manubrium was generally not at the center of the bell (Fig. 2f). Microbasic mastigophore nematocysts (14.5-17 x 5-6 μm) were more densely distributed on the basal half of the exumbrella, but absent in a narrow band near the margin (there were no pseudostenoteles in medusoids). The exumbrellar and subumbrellar epidermis – the latter densely packed with concentric striated muscular fibers (Fig. 2g) – joined at the umbrella margin where there was a ring of large cells enclosing refringent corpuscles of various shape into wide vacuoles (Fig. 2h). There were either one or several corpuscles of different sizes in one cell and these cells were provided with long cilia (seen by phase contrast microscopy). Under a polarizing microscope, the corpuscles appeared to be composed by an isotropic substance that did not polarize. They presented concentric layers indicating growing stages, and under pressure they broke from the center along more or less perpendicular

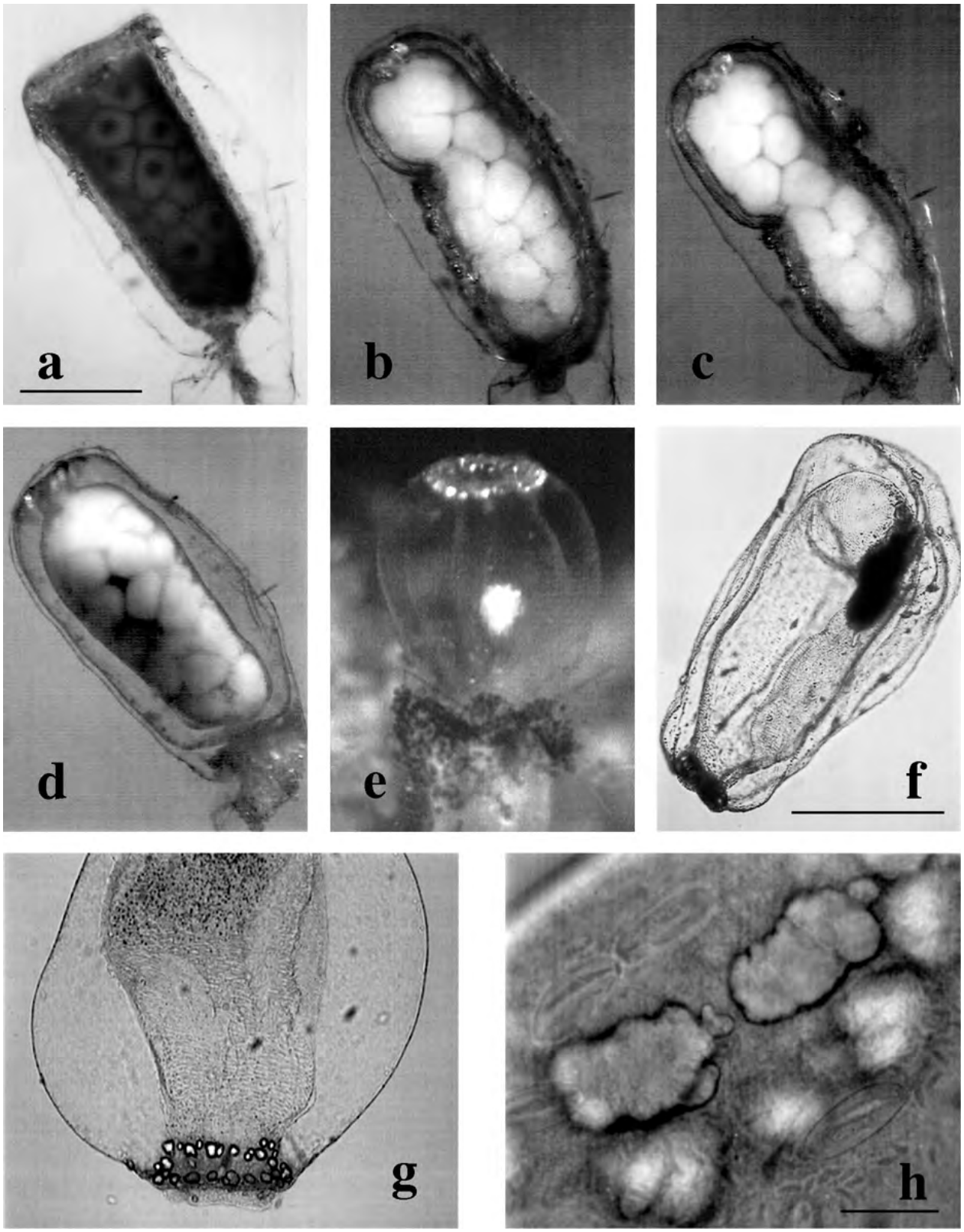

FIG. 2 – Medusoid of *Nemalecium lighti*. a-d, female medusoid inside the gonotheca: a, migrating phase with the mantle surrounding completely the medusoid; b-d stripping phase with retraction of the mantle (beginning, middle and end); e, spent female medusoid still attached to the gonotheca, with well visible circle of refringent corpuscles; f, spent male medusoid soon after liberation, with eccentric manubrium; g, spent male medusoid on slide, clearly showing extruded velum, corpuscles, and circular muscle fibers in the subumbrellar ectoderm; h, corpuscles and nematocysts (pseudostenoteles and microbasic mastigophores) visible at the top of the gonotheca before the release of the medusoid. Bar scales: a-e, 0.25 mm; f-g, 0.25 mm; h = 0.05 mm.

lines. They were quickly destroyed by fixatives (Bouin, alcohol, formaldehyde) but were conserved in seawater.

Each medusoid spawned 40-62 milky-white, spherical eggs, measuring 120-135 μm in diameter. The spermatozoa were rounded and each had a long flagellum. The larval development occurred rapidly; in about 12 hours planulae were already formed, alternating periods of swimming actively in the water column with periods of slowly gliding on the bottom of the culture dish. The planula was elongated with the anterior pole larger and rounder, and with thicker ectoderm than the posterior.

Fully developed male and female gonothecae were identical: curving and tapering basely, elongated, with walls weakly divergent and maximum diameter at the truncated top, and perisarc either smooth or with a few undulations.

The specimens from São Sebastião and La Réunion were apparently identical, except in shape and size of the refringent corpuscles of the medusoid, which differed slightly.

DISCUSSION

The morphology of the medusoid of *Nemalecium lighti* is very similar to those described for species of Sertulariidae, Plumulariidae and Aglaophenidae (Migotto, 1998; Migotto and Marques, 1999). They all share distinctive features, including behavior, shape of the bell, eccentric manubrium and distinct velum; and lack mouth, tentacles and tentacle bulbs, radial and circular canals, and sensory structures like ocelli and statocysts. But the presence of striated muscle fibers in the subumbrellar epidermis, typical of a true medusa, which enables swimming and expulsion of gametes, leads us to consider the medusoids as reduced medusae instead of fixed gonophores which became released (as proposed by Boero and Bouillon, 1989 and named swimming gonophores). Such fibers have been already reported for the medusoid of *Plumularia obliqua* (Johnston, 1847) (= *Monotheca obliqua*) (see Motz-Kossowska, 1907). According respectively to Motz-Kossowska (1907) and Bouillon (1986) there is a vestigial circular canal in the gonophores of *Monotheca obliqua* and *Nemalecium lighti*, but this canal was not described for the other similar medusoids, possibly because they were seen in histological preparations and are not visible in live material. However, in histological sections from mature male gonangia from La Réunion, no vestigial circular canal was observed at the top of the gonophore but two lateral cavities, probably due to the dissolution of the corpuscles into fixatives, were present.

The refringent corpuscles at the margin of the medusoid is a character already described only for species of Plumularioidea: the aglaopheniid *Macrorhynchia philippina* (Kirchenpauer, 1872) by Gravier (1970) and Migotto (1996), and the plumulariid *Dentitheca bidentata* (Jäderholm, 1920) by Migotto (1997) and Migotto and Marques (1999). These corpuscles, previously misinterpreted as lipid droplets by Boero and Bouillon (1989) and Migotto (1998), play the role of a ballast, maintaining the medusoid in a vertical position and carrying it towards the bottom (Gravier, 1970), an observation confirmed recently (Migotto and Marques, 1999). The presence of cilia in the cells enclosing the refringent corpuscles indicates they have a sensorial function, maybe being homologous to statoliths.

The refringent corpuscles are also present in the fixed gonophores of the aglaopheniid *Aglaophenia latecarinata* Allman, 1877 and in the gonophores (possibly short-lived medusoids) of the kirchenpaueriid *Ventromma halecioides* (Alder, 1959) and of the plumulariid *Monotheca margaretta* Nutting, 1900 (Migotto and Marques, unpublished data). In the case of *Aglaophenia latecarinata* the presence of an equilibrium organ seems unnecessary, except if the fixed gonophores could alternatively become free medusoids in response to seasonal environmental changes. Some species of campanulariids, for instance, can have colonies that produce either fixed gonophores or free medusoids if settled, respectively, in calm or rough waters (Stefani, 1956; Gili and Hughes, 1995).

Marginal corpuscles have been noted on the medusoid of *Gymnangium ferlusi* (Billard, 1901) (unpublished data of N. Gravier-Bonnet). For Aglaopheniidae, they are known to be present in eleven species: nine cited by Gravier (1970), plus *Gymnangium hians* (Busk, 1852) from La Réunion and *Macrorhynchia* sp. from Madagascar, where, as in *M. philippina*, their shape is rounder and more regular than in *N. lighti* (unpublished data of N. Gravier-Bonnet). Corpuscles have to be confirmed in two species of Plumularioidea that are respectively known to produce short-lived medusoids: *Aglaophenia* sp. referred by Boero and Bouillon (1989) and *Monotheca obliqua* referred by Motz-Kossowska (1907).

These findings indicate that these structures are probably more widespread within the families

Aglaopheniidae, Kirchenpaueriidae, Plumulariidae and Haleciidae, either associated with free medusoids or not. As the corpuscles disappear in fixed material, their presence can only be ascertained by working with living specimens.

About the role of the "plateau terminal", questioned by Bouillon (1986), it is possible, at the light of these new results, to eliminate any sort of implication in fertilization. This mass of cells, present during the growing phase and absent in the mature gonangium, is at least partly involved in the gonotheca formation.

Sexual dimorphism of *N. lighti* medusoids is very weak, which seems to be usual in hydroids and common among Leptomedusae (Gili and Hughes, 1995). Conversely hermaphroditism has probably been underestimated, at least for some families. Already checked among aglaopheniids (Gravier, 1970; the term dioecious has been wrongly used in the text of that article instead of hermaphrodite, however well documented by the description and illustration) and plumulariids (Gili and Hughes, 1995; Migotto, 1997; Migotto and Marques, 1999), it has been seen in several species of these families (unpublished data of Gravier-Bonnet from Indian Ocean specimens) and it is here and already reported for the haleciids (Gravier-Bonnet and Mioche, 1996). No data are still available on an eventual role of environmental parameters on the sex differentiation.

The diagnosis of the genus *Nemalecium*, recently reviewed by Calder (1991), has to be emended concerning the production of short-lived medusoids instead of fixed gonophores. Up to now the genus is monotypic. But the differences in shape and size of the corpuscles between specimens from São Sebastião and La Réunion could indicate that we are not dealing with one widespread or near circumtropical species, but rather with two or more sibling species. Some characters – like the shape of the gonotheca and of the hydrotheca, and the number and position of the tentacles – differ slightly between specimens from La Réunion and those described by Bouillon (1986) and Calder (1991). In order to clarify this issue, material from different localities should be morphologically compared, taking into account the whole characters of the skeleton and of the coenosarc (hydranth, gonangium, medusoid), and a detailed study of the nematocysts.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Environmental patterns and biomass distribution of gelatinous macrozooplankton. Three study cases in the South-western Atlantic Ocean*†

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SUMMARY: Periodic swarms or blooms of gelatinous macrozooplankton have a negative effect on many human activities such as tourism, fisheries, and industry, but for several reasons (sampling procedures, underestimation of their real abundance, etc.), they have often been neglected in the local literature. The "high spatial resolution" exercise of the South-western Atlantic anchovy *Engraulis anchoita* Recruitment Project (SARP) was therefore also suitable for estimating standing stocks of "jelly" macrozooplankton, attempting to establish particular environmental patterns exerting control on the spatial distribution of these facultative carnivorous predators in coastal frontal environments. These studies were carried out through a sampling programme on board the German R/V "Meteor" in three different systems, convergence and divergent, in the South-western Atlantic Ocean: Region A (42°S) on the Argentine shelf, characterised by tidal mixing fronts; Region B (36°S), the freshwater outflow from Río de la Plata; and Region C (28°S), under upwelling events in subtropical waters on the Brazilian shelf. In general, a dominance of gelatinous macrozooplankton, compared with the other fraction of macrozooplankton and micronekton was observed. Mean standing stock of the gelatinous zooplankton was always greater than 50% of organic carbon (org. C) in every section analysed. The lobate ctenophore *Mnemiopsis leidyi* dominated the zooplankton biomass in Region A, Argentina. It represented 60% of total org. C and was more abundant at the stratified zone of the front. Ctenophores were also dominant in Region B, Río de la Plata, where the related species *Mnemiopsis mccradyi* and the cydippid ctenophore *Pleurobrachia pileus* comprised 81% of total org. C. *Mnemiopsis* was most common in areas of vertical thermal and saline stratification, while *Pleurobrachia* was dominant in the less stratified areas. Gelatinous zooplankton was also the principal component of the macrozooplankton biomass in Region C, Brazil. The hydromedusae *Rhacostoma atlantica* and *Olindias sambaquiensis* dominated both the total and gelatinous biomass (68% and 7% of total org. C), being always more abundant under lower thermal stratification. It was found that, both in convergent and divergent local systems, gelatinous plankton tended to aggregate in areas where the presence of isolines outcropping to the surface (associated with production processes) was observed. These results are discussed in the context of existing hypotheses regarding ecosystem production and food webs.

Key words: Hydromedusae, Ctenophora, biomass distribution, gelatinous organisms, neritic Macroplankton and micronekton, hydrographic structures, South-western Atlantic Ocean.

INTRODUCTION

It is well known that gelatinous macrozooplankton (this includes members of the phyla Coelenterata, Ctenophora and Tunicata), reach important concentrations in certain areas of the world. Aggregations and blooms seem to be usual phenomena for "jelly plankton" (Boero, 1991) and their periodic swarms have a noxious effect on many human activities such as tourism, fisheries, and industry (see Mianzan and Cornelius, 1999). Good examples of these kinds of problems include: *Pelagia noctiluca* stinging swarms in the Mediterranean Sea (Rottini

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Sandrini and Stravisi, 1981; Bernard, 1984; Rottini Sandrini and Avian, 1991); Black Sea losses of millions of dollars of pelagic fisheries due to competition and predation by the ctenophore *Mnemiopsis leidyi* (Vinogradov *et al.,* 1989; Zaika and Sergeyeva, 1990; GESAMP, 1997; G.R. Harbison, pers. comm. 1993), and clogging on the refrigeration inlet of power plants in the Baltic Sea due to swarms of *Aurelia aurita* (Möller, 1984).

The above comments also reflect the fact that the gelatinous plankton is well prepared to impact resources of a system under stress conditions, such as anthropogenic processes (overfishing, eutrophication of semi-enclosed areas), natural deviation from normal physical and climatic conditions, etc (Legovic, 1991; Parsons, 1993, 1996; G.R. Harbison, pers. comm. 1993).

The "jelly" plankton is known to generate enormous populations and can even seasonally dominate the zooplankton biomass in bays and enclosed seas (Papathanassiou *et al*., 1987; Möller, 1978, 1980; Shushkina and Musayeva, 1983). Biomass estimations provide an indirect way to identify production of a population or an ecosystem (Zaika, 1972), even though biomass estimations for "jelly" organism are still lacking for several parts of the world due to several methodological reasons (see Arai, 1988). This gap in knowledge and its consequence makes it hard to define the ecological role of the gelatinous plankton in the processes of energy transfer in the ocean.

Greve and Parsons (1977) and Parsons (1979) suggested two major pathways for the transfer of energy up the food web of the sea. One of these pathways, identified for upwelling (highly productive) ecosystems tends toward food production favouring carnivorous fishes. The other one, for convergent (less productive) ecosystems, tends toward food chains favouring carnivorous jellyfishes (medusae and ctenophores).

Several objections to this hypothesis have been published, based on its generalization (see Arai, 1988; Smayda, 1993) and recently Mills (1995) modified the endpoint of the hypothesis by stating that jellyfishes are present nearly to the same extent in convergent ecosystems as in upwelling ones.

Both hypotheses (Greve and Parsons, 1977; Mills, 1995) refer to ecological rules on a global scale. At meso-scale (100-1000 km), very little is known about jellyfish distribution, since it is mainly affected by physical processes (bottom-up control) such as local upwelling (divergence), river plume fronts, tidal fronts (convergence) (see Arai, 1992). These types of coastal systems are present in the south-western Atlantic Ocean (Bakum and Parrish, 1991).

The "high spatial resolution" exercise of the Southwest Atlantic anchovy *Engraulis anchoita* Recruitment Project (SARP) was therefore suitable for also estimating standing stocks of jelly plankton and provided the first possibility to test the above - mentioned hypotheses. The studies were carried out in three different regions (Fig. 1) in the South-western Atlantic Ocean (Alheit *et al*., 1991) and comprised:

a- An area in the Argentine shelf, Region A (42°S), characterized by tidal mixing fronts off Península Valdés (Carreto *et. al*., 1986a; Glorioso, 1987).

b- An area under the influence of the Río de la Plata freshwater outflow, in the Argentine-Uruguay shelf, Region B (36°S) (Carreto *et al*., 1986b; Elgue *et al*., 1986; Guerrero *et al*., 1997a,b), and

c- An area characterized by upwelling events in subtropical waters on the Brazilian shelf off Cape Santa Marta Grande, Region C (28°S) (Emilson, 1961; Matsuura, 1986).

The main purpose of this paper is to analyse the biomass of gelatinous, facultative carnivorous predators and the main hydrographic features associated with the study areas, in order to establish environmental patterns exerting control on the spatial distribution of jelly plankton.

OCEANOGRAPHIC FEATURES AND PRODUCTIVITY OF THE STUDY AREAS

Longhurst *et al*. (1995) described the Coastal Domain of the South-western Atlantic as one of the most productive regions of the World Ocean. However, within this large ecosystem, productivity is not a homogeneous feature, but is increased locally by several physical processes. The three main scenarios selected are:

Region A: The Patagonian Continental Shelf has high levels of dissipation of tidal energy (Simpson and Bowers, 1981; Glorioso, 1987). The mixing induced by tidal currents contributes to 8.5% of total tidal energy dissipation of the world ocean (Miller, 1966). The tidal front off Península Valdés is a thermal front, observed in spring and summer, that defines the boundary between stratified (offshore) waters and a coastal, vertically mixed body of water. The stratification of shelf waters is induced by surface warming during spring and summer periods,

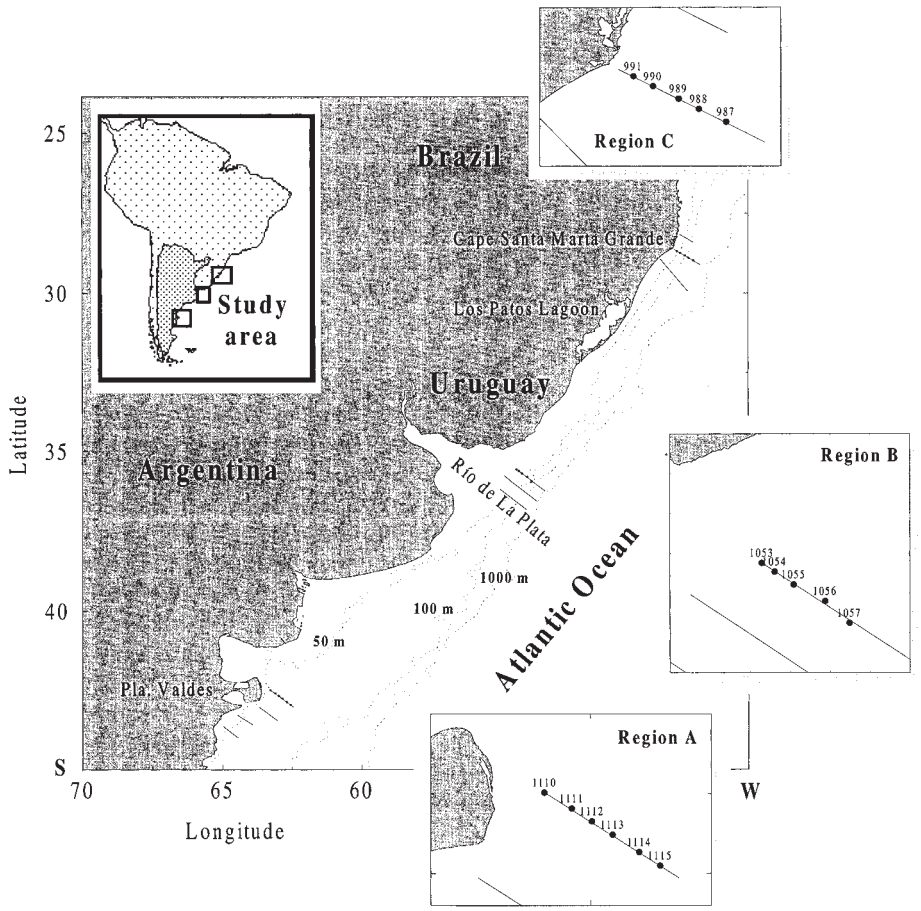

FIG. 1. – Sampling sections and station location of the survey carried out in three hydrographic systems (Region A: Argentina; Region B: Río de la Plata and Region C: Brazil), in the South-western Atlantic Ocean, with the R/V Meteor 11/3 in November-December 1989.

and the mixing of the coastal water is forced by vertical shear induced by tidal currents at particular topographic shoals south-east and north-east of the peninsula (Carreto *et al*., 1986a; Glorioso, 1987). Carreto *et al*. (1986a) demonstrated that this front is highly productive during the spring and summer.

Region B: The Río de la Plata is a coastal plain estuary, draining fresh/brackish water over the continental shelf through a 230 km width open mouth (Punta Rasa, Argentina to Punta del Este, Uruguay). Stratification is consequently controlled by the confluence of a high buoyancy continental discharge advecting offshore, lying on denser shelf water that intrudes into the estuary as a topographically-controlled wedge (Guerrero *et al*., 1997a). The external limit of the surface salinity front, where horizontal salinity gradients indicate the mixing limit between both systems, is observed in the study area (Guerrero *et al*., 1997b). Associated with this front, and limited to the upper few meters, a high chlorophyll-*a* concentration has been reported by the Servicio de Hidrografía Naval (1968), Hubold (1980) and Carreto *et al*. (1986b). A high-productivity band along the front is maintained by injection of river nutrients and particulate matter from estuarine waters (re-supplied by bottom-up fluxes of shelf water), which is advected as a surface plume over the shelf regime.

Region C: Shelf water off Cape Santa Marta Grande is evenly stratified, with warm saline water at the surface driven by the SW Brazil Current overlying much cooler, fresher, and nutrient-rich subtropical water. The bottom is rather steep and narrow, as the shelf is just slightly over 100 km wide. The summer season is characterised by moderate dominant NE winds that force an upwelling condition at the coast (Matsuura, 1986). Less frequently, a downwelling condition is observed when winds reverse to a SW direction. Under either condition (up- or downwelling), a subsurface chlorophyll-*a* maximum layer is present at coastal and near-coastal stations. This layer is maintained primarily by bottom-driven turbulence that injects South Atlantic Central Waters, rich in nitrate, to the euphotic zone (Odebrecht and Djurfeldt, 1996).

MATERIALS AND METHODS

The field sampling was carried out on board the German R/V "Meteor" during November-December 1989 (Cruise 11/3). Macroplankton and micronekton were collected using a rectangular midwater trawl (RMT) (2 x 4m, 1mm-mesh size in the cod end) in the three areas from Brazilian, Uruguayan and Argentine Atlantic waters. A total of 70 hauls were made with this gear: 17 in the Argentine Region A, 12 in the Río de la Plata Region B and 41 in the Brazilian Region C (Figure 1). The samples were taken in sections of about 5 stations each. Information about cruise design, station work and hydrographic conditions are given in Nellen (1990) and Alheit *et al*. (1991). The average filtered volume was 9114.9 m^3 (range 2765,7-20989 m^3). Many sections were not completed because of bad weather conditions or excessive quantities of jelly plankton. In consequence, only one section in Regions A (Stations 1110-15) and B (Stations 1053-57) and 3 sections (15 stations: 982-996) in Region C were completed, the last being analysed as an average of the three sections. Identification was done "*in situ*", sorting, counting and weighting the large-sized material (> 1cm). Small-sized material (< 1cm) was fixed in formalin or alcohol for different purposes and was later analysed in the laboratory. Unidentifiable and damaged specimens were also stored in 4-8% formalin seawater solution for further identification in the laboratory.

For biomass analysis we followed the three categories used by Larson (1986a): gelatinous (Hydromedusae, Scyphomedusae, Siphonophora, Ctenophora and Salps); semigelatinous (Tomopteridae, Pteropoda, Heteropoda and Chaetognatha) and non-gelatinous (Euphausiida, Stomatopoda, Caprellida, other Decapoda, Cephalopoda and Pisces). Standing stock was estimated by converting wet weight (ww) into org. C using the converting factors of Omori (1969), Baker (1973), Larson (1986a,b) and Schneider (1989). The results are expressed as mg org. C per 100 m^3, to make them comparable with previous estimates of medusa biomass (Hay *et al.,* 1990). Identification of gelatinous taxa is based on Mayer (1912), Kramp (1961), Alvariño (1981), Alvariño *et al*. (1990), Esnal (1981), Larson (1986b), Harbison (1986), Mianzan (1986a,b; 1989a; 1999) and Mianzan *et al*. (1988).

Temperature and salinity data were collected with a Mark III and a Smart Neil Brown CTDs (the last instrument used in the Brazil Region only) leading to a final vertical resolution of 1m. A total of 239 vertical profiles were obtained: 45, 107 and 67 profiles in Regions A, B and C respectively (Alheit at al. 1991). The CTDs performed at each mid-water trawl (RMT) station were arranged along sections and were correlated with the associated biomass distribution. Temperature was used for describing the coastal front systems (off Brazil and Argentina) as this parameter determines the density stratification (Matsuura, 1986; Glorioso, 1987). Salinity was the parameter used for the estuarine system, as it controls density distribution (Guerrero, *et al*. 1997a).

RESULTS

In general, dominance by gelatinous macrozooplankton, compared with the other fraction of macrozooplankton and micronekton, was observed. Although specific composition was different for the three regions, mean standing stock of the gelatinous organisms was always greater than 50% of org. C along every section analysed.

The lobate ctenophora *Mnemiopsis leidyi* A. Agassiz, 1865, dominated the zooplankton biomass in the tidal front off Península Valdés (Region A,

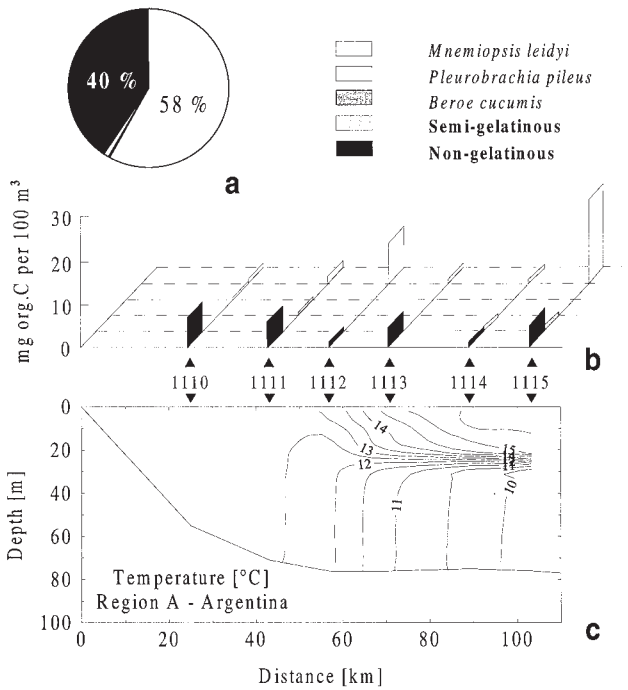

FIG. 2. – a: % of biomass contribution (mg org. C per 100 m^3) of major components of the macrozooplankton; b: distribution of major components of the macrozooplankton (mg org. C per 100 m^3) and c: temperature vertical section along the northern section of Region A, off Argentina, during December 1989.

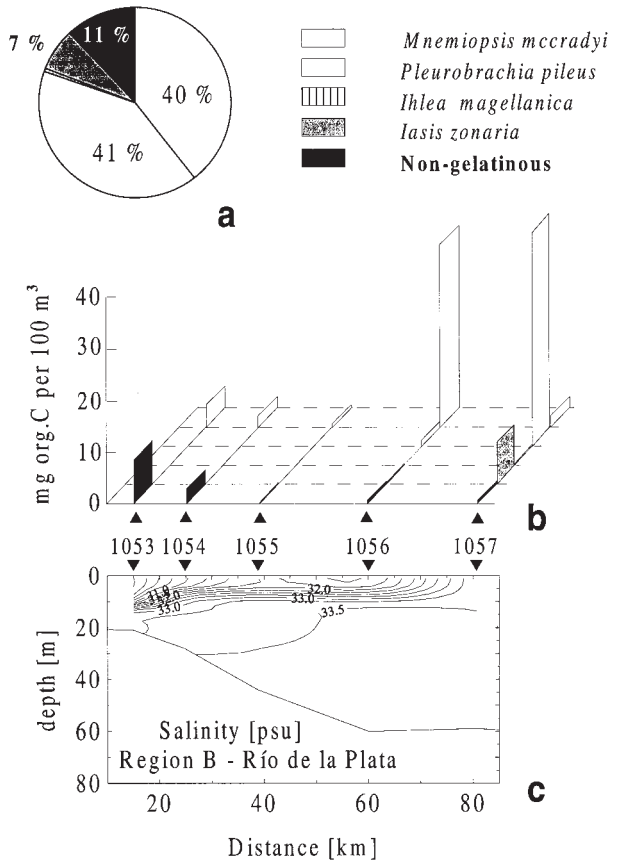

FIG. 3. – a: % of biomass contribution (mg org. C per 100 m^3) of the major components of the macrozooplankton; b: distribution of major components of macrozooplankton (mg org. C per 100 m^3) and c: salinity vertical section along the northern section of Region B, off Río de la Plata (Argentina-Uruguay), during November 1989.

Argentina). It represented almost 60% of total org. C, and reached 20.2 mg org. C per 100 m^3 (Fig. 2a). It was more abundant in the deeper sampling stations of the section (Fig. 2b). The maximum value was observed at the highly stratified station (St.1115), and a secondary maximum was located at Station 1112, where the isotherm outcrops to the surface (Fig. 2c). Vertical temperature gradients at the stratified side were 0.5°C/m (15 to 10°C). Horizontal gradients were not strong (0.6°C/10km). Small biomasses of the ctenophores *Pleurobrachia pileus* (O.F. Müller, 1776) and *Beroe cucumis* Fabricius, 1780, were also obtained here.

Non-gelatinous zooplankton was nearly evenly distributed across this section, represented by the crustacean *Pterosquilla* sp., euphausids, pycnogonids, tomopterids, the cephalopods *Rossia tenera* (Verril, 1880) and *Illex* sp. and fish larvae of *Engraulis anchoita* Hubbs and Marini,1935, *Merluccius hubssi* Marini, 1933 and *Genypterus blacodes* (Schneider, 1801).

Ctenophores were also dominant in the surface salinity front (Region B, Río de la Plata) in the northern section. The lobate *Mnemiopsis mccradyi* Mayer, 1900 and the cydippid *Pleurobrachia pileus* comprised 40% and 41% of total org. C (Fig. 3a) and reached maximum values of 35.25 and 42.9 mg org. C per 100 m^3 respectively. The presence of maximum concentrations of *Mnemiopsis mccradyi* was related to those stations located at the surface outcropping of the halocline (in the surface salinity front: St. 1053 and 1056), while *Pleurobrachia pileus* and the salp *Iasis zonaria* (Pallas, 1774) were dominant at the station 1057 that defines the outer limit of this outcropping (Figs. 3b, 3c). Another gelatinous member rarely collected, the salp *Ilhea magellanica* (Apstein, 1894), was also found here.

Horizontal salinity gradients of 0.1 psu/km and vertical gradients greater than 0.5 psu/m indicate, as expected, that salinity distribution controlled total stratification, even though a strong vertical temperature gradient was present (0.5°C/m). Horizontal temperature gradients were 0.64°C/10km (Fig. 3c).

Non-gelatinous zooplankton dominated the inner estuarine regime, represented by crustaceans including stomatopods, caprellids, euphausiids, mysids and others, cephalopods and pisces *Engraulis anchoita* and other unidentified fish larvae.

Gelatinous zooplankton was also the main component of the macrozooplankton biomass in the upwelling at Cape Santa Marta Grande (Region C, Brazil). In this case, the hydromedusae *Rhacostoma atlantica* L. Agassiz, 1850 and *Olindias sambaquiensis* Müller, 1861, dominated the total biomass (68% and 7% of total org. C respectively) and reached very high biomass values. These values are one order of magnitude higher than in the other Regions (averages values 335 and 39 mg org. C per 100 m^3; maximum values: 572 and 80 mg org. C per 100 m^3 respectively). These two species were aggregated at coastal stations, where the 22° and 23°C isotherms intersect the surface (Figs. 4b and 4c). These stations also showed the coolest temperature and shallowest thermoclines of the section. Calycophoran siphonophores *Sulculeolaria quadrivalius* Blainville, 1834, *S. monoica* (Chun, 1888), *Lensia subtilis* (Chun, 1886), *Lensia hardy* Totton, 1941, *Agalma elegans* (Sars, 1846), *Diphes bojani* (Eschscholtz, 1829), *D. dispar* Chamisso and Eisenhardt, 1821, *Diphyopsis mitra* (Huxley, 1859), *Chelophyes appendiculata* (Eschscholtz, 1829), *Muggiaea kochi* (Will, 1844), *Abylopsis eschscholtzi* (Huxley, 1859), *A. tetragona* (Otto,

1823), *Bassia bassensis* (Quoy and Gaimard, 1833), and *Enneagonum hyalinum* Quoy and Gaimard, 1827 and the salps *Thalia democratica* (Forskål, 1775), *Salpa maxima* Forskål, 1775 and *S. fusiformis* Cuvier 1804 were the most abundant quantitatively, but their total biomass only reached 4%. The scyphomedusae *Aurelia aurita* (Linné,1758) and *Pelagia noctiluca* (Forskål, 1775) contributed a further 2% to the total org. C. Other organisms collected in very small quantities were the ctenophores *Beroe ovata* Chamiso and Eysenhardt, 1821, *Cestum veneris* Lesueur, 1813, *Pleurobrachia pileus* and *Mnemiopsis mccradyi*, and the floating athecate hydroid *Velella velella* (Linné, 1758). Among the semi-gelatinous species, chaetognaths and pteropods were found. In the non-gelatinous category, we found stomatopod and caprellids crustaceans, pisces *E. anchoita* larvae, and the cephalopod *Argonauta* sp.

Along each section a different degree of stratification was observed. The stronger horizontal changes in the parameters considered here, off Peninsula Valdés (Region A, Argentina) and at the Río de la Plata (Region B, Argentina-Uruguay), define the tidal and estuarine fronts respectively at the outcropping of the isolines (Figs. 2c and 3c). The upwelling at Cape Santa Marta Grande (Region C, Brazil) exhibits a relatively weaker front at the coastal stations (Fig. 4c). The tidal front off Peninsula Valdés was the only system with a purely homogeneous water column. The maximum biomass of *Mnemiopsis leidyi* was observed in the deeper station with the maximum stratification and a second biomass maximum was located at a frontal station. At the Río de la Plata estuary, the front is situated between Stations 1056 and 1057, associated with the maximum aggregation of *Mnemiopsis mccradyi* and *Pleurobrachia pileus*. The Brazilian Region showed a regime going from total to partial stratification at the coast. For all three sections performed, those stations with a relative maximum in hydromedusan biomass were situated at minimum stratification stations (onshore), where the isotherms outcrop to the surface.

DISCUSSION

Ctenophores and hydromedusae were found to dominate the gelatinous as well as total macroplanktonic biomass within the three hydrographic systems analysed here.

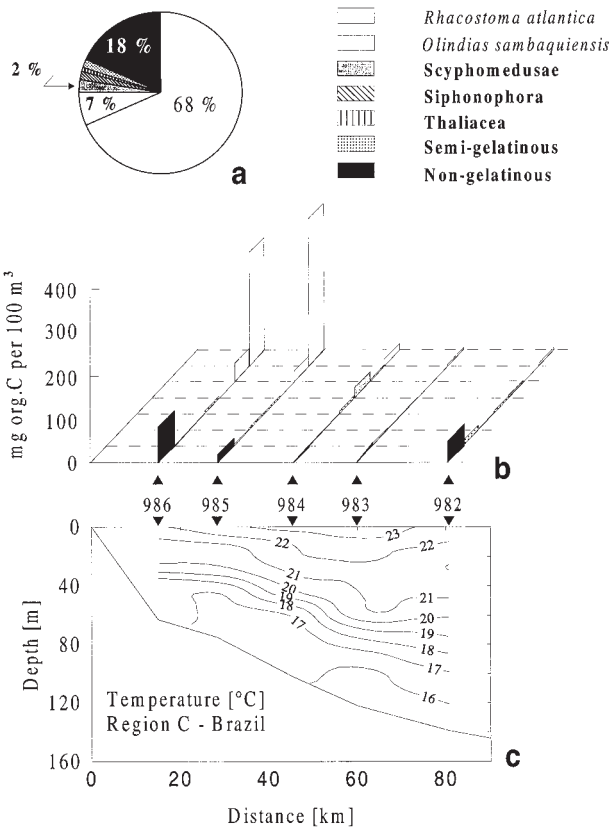

FIG. 4. – a: % of biomass contribution (mg org. C per 100 m^3) of major components of the macrozooplankton; b: distribution of major components of the macrozooplankton (mg org. C per 100 m^3) and c: vertical temperature section along the central section of Region C, off Brazil, during November 1989.

Most previous papers stating that certain species of the gelatinous community reach important biomass are referring to scyphomedusae, usually having high individual weights. Shenker (1984) found that swarms of *Chrysaora fuscescens* Brandt, 1835 off the coast of Oregon contained at least 80% as much carbon as the densest concentration of copepods there. In the fall, *Aurelia aurita* has been found to reach a wet weight 20 times greater than that of the entire remaining plankton in the Black Sea (prior to the arrival of *Mnemiopsis* there) (Shushkina and Musayeva, 1983). However, biomass estimations of hydromedusae and moreover of Ctenophora are less abundant, and reference to their biomass contribution is sometimes underestimated.

In the tidal front off Península Valdés (Region A, Argentina), the ctenophore *Mnemiopsis leidyi* dominated the total biomass, although the values are small in comparison with others reported from the same location and cruise. As previously reported by Alheit *et al.* (1991) using a BIOMOC sampler, this species reached 20 times greater biomass (expressed

as volume) in the stratified zone of a southern section. The RMT sampler was not used in areas with great concentrations of ctenophores due to its possible loss, so only the northern section (Stations 1110-15) was completed. There, the population of this ctenophore was in a juvenile stage (individuals of about 1cm length) and we can assume that we sampled during the initial steps of development of the population. In consequence, a much higher gelatinous biomass should be expected within a short time. In the Azov Sea (Volovik *et al*., 1993) similar values for *Mnemiopsis* during the beginning of a bloom were reported (10.5 mg C org. per 100 m^3).

In the river plume front of the Río de la Plata (Region B), gelatinous plankton also dominated the biomass and the ctenophore *Mnemiopsis mccradyi* was responsible for the aggregations observed. In the Río de la Plata area, aggregations of jelly plankton were also reported to dominate gelatinous and total biomass in Samborombón Bay (36°S, 57°W). This ctenophore and the hydromedusa *Liriope tetraphylla* (Chamisso and Eysenhardt, 1821) peaked during spring and summer respectively, and represented 32% and 11% of the total biomass (15 and 4 mg org. C m^{-3}) (Sorarrain, 1998). This hydromedusa was not collected in significative numbers in the present study, probably because of its small number during this season and also due to the gear employed. *Pleurobrachia pileus*, another important component of the studied community, is more oceanic. It was found in association with several salps, usually in the Malvinas Current. A related species *Pleurobrachia bachei*, also reaching important biomass ranked first in Saanich Inlet, British Columbia (Larson, 1986c).

Off Cape Santa Marta Grande (Region C, Brazil) two hydromedusae were significantly abundant. *Rhacostoma atlantica* is a common neritic and mesopelagic species, well documented for the South-western Atlantic Ocean (Bouillon, 1999). *Olindias sambaquiensis*, also a neritic species, affects summer recreational activities on the Argentine coast (Mianzan, 1989b; Mianzan and Ramírez, 1996). High concentrations of both of these species have not been reported previously for these waters, even though it has been established that off shore winds increase the probability of finding *Olindias* on the beaches, giving upwelling condition (Mianzan and Zamponi, 1988).

According to Arai (1992), aggregations of hydromedusae are due to a combination of the effects of physical forces and behavioural responses, being often difficult to distinguish between passive and active factors. A passive factor contributing to gelatinous aggregation in Regions A (tidal front) and B (Río de la Plata estuary) is the vertical stratification of the water column. In Region C (Cape Santa Marta Grande), aggregation observed in the less-stratified sector seems to be controlled by the upwelling of deeper offshore waters driven by NE winds. A common feature found in all three systems was the presence of isolines emerging to the surface. This outcropping indicates a break of the stratification and the formation of a front that separates a stratified regime from a homogeneous one. These fronts are associated with the convergence of waters and accumulation of particulate matter and planktonic organisms (see Bowman and Esaias, 1977). Intensification of mixing between both regimes also characterizes the frontal area, as does a vertical enhancement of nutrient flux and increasing of primary productivity (Pingree *et al*., 1978; Kiørboe, 1993; Odebrecht and Djurfeldt, 1996).

There is a general lack of information on trophic processes in the study area. However, in terms of some active factors, available literature on spatial distribution of phyto-, micro- and mesozooplankton permits us to infer that feeding requirement of gelatinous plankton could be fulfilled in each of the three hydrographic systems under study.

The tidal front is the main feature analysed in Region A, Argentina. The stratified zone of the front is dominated by the dinoflagellate *Alexandrium tamarense (= Gonyaulax excavata* (Braarud)), whereas the homogeneous sector was dominated by chain-forming diatoms (Carreto *et.al*, 1986a). The horizontal distribution of microzooplankton (mainly copepods eggs and nauplii) showed the highest values of abundance at the transitional zone, in coincidence with the maximum values of chlorophyll-*a* (Viñas and Ramírez, 1996). Mesozooplankton (e.g. copepods, mainly calanoids) has its maximum values in the stratified zone (Möhlenkamp, 1996). *Mnemiopsis leidyi* was found in the stratified zone of the front, in agreement with the hypothesis of Greve and Parsons (1977).

The Río de la Plata surface salinity front (Region B) showed a maximum of chlorophyll-*a* (Carreto *et al*., 1986b). According to Gayoso (1996), dinoflagellates and coccolitophores dominated the phytoplankton assemblages in the outer area. Information on micro- and mesozooplankton of the area is scarce. Sorarrain (1998) found that *Mnemiopsis mccradyi* peaked in concordance with the increase

of *Acartia tonsa*, the dominant copepod species within Samborombón Bay. *Mnemiopsis* spp. elsewhere also show a strong association with warm temperatures and waters dominated by the copepod *Acartia tonsa*, a fast-growing, ubiquitous, small-sized species typical of warm coastal environments (Kremer, 1994). Ctenophores are known to swarm in localised patches elsewhere, and it has been postulated that they may be dependent on dense aggregations of prey to support high metabolic demands (Larson, 1987). Mianzan and Sabatini (1985) established that the *Mnemiopsis* peaks observed at Blanca Bay (39°S, 61°W) were related to food availability. Modelling studies seem to support this statement, having shown that food availability is the key factor in determining maximum ctenophore biomass (Kremer, 1994). A trophic pathway ending in gelatinous plankton corresponds to patterns proposed by Greve and Parsons (1977).

In Region C (Brazil), analysis of material from the same cruise as studied here states that primary production is very high at the on-shore stations, during both, upwelling and downwelling conditions. This production is sustained by large diatom chains generating the most important phytoplankton biomass along the section (Odebrecht and Djurfeldt, 1996). Matsuura and Kitahara (1995) stated that eggs and small larvae of the anchovy *Engraulis anchoita* were found predominantly in warm, nearshore waters above the thermocline. The species of hydromedusae that dominated the biomass in this area, *Rhacostoma atlantica* and *Olindias sambaquiensis*, are active predators. *Olindias* in particular is capable of consuming fishes as big as its bell diameter (Zamponi and Mianzan, 1985) and a species related to *Rhacostoma*, *Aequorea* sp., includes eggs and fish larvae in its diet (see Alvariño, 1985; Purcell, 1989). During upwelling conditions, it is probably not by chance that these two species are advected to the coast by wind-induced currents (Mianzan and Zamponi, 1988), as their shapes resemble a parachute that undoubtedly contributes to such movement. It is also possible that they were neglected in previous works because the gear employed to collect plankton (Bongo net, Nackthai, multinet, etc) is often inappropriate for sampling very large hydromedusae.

Higher jellyfish biomasses were found in the tidal front off Península Valdés and the Río de la Plata outflow, as well as in the upwelling of Cape Santa Marta Grande, so when one compares different kinds of systems (convergent and divergent areas), this results seems to be collectively coincident with Mills (1995). However, when one considers each of the systems separately, the trophic pathways proposed by Greve and Parsons (1977) seem to be observed in each of them. The spatial distribution of gelatinous plankton biomass analysed along sections for the three areas was not homogeneous, as jellyfishes and ctenophores tended to aggregate in particular areas of each hydrographic feature. These areas of aggregation corresponded mainly to the presence of isolines outcropping to the surface, where higher production processes occurred, satisfying metabolic requirements of the aggregation of gelatinous populations maintained there.

As a general conclusion, it is suggested that local environmental patterns exert control on the distribution of biomass of gelatinous predators. Consequently it is suggested that further work on vertical distribution and succession patterns, as well as better spatial coverage of the sections of the different regimes, is needed in order to properly asses those hydrographic features.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

A new species of *Pachycordyle* (Hydrozoa, Clavidae) from Lake Biwa (Japan), with remarks on this and related Clavid genera*

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SUMMARY: The history of research on species of *Pachycordyle* and related genera is discussed. A description and differential diagnosis of a new species, *Pachycordyle kubotai*, is presented. Hydroid colonies, hydranths, and gonophores of this species are described in detail. Peculiarities of medusoid development and oocyte maturation are analyzed. The genus *Pachycordyle* is rediagnosed and an identification key to species assigned to it is provided. *Clavopsella* is regarded as congeneric with *Pachycordyle*. *Thieliana* is established as a new genus for species subsequently and erroneously assigned to *Clavopsella*. The taxonomic status of species referable to *Thieliana* is discussed. Justification is provided for our position that these genera belong to the family Clavidae. Characteristics of genera assigned to the family Clavidae are summarized. Data on the geographic distribution and ecology of the species of *Cordylophora*, *Pachycordyle*, and *Thieliana*, referred here to the subfamily Cordylophorinae, are presented.

Key words: freshwater and brackish Cnidaria, morphology, life history, nematocysts, karyotypes.

INTRODUCTION

The genus *Pachycordyle* and its type species, *P. napolitana*, were described at the end of the 19th century (Weismann, 1883). However, the primary interest of August Weismann, a well-known German professor of zoology at the University of Freiburg and famous evolutionist, was research on the origin and maturation of germ cells in various hydroid and siphonophore species from the Bay of Naples. Unfortunately, his description of *P. napolitana* was incomplete because he gave neither a full diagnosis nor full illustrations of colonies, hydranths, or gonophores. The single illustration that he provided portrays a longitudinal section of a fragment of a pedicel, the gastroderm of which contained developing spermatoblasts (no female gonophores were illustrated). Major features used by Weismann as a basis for the establishment of a new genus, and for the new species *P. napolitana*, were the sparse branching of the colonies, "one whorl" of tentacles at the base of the hydranth hypostome, and attached gonophores that developed into medusoid-like nodules on the stem or branches. These attributes are of

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limited use in distinguishing *P. napolitana* from related species. Much attention was paid by Weismann to the layer in which germ cells developed. He assumed that germ cells had an ectodermal origin whether they matured in the endoderm (*Pachycordyle*) or in the ectoderm (*Cordylophora*). Much more important, in our estimation, is the formation and development of the gonophores. Unlike in *Pachycordyle*, those of *Cordylophora* do not form medusoid nodule but are transformed into sporosacs.

Hargitt (1904) described colonies of another hydroid of this genus from the Bay of Naples, with female gonophores, which he named *P. weismanni*. Medusoids of this hydrozoan developed the rudiments of a velum, marginal tentacles, and a ring canal. Hargitt found no radial canals and no mouth on the manubrium of the medusoids. According to the terminology of Kuhn (1913), such a gonophore should be termed a "eumedusoid". Hargitt observed that the medusoid of *P. weismanni* was liberated as an ephemeral, free-living (no more than 1-2 hours), abortive medusa. Its manubrium bore numerous eggs, which were shed into the water shortly after release of the medusa.

Hargitt (1904: 555, 556) believed that germ cells were formed in the ectoderm and he provided a relatively complete diagnosis of the hydroid that he named in honour of Weismann. The same year, Mayer (1904) described *Parvanemus degeneratus* from the Bahamas, later assigned (Mayer, 1910) to *Pachycordyle* as *P. degeneratus*. An additional nominal species, *P. annulata*, was described from the Mediterranean by Motz-Kossowska (1905). Like *P. weismanni*, it has been included in the synonymy of *P. napolitana* by most researchers. *Parvanemus* was included, with some doubt, in the synonymy of *Pachycordyle* by Calder (1988).

Notwithstanding the rather distinctive characteristics of *Pachycordyle*, many specialists have regarded it as a synonym of *Cordylophora* (Picard, 1958; Morri, 1980, 1981). The diagnosis of the latter was broadened (Morri, 1980) to include species in which gonophores varied in degree of reduction from sporosacs to free medusae.

Stechow (1919, 1921) twice repeated a proposal of the genus name *Clavopsella* for *C. weismanni* and *C. annulata*. The principal attributes of the genus, according to Stechow, were: (1) tentacles of polyps restricted to the apical part of the hydranth, and (2) gonophores medusoid, lacking radial canals and a mouth on manubrium but with a ring canal and rudiments of a velum. *Pachycordyle weismanni* was designated the type species of *Clavopsella*. Stechow did not retain *Pachycordyle* and ignored *P. napolitana*, the type species of *Pachycordyle*.

Thiel (1962) described as *Clavopsella quadranularia* a hydroid from the Kiel Canal. Millard (1975) concluded that the species was identical with *Rhizorhagium navis*, described by her earlier (Millard, 1959) from South Africa. Also part of this group, in our opinion, is *Cordylophora inkermanica* (Marfenin, 1983) from the Black Sea. It seems likely that all are conspecific. Thiel (1962) was the first to describe in detail the very peculiar life cycle in this species. He established a new family, Clavopsellidae, for it and for the related genus *Balella*.

There is some uncertainty about the taxonomic affinities of these hydrozoans. Weismann (1883) compared *Pachycordyle* with *Corydendrium* and *Cordylophora* (family Clavidae). Stechow (1923) included *Clavopsella* in the same family, although he earlier (Stechow, 1919) had assigned it to the Bougainvilliidae. Millard (1975), Calder (1988), and others included *Pachycordyle* in the Bougainvilliidae (but *Cordylophora* in the Clavidae). Thiel (1962) referred the genus to the family Clavopsellidae. Our arguments in favour of assigning *Pachycordyle* to the family Clavidae are presented at the end of this paper.

MATERIALS AND METHODS

The benthic biota of Lake Biwa (central Japan) was sampled as part of biodiversity studies during joint Russian and Japanese expeditions in 1996-97. Small epibiotic colonies of hydroids (Fig. 1A) were found by Dr. O. A. Timoshkin along the western shore of the southern part of the lake in front of the site of the Center for Ecological Research, Kyoto University (the Center has since moved). The samples were from a depth of 0.5-2.0 m. Such polyps are previously unreported as part of the fauna of the lake. The morphology and histology of the hydroids were studied in detail based on live and preserved material. Photographs (black-and-white and colour) were also taken of live specimens. Nematocyst morphology and structure of medusoids (both external and histological) were examined and photographed using a stereoscope (MBI) and a compound microscope (Amplival, with phase contrast optics and an automatic camera). Chromosome preparations were made using an air-drying technique (Ovanesyan and

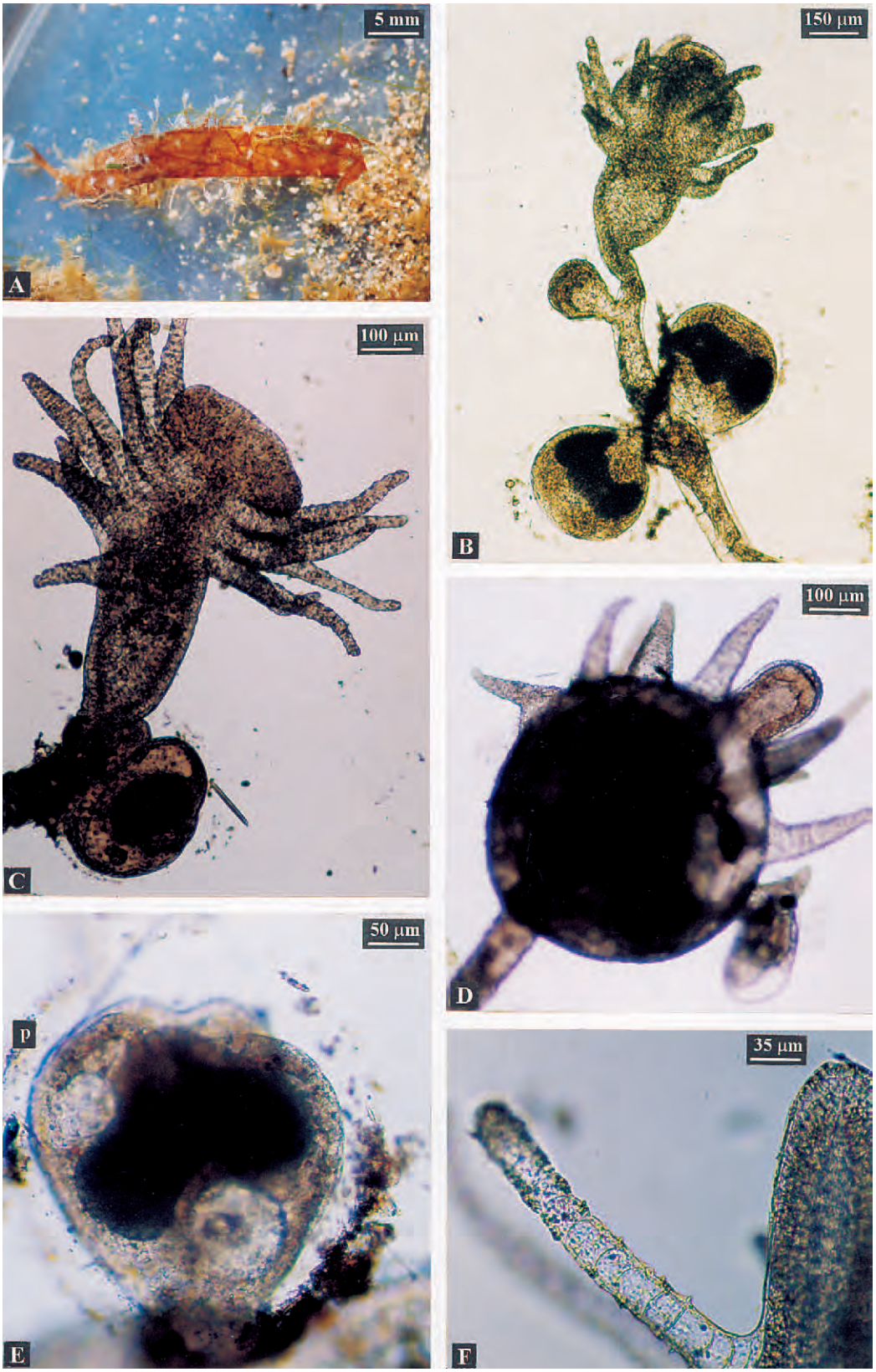

FIG. 1. – Type colony of *Pachycordyle kubotai* sp.n. from Lake Biwa (photographs by O. Timoshkin); A, fragment of colony collected 15.07.1997 on western coast of south Lake Biwa; B, stem with terminal hydranth and 3 gonophore buds; C, contracted hydranth with imprecise whorls of tentacles and opened mouth of hypostome; D, head of a satiated hydranth with contracted tentacles; E. Eumedusoid, p - perisarc; F, solid polyp tentacle showing its vertical row of gastrodermal cells.

Kuznetsova, 1995). Specimens were incubated in 0.4% sodium citrate and fixed in an ethanol-acetic acid mixture (3:1). Specimens were then placed individually on a slide and macerated in 70% acetic acid. Thereafter, slides were air-dried and stained by the conventional Giemsa technique.

Morphological comparison of the specimens with accounts of related species indicated that these hydroids constitute a new species. We name it *Pachycordyle kubotai*, in honour of the hydrozoan specialist Dr. Shin Kubota (Kyoto University), who carried out investigations on freshwater Hydrozoa of Japan for his undergraduate thesis.

RESULTS

Pachycordyle kubotai sp. n.

Holotype: Colony composed of a hydrorhiza growing on a small piece of wood; western coast of southern Lake Biwa (central Japan), in front of the then site of the Center for Ecological Research, Kyoto University; depth 0.1-1.0 m, 5-11.07.1997; 24.7-25.1 C; ZIN RAS (Zoological Institute, Russian Academy of Sciences) nº 10302/1. A fragment is also deposited in the Lake Biwa Museum (slide in Canada Balsam).

Paratypes: nº 1: colony composed of small, unbranching or irregularly branching stems arising from stolonal hydrorhiza; collection as for holotype except depth ca. 1.0 m; 5-17.07.1997; ZIN RAS nº 10303/2. nº 2: small, sterile colony composed of unbranching stems, collection data as for Paratype nº 1; ZIN RAS nº 10304/3. nº 3: unbranched, sterile stems rising from hydrorhiza covering a fragment of a plant; locale as above; depth unrecorded; ZIN RAS nº 10305/4.

Other Material: In ZIN RAS collections, a fragment of a colony with hydranths tangled in algal thalli, in poor condition, hydranths small and contracted. Colony fragments from holding tank nº 4 of aquarium of Lake Biwa Museum; 26.03.1988; coll. Dr. Mark J. Grygier, Mr. Hiromitsu Akiyama, and Dr. Yasushi Kusuoka. The colony from this tank is currently alive in the aquarium of Boris Anokhin (ZIN RAS, St. Petersburg).

Description of holotype: Colony composed of many small stems (not more than 2.2 cm each) rising vertically from filiform hydrorhiza. Each stem with a terminal hydranth having a gonophore-budding zone beneath hydranth base. Colonies typically with one mature gonophore (rarely two) and several (not more than 4-5) immature ones at different stages of development. Hydrorhiza and stems covered with rather dense, light brown perisarc annulated along almost entire length, reaching base of hydranth where annulation is particularly distinct. Hydranths of living colonies elongate-fusiform, with hypostome elongate-conic (when polyp is hungry) or nearly spherical (when polyp is satiated) (Fig. 1D). Mouth in form of an opening into canal of hypostome, open or turned inside out (if polyp is ready to swallow food) (Fig. 1C). Tentacles solid, with a central core of gastrodermal cells lying one under the other (Fig. 1F). Tentacles located on upper half of hydranth around and slightly below hypostome, with 3-4 indistinct whorls, appearing randomly arranged ("Cordylophora-like") in hungry polyps (Fig. 1C, D). Tentacles varying in number from 11-14 (rarely to 17). Erect tentacles of hungry polyp fairly long, extending beyond hypostome. Tentacles of satiated polyps contracted, about 1/4 length of those above, whorls appearing coalesced and merged almost into one ring.

Gonophores budding on stem below hydranth (Fig. 1B, E). Oocytes developing in ectoderm (Fig. 2), rather large early in development of medusoid bud. Each gonophore with two oocytes, with a large gastric cavity and four radial canals. Gastroderm of gastric cavity and canals with cubical cells. Lower part of gonophore with well developed mesoglea. Ring canal, velum, and marginal tentacles lacking. Gonophore eumedusoid, surrounded by transparent, spongy, thin, mucous-like perisarc (Figs. 1E, 2C, 6A). The maturity of oocytes (July - see p. 000) indicated that their release was imminent and that the medusoids were nearly ready for release, assuming that they are liberated from the hydroid.

Measurements (in mm). Height of the stem 10.17-20.21; diameter of perisarc stem tube 0.052-0.060; height of polyp head 0.65-0.78; maximal width of polyp head 0.42; length of polyp hypostome 0.09-0.13; width of polyp hypostome 0.07; length of tentacles 0.13-0.20; height of developed gonophore with pedicel 0.39; length of gonophore without pedicel 0.32-0.33; diameter of developed gonophore 0.29.

Nematocysts (examined in live specimens, in mkm): Microbasic euryteles 6.5-7.0 x 3.0-3.5 and desmonemes - 4.5 x 3.0-3.2 (Fig. 3).

Karyotype: 2n = 30. Consists of 30 isomorphic chromosomes forming a gradually decreasing size row. Each chromosome of the fourth pair carries a distinct negatively heteropycnotic region (Fig. 4).

Description of paratypes: (Nos. 1, 2, 3). Paratype colonies differing little from holotype morphologically except for structure of colonies proper (in paratype nº 1, stems can form branches of the first and second orders). Hydranths of paratype colony nº 3 evidently satiated at moment of fixation because their hypostomes almost indistinct, and the heads of

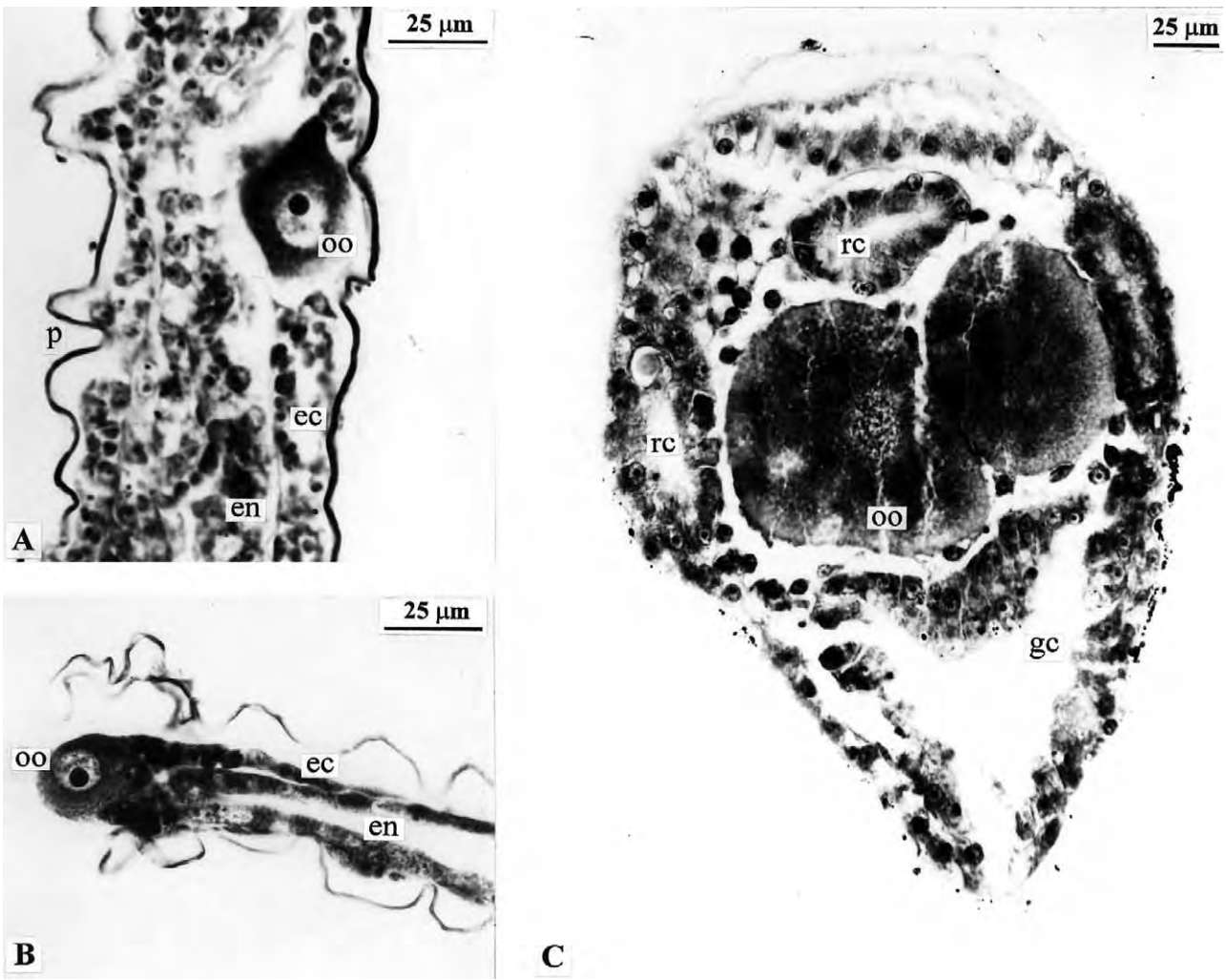

FIG. 2. – *Pachycordyle kubotai*, stages of gonophore formation. A, zone of stem below hydranth with developing oocytes; B, early stage of gonophore bud formation; C, mature eumedusoid. ec - ectoderm; en - entoderm; rc - radial canals; gc - gastral cavity; oo - oocyte

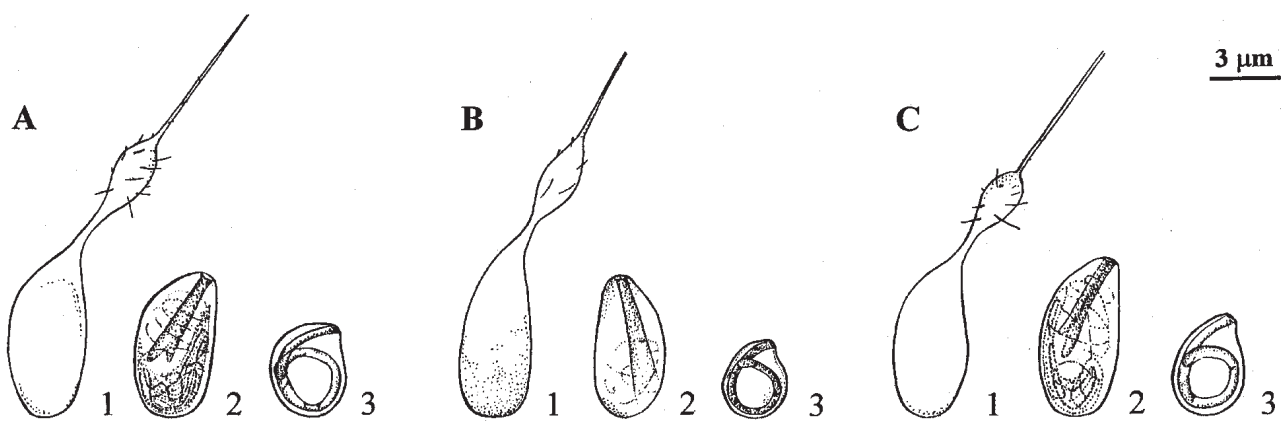

FIG. 3. – Nematocysts of three representatives of the subfamily Cordylophorinae. A, *Pachycordyle kubotai*; B, *Thieliana inkermanica*; C, *Cordylophora* sp. 1,2 - microbasic euryteles: discharged capsula (1) and undischarged capsula (2); 3 - undischarged desmoneme.

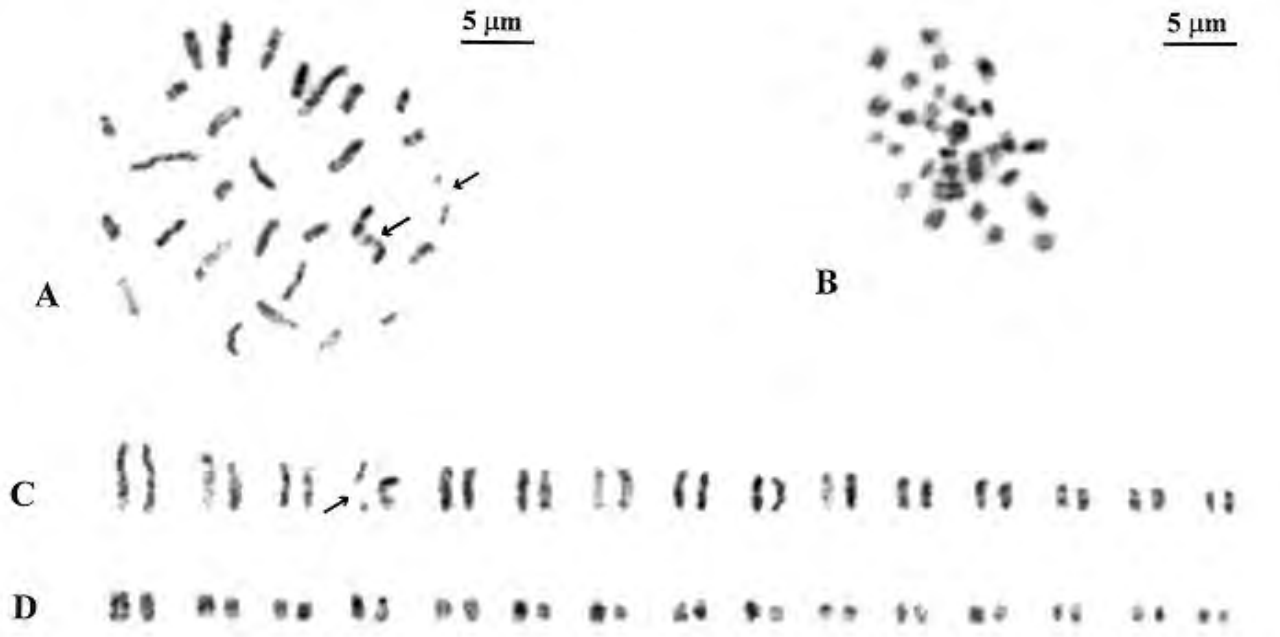

FIG. 4. – Mitotic chromosomes of representatives of two genera of Cordylophorinae. A, *Pachycordyle kubotai*, mitotic metaphase, 2n = 30; B, *Cordylophora* sp., mitotic metaphase, 2n = 30; C, karyogram of *Pachycordyle kubotai*; D, karyogram of *Cordylophora* sp. The negatively heteropycnotic regions on the fourth chromosome pair of *Pachycordyle kubotai* are visible (arrows).

polyps nearly round; their length and width nearly the same - about 0.65 mm.

DISCUSSION

Pachycordyle kubotai is related to and believed congeneric with *P. napolitana* (and its synonyms *P. weismanni* and *P. annulata*). Attributes of the species that conform with the diagnosis of the genus include the following: (1) tentacles of hydranth restricted to distal end, and (2) gonophores eumedusoid. We do not share the opinion of some authors that the degree of medusa development or reduction has no taxonomic significance (Petersen, 1990, as applied to Capitata; Boero, Bouillon, and Piraino, 1996, as applied mainly to Campanulariidae; Boero, Bouillon, and Piraino, 1998, as applied to Hydractiniidae). Our position, outlined in discussions of Corymorphidae (Stepanjants and Svoboda, 1999) is that gonophore development in combination with other attributes (e.g., gonophore position, blastostyle construction, etc.) may be of taxonomic value.

Pachycordyle kubotai possesses some peculiarities distinguishing it from *P. napolitana*:

1. The perisarc in colonies of *P. napolitana* is irregularly wrinkled, whereas the perisarc of *P. kubotai* is uniformly wrinkled.

2. The number of tentacles on polyps of *P. napolitana* varies from 8-20, while those of *P. kubotai* are no less 11 and no more than 17.

3. The female gonophore is elongate-oval in *P. napolitana* and almost rounded in *P. kubotai*.

4. There are 1-4 oocytes in developing female gonophores of *P. kubotai*; this number is larger in *P. napolitana*.

5. Female gonophores of *P. napolitana* have a ring canal and a velum, but no radial canals; female gonophores of *P. kubotai* have 4 radial canals and no ring canal and velum.

6. Both species have the same two types of nematocysts: desmonemes and microbasic euryteles, but the capsules of the latter are slightly larger in *P. napolitana* (10 x 4.5 mkm) than in *P. kubotai* (7 x 3.5 mkm).

7. *Pachycordyle napolitana* inhabits marine basins or bodies of water with reduced salinity (more often about 20‰: Morri, 1980), but *P. kubotai* is found in freshwater only.

Colonies similar in form to this hydroid were found earlier in brackish waters of Japan and described as new species: *Cordylophora japonica* by Ito (1951) and *C. mashikoi* by Ito (1952). Ito provided descriptions and differential diagnoses of these hydroids, comparing his new species mainly with *Cordylophora lacustris*, but distinctions between these species and *C. lacustris* were not

clearly presented by the author. *Cordylophora japonica* is clearly not referable to *Pachycordyle* because its hydranths have tentacles that are irregularly distributed over its surface and its gonophores are sporosacs rather than medusoids. However, *C. mashikoi* may be referable to *Pachycordyle* because its tentacles are located at the distal end of the hydranth just below the hypostome. Female medusoids are unknown for the latter species, and the pattern of male gonophores is poorly described. Nevertheless, *C. mashikoi* differs from *P. kubotai* by its irregular branching of colonies and larger number of gonophore buds (each gonophore is approximately 0.3 mm long), distributed in a budding zone on the ramuli (up to 12 buds on each ramulus).

Negoro (1982) reported the discovery of medusae in the southern part of Lake Biwa, near the Seta River entrance, during October 1977. The medusae were very small (about 600 mkm in diameter) with a manubrium, radial canals, and rudiments of marginal tentacles (judging from illustrations in his atlas). As the illustrated medusa does not resemble the well-known *Craspedacusta*, this medusa might be a species of *Pachycordyle*. However, verification is needed to be sure of the identity. Discovery of the medusa of *P. kubotai* would contribute to knowledge of the life cycle of the species and resolve questions about the family affinities of the taxon.

Stechow (1919) established *Clavopsella* as a new genus, with *Pachycordyle weismanni* as its type species. He did not accept *Pachycordyle* as valid, believing that *P. napolitana* should be referred to *Rhizorhagium*. We do not accept Stechow's view because *Rhizorhagium* (type species *R. roseum*) is a typical bougainvilliid with one whorl of filiform tentacles, a pseudohydrotheca, and gonophores as sporosacs. We consider *Clavopsella* a junior synonym of *Pachycordyle* and retain the older name, which has priority. The spelling *P. neapolitana* used by some authors for the type species is an incorrect subsequent spelling (Calder, 1988).

Genus ***Pachycordyle*** Weismann, 1883

Type species: *Pachycordyle napolitana* Weismann, 1883, by monotypy.

Diagnosis: Clavid colonies characterized by small unbranched or little-branched stems arising from a filiform hydrorhiza. Coenosarc of hydroid colony covered by light-yellow or brown, smooth or partly or fully wrinkled perisarc. Stem and branches terminating in hydranths. Tentacles of hydranths filiform, solid, arranged in several (3-4) whorls on upper half of hydranth around hypostome. Stem or branches with a gonophore budding zone immediately below base of hydranth. Mature gonophore eumedusoid, with radial or ring canals present; velum and marginal tentacles more or less developed. Medusa, if liberated, short-lived and living in plankton not more than several hours. Oocytes from 1-12 in female eumedusoids.

Representatives of the genus inhabit brackish and freshwater environments.

Key to known species of *Pachycordyle*

1(2). Gonophore buds forming on ramuli,
    several on each ramulus .....*P. mashikoi*
2(1). Gonophore buds not on ramuli,
    arising one by one on stem or branch.
3(4). Ring canal and velum present;
    radial canals lacking in eumedusoid;
    up to 10 or more oocytes.....*P. napolitana*
4(3). Radial canals present; velum and ring canal
    lacking; not more than 4 oocytes .....*P. kubotai*

After Stechow (1919) proposed *Clavopsella*, two species were added to the genus: *C. navis* (Millard, 1959) and *C. quadranularia* Thiel,1962. The latter was subsequently referred to the former as a junior synonym (Millard, 1975). A peculiarity in their life cycles is a common attribute of both of these hydroids: developing oocytes are concentrated in the apical part of the medusoid and transform there into planulae before being released into the water by twisting themselves out of the gonophore. In this regard, *C. navis* differs markedly from *P. weismanni*, for which Stechow erected *Clavopsella*. As already noted, *Clavopsella* is a junior synonym of *Pachycordyle*. *Clavopsella navis* and *C. quadranularia*, with cryptomedusoid gonophores and different life cycles, do not belong in this genus. We establish a new genus, *Thieliana*, after H. Thiel, who provided a comprehensive description of the peculiarities of the representatives of this genus in his account of *C. quadranularia*.

No colonies of *Thieliana* quadranularia or *T. navis* were available to us, and it was thus impossible to determine whether the nominal species are conspecific, as Millard (1975) contended.

Besides the above-mentioned two species of *Clavopsella*, we also assign to *Thieliana* a species

from the Black Sea described by N. N. Marfenin (1983) as *Cordylophora inkermanica*. The differential diagnosis of *C. inkermanica* did not mention *Clavopsella navis* and *C. quadranularia*, despite a certain morphological similarity among all three species. Their similarity lies in the same kind of stem branching; a similar perisarc pattern, generally smooth but annulated in some parts; the structure of the hydranths and medusoids, and the size of the latter; the sizes of the planulae emerging from the gonophores; and the sizes of the nematocyst capsules (Figs. 3B, 5D, 6B).

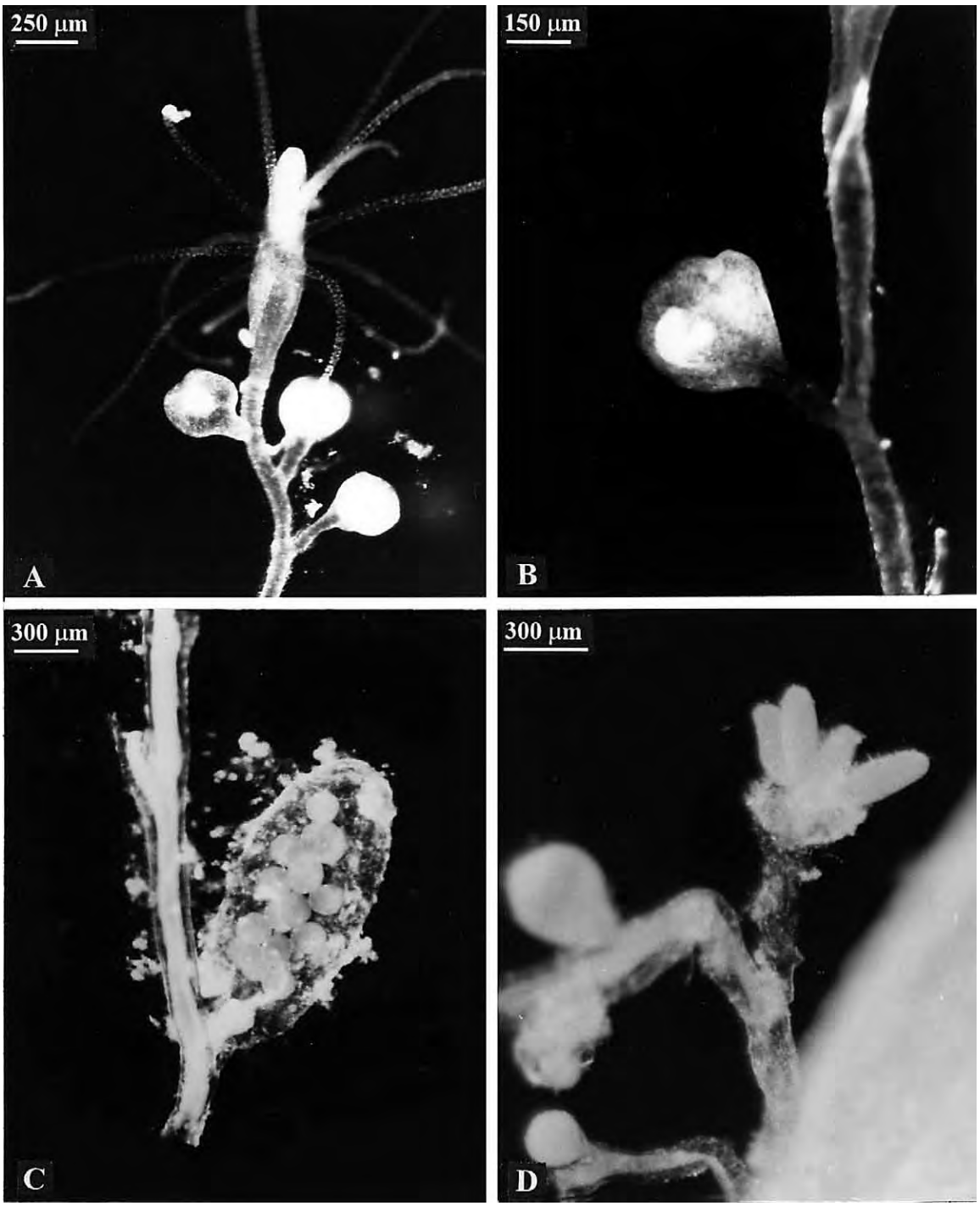

FIG. 5. – Representatives of the subfamily Cordylophorinae (photographs by B. Anokhin). A, fragment of living colony of *Pachycordyle kubotai* from aquarium of Zoological Institute RAS, St. Petersburg: hydranth with gonophores (A) and female eumedusoid (B); C, gonophore of living colony of *Cordylophora* sp. From aquarium of Zoological Institute RAS, St. Petersburg; D, fragment of colony of *Thieliana inkermanica* (Marfenin) with gonophores and mature planulae (collection of Zoological Institute RAS).

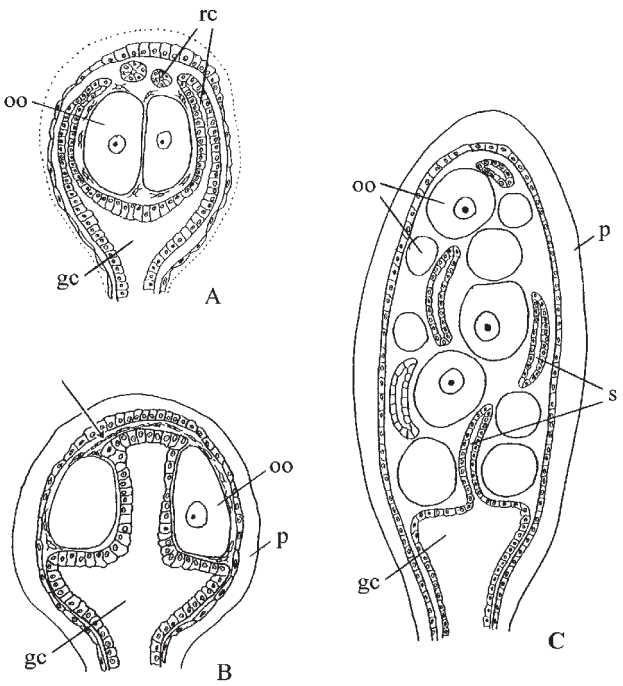

FIG. 6. – Diagrams of female gonophore morphology of representatives of Cordylophorinae. A, eumedusoid of *Pachycordyle kubotai*; B, cryptomedusoid of *Thieliana inkermanica*; C, sporosac of *Cordylophora* sp. oo - oocytes; gc - gastral cavity; s - spadix; p - perisarc; rc - radial canals; subumbrellar cavity rudiment (arrow).

Genus ***Thieliana*** gen.n.

Type species: *Clavopsella quadranularia* Thiel, 1962, by original designation herein.

Diagnosis: Colonies in form of unbranching or branching stems rising from creeping hydrorhiza. Coenosarc of stem and branches covered by smooth or segmented perisarc. Stems and branches terminating in hydranths. Hydranth head ending in hypostome with two whorls of filiform tentacles around its base. Lower part of polyp head lacking tentacles. Budding zone of gonophores on stem or branch below hydranth base. Solitary gonophores on pedicels. Gonophores as cryptomedusoids lacking radial and ring canals, velum, and tentacle rudiments; subumbrellar cavity well-defined (Fig. 6B). Formation of female gonophores very peculiar: oocytes become concentrated in apical part of gonophore in one or two rows, grow into planulae within gonophore, and then leave it (Fig. 5D).

The representatives of this genus are euryhaline marine organisms, which can survive at salinities from 7% up to 35%.

Representatives of *Pachycordyle* have been assigned by different taxonomists to either Clavidae (Stechow, 1923; Morri, 1980), Bougainvilliidae (Millard, 1975; Bouillon, 1985; Calder, 1988), or Clavopsellidae (Thiel, 1962). The last assignment is untenable since *Clavopsella* is considered by us to be a synonym of *Pachycordyle*.

What are the diagnostic characters of the Clavidae and Bougainvilliidae, and where might *Pachycordyle* best be assigned?

The family Clavidae is characterized by solitary or colonial hydroids, the coenosarc of which is covered by perisarc while the hydranths are always devoid of an exoskeleton (i.e., no hydrotheca or pseudohydrotheca is present). Hydranths are fusiform with an elongate-conic hypostome. Tentacles are solid and filiform and either randomly arranged over the hydranth or in indistinct whorls. The tentacle arrangement is best observed in living colonies. Those of a living, hungry polyp appear to be scattered, although they often gather in indistinct whorls after fixation. This sort of tentacle arrangement is seen in *Turritopsis*, *Rhizogeton*, and *Tubiclava*, and also in *Pachycordyle* and *Thieliana*, but to a greater extent. Gonothecae occur on stems or branches, on the body of the hydranth, or rarely on the hydrorhiza. Gonophore development varies from free medusae (*Turritopsis*) to styloid gonophores (*Clava*). Both facultative (*Pachycordyle*) and complete (*Turritopsis, Oceania*) detachment of free medusae are known. The characteristic feature of these medusae is the presence of vacuolated cells in the apical part of the stomach gastroderm. Other features of the medusa are numerous marginal tentacles and a mouth with four lips.

Members of the Bougainvilliidae are exclusively colonial hydroids. Their coenosarc is covered by perisarc generally extending to the hydranth, or even covering it as a pseudohydrotheca. Tentacles are solid, filiform, and commonly arranged in a single whorl around the hydranth below the hypostome. The arrangement is orderly in both living and preserved specimens. Free medusae are characterized by the presence of oral tentacles and groups of marginal tentacles. Gonophores develop on the hydrorhiza, stem, and branches (sometimes on special blastostyles), and vary in development from medusae to styloid gonophores.

Nematocysts are of two categories: microbasic euryteles and desmonemes.

Basaed on the attributes listed above for the two families, we assign *Pachycordyle* and *Thieliana* to the Clavidae.

The family Clavidae includes about 10 genera (Bouillon, 1985), some of which have well devel-

oped free medusae (*Oceania, Turritopsis*). Representatives of other genera have gonophores of varying degrees of degeneration. Some have solitary hydranths arising from the hydrorhiza (*Clava, Tubiclava, Rhizogeton*), and *Hataia* has solitary polyps without a hydrorhiza. *Merona* is unique in the group in having nematothecae. *Corydendrium* differs in having branched colonies in which the branches fuse with the stem, and in having gonophores arising under the perisarcal tubes of the branches. *Cordylophora* appears closest to *Pachycordyle* and *Thieliana*.

How are *Cordylophora*, *Pachycordyle*, and *Thieliana* alike, and how do they differ? First, they are alike in:

1. colony form, with stems arising from a filiform hydrorhiza and branches irregular when present;

2. skeletal form, with perisarc over coenosarc of stem and branches being smooth or more or less wrinkled;

3. growth form (monopodial with terminal hydranths);

4. hydranth shape, being elongate-conic with a conical hypostome (hydranths of satiated hydroids being almost globe-shaped);

5. tentacle arrangement, with scattered filiform tentacles over hydranth;

6. arrangement of gonophores, arising from stems and branches below hydranth bases and not from hydrorhiza;

7. gonophore type, with reduced medusoids or fixed sporosacs rather than free medusae.

These genera differ in the following characters:

1. colony branching pattern and overall height, with *Cordylophora* having stronger, more extensively branched colonies that may reach 10 cm high (we consider *C. caspia* and *C. lacustris* to be distinct and valid species); colonies of *Pachycordyle* and *Thieliana* are smaller (2-3 cm) and less branched;

2. tentacle arrangement, with those of *Cordylophora* being scattered over the hydranth and those of *Pachycordyle* and *Thieliana* being concentrated in several indistinct whorls at the distal end of the hydranth;

3. gonophore morphology, with fixed sporosacs and many oocytes in *Cordylophora* and a different gonophore type in the other two genera (Fig. 6). As noted above, data from Weismann (1883: 30) indicate that sporosac development in *Cordylophora* bypasses the medusoid nodule stage.

We investigated the nematocysts and karyotype of young *Cordylophora* from the Gulf of Finland. Nematocysts of representatives of all three genera, and karyotypes of *Pachycordyle kubotai* and *Cordylophora* sp., are shown in Fig. 3.

The clear similarities listed above indicate that *Cordylophora*, *Pachycordyle*, and *Thieliana* are closely related, and we combine the three in the clavid subfamily Cordylophorinae von Lendenfeld, 1885.

We here redefine the subfamily as follows:

Diagnosis. Colonies with creeping hydrorhiza and with unbranched or irregularly branched stems; branches many or few; colony height from 1-2 cm to >10 cm; coenosarc of stems and branches covered by smooth or wrinkled perisarc; pseudohydrotheca lacking; growth monopodial with terminal hydranths. Hydranths bearing solid filiform tentacles; tentacles scattered or in indistinct whorls. Gonophores arising from a budding zone beneath hydranth base, solitary on pedicels or a few on a ramulus. Gonophore development varying from styloid, cryptomedusoid to eumedusoid, with radial canals, ring canal, reduced marginal tentacles, velum, gastric cavity, and subumbrellar cavity sometimes present. Eumedusoids, when present, may become free and survive for several hours in the plankton. No fully developed free medusae known.

Other groups of Clavidae have representatives with one or more of the following characteristics: (1) free medusae; (2) spreading colonies; (3) solitary polyps; (4) nematothecae; (5) gonophores inside perisarcal tubes of branches.

Key to Cordylophorinae

1(2). Tentacles scattered over surface of hydranth; gonophores styloid.....*Cordylophora*
2(1). Tentacles in several indistinct whorls on upper half of hydranth; medusoids not styloid.
3(4). Tentacles in 3-4 whorls; gonophores eumedusoid, no planulae developing inside gonophore .....*Pachycordyle*
4(3). Tentacles in 2 whorls; gonophores cryptomedusoid, planulae developing inside apical part of gonophore.....*Thieliana*

Ecology

Representatives of the Cordylophorinae are mostly eurybionts. Representatives of *Cordylophora*

are widespread in marine, brackish, and even fresh waters. In terms of geographic range, they have been reported from the North Atlantic; the Mediterranean, Black, Caspian, and Azov seas; continental waters of Europe; and brackish and fresh waters of Japan, Australia, and New Zealand (Bouillon et al., 1995; Schuchert, 1996). They have been found at salinities from 0-35‰ (optimal 5-15‰), and at temperatures from 2-30°C (optimal 6°C) (Kinne, 1963, 1964; Morri,1980; Calder,1990).

Cordylophora occurs on algal thalli, mollusc shells, and pieces of wood.

Pachycordyle has been found in shallow waters of the Bay of Naples and other parts of the Mediterranean (Adriatic and Aegean Seas); the western Atlantic (Wedler and Larson, 1986); Bermuda (Calder, 1988); possibly Nassau Harbour, Bahamas, as abortive medusoids (Mayer, 1910); and lakes of Japan (including the entirely freshwater Lake Biwa as well as the brackish Ono River – Lake Kahokugata, connected to the Sea of Japan). They occur mainly at salinities of 0-20 ‰, but in Bermuda, near Harrington Sound, Calder (1988) found a 2 mm high colony at 2 m depth in water with a salinity 35-36‰. Their temperature range is the same as for *Cordylophora*. Substrates include algae, *Zostera*, mollusc shells, crustacean carapaces, and pieces of wood.

Hydroids of *Thieliana* have been found in Table Bay, South Africa, on hulls of ships; in the Kiel Canal; and in the Black Sea (Inkerman and Jarylgachsky Bays) at depths from 20 cm to 6 m. The reported salinity range is 7-35‰, and the temperature range, 2-20°C. Substrates include mollusc shells, stones, plant debris, pieces of wood, and hulls of ships.

CONCLUSIONS

1. Detailed investigations were made on a new species of hydroid, *Pachycordyle kubotai*, from Lake Biwa, Japan.

2. Careful consideration of its characters, and those of related species, indicates that it belongs to the genus *Pachycordyle*. A new diagnosis of this genus and a key to the species included in it are provided.

3. Morphological studies show that the nominal genus *Clavopsella* is a junior synonym of *Pachycordyle*, while some of its included species are generically distinct. The new genus *Thieliana* is erected for these latter species.

4. Contrary to a number of recent publications, *Pachycordyle* and *Thieliana* are assigned to the family Clavidae rather than the Bougainvilliidae.

5. *Pachycordyle* and *Thieliana* are shown to be most closely related to *Cordylophora* within the Clavidae. The three genera are included in the subfamily Cordylophorinae. An identification key is provided to these genera.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Additional notes on *Clathrozoella drygalskii* (Vanhöffen, 1910) (Cnidaria, Hydrozoa)*

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SUMMARY: Study of the material of *Clathrozoella drygalskii* (Vanhöffen, 1910), a hydroid initially referred to the thecate hydroids but probably better classified in athecate hydroids along with such families as Hydractiniidae and Solanderiidae, has been continued with the help of sectioned material. Owing to the conditions of the material available, sectioning proved to be quite difficult; this combined with the poor condition of preservation provided inadequate and inconclusive results, as a result of which the taxonomic position of this enigmatic genus could not be definitely clarified. However, available evidence seems to suggest that the genus *Clathrozoella* Stechow, 1921, contains at least three species of which one, *C. drygalskii*, occurs in Antarctic, New Zealand and Australian waters, a second to the south of Stewart Island south of New Zealand, and a third exclusively (so far) in the Antarctic. Histological details of the two additional species are still lacking and information on the cnidome of the three species is still insufficient.

Key words: Antarctic, Cnidaria, Hydrozoa, Clathrozoidae, *Clathrozoella*, *Clathrozoon,* morphology.

The re-discovery of the rare hydroid *Clathrozoella drygalskii* (Vanhöffen, 1910), found in the collections of the New Zealand Oceanographic Institute (NIWA), Wellington, New Zealand and subsequently in those of the Museum of Victoria, Melbourne, Australia, was reported previously (Vervoort and Watson, 1996). This species, originally described as *Clathrozoon drygalskii* Vanhöffen, 1910, after Antarctic material collected by the German South Polar Expedition, was initially referred to the family Clathrozoidae Stechow, 1921, because of similarity with *Clathrozoon wilsoni* Spencer, 1890. This resemblance is based on the presence, in both species, of 'false hydrothecae', composed of perisarc tubes with a core of coenosarc. *Clathrozoella drygalskii* differs by the presence of a bottom in that 'false hydrotheca', formed by part of the wall of the preceding 'false hydrotheca'. The polyp is attached to that bottom, communicating with the coenosarc in the tubules. In *Clathrozoon wilsoni* and its relatives the one-layered 'false hydrotheca' is fully embedded in perisarc tubes; the hydrothecal bottom is open and the various hydranths are in direct contact through a system of coenosarc tubes in the interior of the colony as occurs in the majority of thecate hydroids. Those structural differences induced Stechow (1921, 1923) to institute the genus *Clathrozoella* for the reception of *Clathrozoon drygalskii*, though maintaining its position in the family Clathrozoidea, close to the Lafoeidae A. Agassiz, 1865. Both Hirohito (1967) and Bouillon (1985, 1995) expressed doubts concerning the correctness of that view, Hirohito even going so far as to remove *Clathrozoella* from the Clathrozoidae, however

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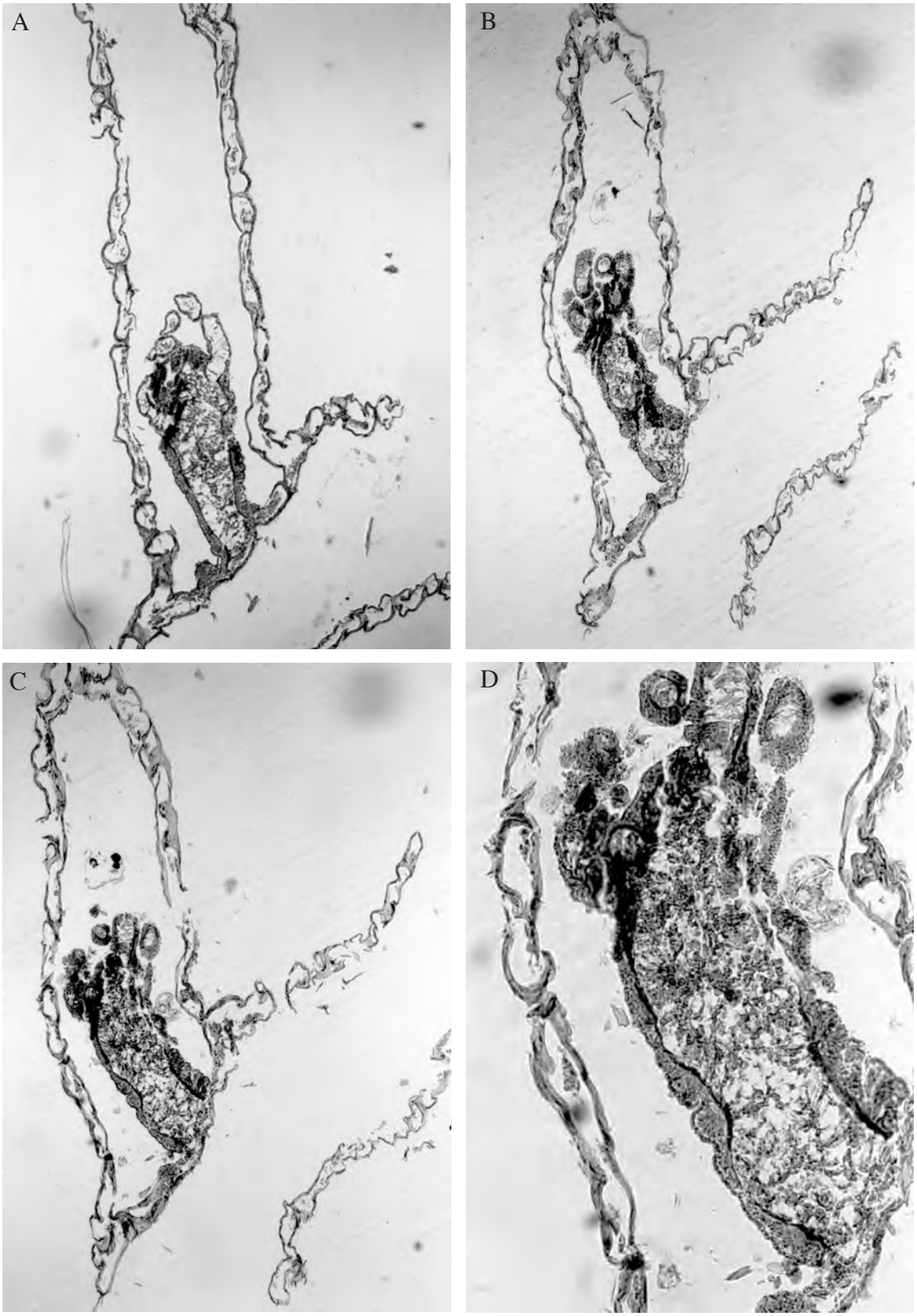

FIG. 1. – A-D, *Clathrozoella drygalskii* (Vanhöffen, 1910), longitudinal sections through a 'false hydrotheca' with hydranth attached to bottom. In A and C the attachment of the gonophore is just visible; in B-D the commensal copepod (?) is visible on the right side of the polyp. Magnifications: A-C, x41; D, x105.

without clarifying its systematic position because of lack of knowledge of the gonosome. That systematic position has remained enigmatic ever since though from the evidence so far available it seems clear that its most likely position is with the Athecate hydroids amongst such families as Hydractiniidae L. Agassiz, 1862, and Solanderiidae Marshall, 1892. Two structural details are essential for a definite solution, viz. knowledge of the gonosome and mode of reproduction, and information on the cnidome. To this end histological sections of some of the colonies were made; the provenance of this material is specified in Vervoort and Watson (1996). Unfortunately the New Zealand material turned out to be badly preserved but sections of the Australian material, prepared in the classical way and stained with Mallory-Heidenhain's triple stain, provided several reasonable longitudinal sections of the polyps and sufficed to confirm Vanhöffen's quite accurate and detailed observations on the gross anatomy of the polyp, its attachment to the bottom of the 'false hydrotheca' and the presence of a strand of tissue in the tubules that build up the 'false hydrothecae' and the colony. The polyp is a simple, sac-like structure with a conical hypostome surrounded by a single whorl of filiform tentacles. Nematocysts are visible in the ectoderm but owing to the method of preservation details are difficult to observe. Two size classes of desmones can be distinguished, but there is no conclusive evidence to suggest also the presence of euryteles or stenoteles, that almost certainly must have been present if we are dealing with an athecate hydroid. In one of the sections a commensal animal, probably a copepod, was observed in the space between polyp and internal wall of the 'false hydrotheca'.

In a series of sections of one of the Australian colonies a small, developing gonophore was observed next to the hydranth, communicating, as the polyp, with the coenosarc of the tubules. This gonophore is a conical structure half as height as the column of the hydranth, containing a single developing oocyte. Unfortunately the best sections of this series are slightly folded.

Close inspection of the New Zealand material has proved this material to be composite: the colonies from NZIO Stn D149, south of Stewart Island (roughly 49°S, 167°E), from a depth of 454 m, mentioned by Vervoort and Watson (1996) as having a "thick cover of small algae and diatoms", differ from those of the remaining two New Zealand stations and the Australian material in colony structure. The latter agrees closely with Vanhöffen's detailed description; the walls of the 'false hydrothecae' and consequently the resulting colony, is formed by a network of anastomosing perisarc tubules with a core of coenosarc. The exterior of the 'false hydrothecae' is covered by small, cylindrical nematothecae that are fairly closely packed and form a quite significant feature of this species. All this material clearly represents *Clathrozoella drygalskii* (Vanhöffen, 1910); it has been compared with the lectotype with which it shows no real differences. The Stewart Island material, however, is different. It strikes at a first glance by the much coarser 'false hydrothecae' with a wider bore; the coarseness in appearance being brought about by a thick layer of foreign bodies on the outside of the 'false hydrotheca', formed principally by sand grains, dead Foraminifera, diatoms and small algae, attached to and cemented together and to the exterior of the colonies by threads and tubules of perisarc and mixed with many short, cylindrical nematothecae that do not protrude beyond the surface of this layer of detritus. This material so far proved unsuitable for sectioning and no polyps could be isolated. It is tentatively considered a second species of *Clathrozoella*.

In material collected by USARP (United States Antarctic Research Program) in 1962 and sent to us for sorting and further study, a species of *Clathrozoella* has been found, collected approximately 58.5°S, 60.5°W (Drake Passage) at 3076 m depth, that has every appearance of being a third species of the genus. It is quite characteristically geniculate as the 'false hydrothecae' are not curved but straight, while the nematothecae are shorter, wider and considerably more dispersed than in *Clathrozoella drygalskii*. So far this species has been only cursorily studied, a study much hampered by the paucity of material. No polyps have so far been found in this material.

In conclusion: Vanhöffen's detailed observations on the gross anatomy of *Clathrozoon drygalskii* [now *Clathrozoella drygalskii* (Vanhöffen, 1910)] have been confirmed. This structure and the presence of a gonophore next to the hydranth at the bottom of the 'false hydrotheca' support the suggestion that the species should be referred to the athecate hydroids rather than to the thecate hydroids; this suggestion is not contradicted by the characters of the cnidome as far as that is known at present. The genus *Clathrozoella* Stechow, 1921, probably contains at least three species.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Parallel, paedomorphic evolutionary processes of the bivalve-inhabiting hydrozoans (Leptomedusae, Eirenidae) deduced from the morphology, life cycle and biogeography, with special reference to taxonomic treatment of *Eugymnanthea**

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SUMMARY: It is hypothesized that bivalve-inhabiting hydroids originated from colonial, free-living eirenid hydrozoans, initially appearing as an *Eutima* species with solitary hydroids producing immature medusae with tentacles and manubrium, and also with derived characteristics of the marginal warts of the mature medusae as the remnants of the tentacular bulbs of the ancestral eirenid, and decreased number of statocysts. The derivate eumedusoid-producing *Eugymnanthea* evolved then. Deduced from the morphology, life cycles, and geographical distributions of this group and of all the known extant *Eutima* species, it is proposed that parallel, paedomorphic evolution took place in the marginal regions of the area of distribution of the ancestral-like *Eutima* at least in the northern hemisphere of both the Pacific and the Atlantic Oceans. *Eugymnanthea* appeared as a polyphyletic taxon as a result of the parallel evolution of different species of *Eutima*. It is pointed out that *Eugymnanthea* and *Eutima* are to be merged into a single genus in the future.

Key words: bivalve-inhabiting hydrozoan, paedomorphic, parallel evolution, eirenid, *Eutima*, *Eugymnanthea*, life cycle, geographical distribution, polyphyly, taxonomy.

INTRODUCTION

The most derived bivalve-inhabiting hydrozoan, *Eugymnanthea*, is distributed disjunctly in the northern hemisphere: *E. inquilina* Palombi, 1935 has been found in the Mediterranean Sea (Palombi, 1935; Cerruti, 1941; Crowell, 1957; Uchida, 1964; Morri, 1981; Gili, 1986; Kubota, 1989; Piraino *et al*., 1994; Bouillon *et al*., 1995; Celiberti *et al*., 1998; Benovic, personal communication) and *E. japonica* Kubota, 1979 in the Noth-West Pacific (Kubota, 1979, 1985a, 1991, 1992b, 1999; Kubota *et al*., 1999). By combinations of the two diagnostic features of the medusae, manubrium and statolith, the two species of *Eugymnanthea* can be clearly distinguished (Table 1). There are no records of introduction of *Eugymnanthea* in any places (Kubota, 1991), differing from the case of their most popular host, *Mytilus edulis galloprovincialis* Lamarck. In Japanese waters, for instance, *M. e. galloprovincialis* from the Mediterranean Sea settled at the beginning of the 20th century, then spread over nearly all the coasts of Japan except for those of Okinawa Prefecture (Kubota, 1989, 1992b;

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TABLE 1. – Morphological differences between the mature medusae of the two species of *Eugymnanthea* and their geographical distributions. Fm: frequency (%) of medusae with manubrium; Nm: number of medusae examined; Fs: frequency (%) of statocysts containing 1-4 statoliths (Stl); Ns: number of statocysts examined; Nl: number of localities examined.

Species	Fm	(Nm)	Fs 1Stl	2Stl	3Stl	4Stl	(Ns)	Nl	Distribution	References
E. japonica	97.9	(4597)	90.2	7.4	1.1	0.1	(12955)	21	Japan (West Pacific)	Kubota, 1991
E. japonica	95.2	(105)	93.0	4.3	0	0	(517)	1	Taiwan (West Pacific)	Kubota, *et al*., 1999
E. inquilina	3.4	(183)	9.4	37.3	35.4	11.7	(1462)	4	Italy (Mediterranean)	Kubota, 1989

Kubota and Hayashibara, 1995; Kubota *et al*., 1995; Kubota, 2000).

Kubota (1987, 1989, 1991), discussing the origin of the two species of *Eugymnanthea*, discarded both the species introduction and the Tethys Sea relict hypotheses, and proposed the parallel, paedomorphic evolution. Bouillon (1985, 1994) ascribed *Eugymnanthea* and bivalve-inhabiting *Eutima* to the Eirenidae, merging the Eutimidae into that family. Monophyly of bivalve-inhabiting hydrozoans is supported also by unpublished data by Kubota, Larson and Migotto who found Atlantic bivalve-inhabiting hydroids producing *Eutima* medusae. If *Eucheilota*-like mature medusae are produced in any bivalve-inhabiting hydroids, they will belong to the Eirenidae as is the same case of the *intermedia* form of *Eutima japonica* that was firstly described as a new species of *Eucheilota*, being then considered as one of the four forms of *Eutima japonica* (Kubota, 1984, 1985b,1992a, 1993, 1997, 1999).

In this paper the evolutionary processes of bivalve-inhabiting hydrozoans leading to the origin of *Eugymnanthea* are deduced from morphology, life cycles, and geographical distributions. Furthermore, the phylogenetic patterns that led to *Eugymnanthea* species allow a re-appraisal of the taxonomic value of this nominal genus.

EVOLUTIONARY PROCESSES OF BIVALVE-INHABITING HYDROZOANS

Early process of evolution

The ancestral form of the bivalve-inhabiting hydrozoans is conceivable as a free-living eirenid resembling *Eirene menoni* Kramp (see Bouillon, 1984) or *E. lactea* (Mayer) (see Brinckmann-Voss, 1973), i.e. a colonial hydroid producing immature medusae with tentacles and manubrium at liberation. Two key character changes took place in this hypothetical ancestor: decrease in number of both the marginal tentacles and the statocysts in the mature medusa (Table 2: 1). It is conceivable that the marginal warts of *Eutima* medusae are remnants of the tentacular bulbs of the ancestral eirenid medusae.

In Japanese waters, records of *Eutima* refer only to the bivalve-inhabiting species, *E. japonica* Uchida, 1925 (Uchida, 1925; Yamazi, 1958; Kubota, 1992a, b, 1998, 1999, unpublished data) and no free-living species have been known. Other free-living eirenids in Japan are referred to *Eirene, Tima* and *Eutonina* (see Kubota, 1998). *Eutima japonica,* representing what could have been the ancestor of *Eugymanthea japonica*, is divided into the four forms that are parapatrically distributed, changing their morphology of the earliest matured medusae step by step (Kubota, 1992a, 1997, 1999). The medusa of *Eutima japonica* was also collected in the central part of the north Pacific (Kramp, 1965), Cochin Backwater, India (Santhakumari *et al*., 1971), and in the Jiulong River estuary near Amoy, China (Zhenzu and Jiachi, 1983). It was never found in the east coasts of the Pacific, from where no bivalve-inhabiting hydrozoans have been recorded yet, in spite of recent surveys conducted in Central and Southern California, USA (Kubota, unpublished data). It is assumed that the water temperature is too low for this group to settle, since the coasts are usually washed by cold currents.

Some planktonic *Eutima* species have been described from the Mediterranean Sea (Boero and Bouillon, 1993; Fig. 1), but ancestral-like bivalve-inhabiting *Eutima* have not been found, even in recent surveys carried out in Italy, Croatia and Spain (Kubota, unpublished data). However, in the west coasts of the Atlantic Ocean, in both northern and southern hemisphere, bivalve-inhabiting *Eutima* have been reported (Mattox and Crowell, 1951; Narchi and Hebling, 1975; Kubota and Larson, 1990; Kubota *et al*., unpublished data), though *Eugymnanthea* has not been recorded from this region.

TABLE 2. – Parallel evolutionary processes (1-3) producing the bivalve-inhabiting hydrozoans, showing some extant representatives. (1) Decrease of number of statocysts and tentacles; (2) Evolution of sucker-like hydrorhiza and disappearance of periderm; (3) Evolution of eumedusoids; * *Eirene hexanemalis* is another possibility.

Region (Oceans and their marginal seas)	Exsistence of ancestral-like free-living *Eirene*	Evolution of free-living *Eutima*	Evolution of bivalve-inhabiting *Eutima*	Evolution of derivative *Eugymanthea* by progenesis
Atlantic	*Eirene lactea*	(1) *Eutima mira*	(2) *Eutima sapinhoa*	(3) *Eugymnanthea inquilina*
Pacific	*Eirene menoni**	(1) ?	(2) *Eutima japonica*	(3) *Eugymnanthea japonica*

The evolution of the most derived bivalve-inhabiting hydrozoan *Eugymnanthea*

From the free-living, colonial *Eutima*, the solitary, bivalve-inhabiting hydroid evolved without drastic modification of the medusa stage, and appeared as a specialized, distinct member in the Eirenidae as mentioned above. The hydrorhiza changed into a sucker-like structure from the stolon in order to attach to the surface of the living, soft body portions of the bivalve hosts. The periderm disappeared in these hydroids in relation to the endosymbiotic life within the mantle cavity of the hosts (Table 2: 2). Such an evolution occurred parallely both in the Atlantic Ocean and in the Pacific and their marginal seas, producing primitive bivalve-inhabiting *Eutima* (Kubota, 1987, 1991). In the Pacific, a different hydroid morphotype, i.e. a branched, solitary one with hydrorhiza penetrating into the host tissues, appeared in specialised wood-boring host bivalves (Santhakumari and Balakrishnan Nair, 1969; Ramachandra *et al*., 1974; Kalyanasundaram, 1975). The medusae of this species were recorded from the Cocin Backwater, India, where the hydroids were also recorded (Santhakumari, 1970), Bombay harbour, India (Santhakumari, *et al*., 1971, 1977), and the Red Sea (Schmidt, 1973). Including *E. commensalis* Santhakumari, 1970, all the bivalve-inhabiting *Eutima* in the Pacific possess cirri in the medusa stage, contrary to the bivalve-inhabiting *Eutima* in the Atlantic Ocean (Kubota, 1987; Kubota and Larson, 1990; Kubota *et al*., unpublished data).

Boero *et al*. (1996) hypothesized another possible origin of bivalve-inhabiting *Eutima*, i.e. from a free-living, solitary, planktonic eirenid hydroid resembling *Eirene hexanemalis* (Goette) (see Bouillon, 1983). Such floating hydroids are rare at present and the only other species with this feature is *Zelounies estrambourdi* (see Gravier-Bonnet, 1992). It is possible that both types of ancestral-like hydrozoans produced independently the special hydrorhizas for the endosymbiotic life with bivalves. The invasive developmental stage of the bivalve-inhabiting hydrozoans is a solitary planula, therefore a particular ability of this larva to settle on the living tissues of bivalves, which are often covered by a mucous layer and moving cilia, should be acquired. However, this was accomplished during the course of evolution of this group like other endosymbiotic hydrozoans with ascidians, *Ascidioclava* (see Kubota and Yamada, 1988) or with bryozoans (Boero *et al*., 2000).

From the bivalve-inhabiting hydrozoans with sucker-like hydrorhiza, *Eugymnanthea*-like species evolved independently by progenesis, at least in the northern hemisphere of the Pacific and of the Atlantic Ocean and its marginal seas, appearing as the most derived and aberrant thecate hydrozoans (Table 2: 3). These hydroids produce eumedusoids without marginal tentacles and ordinary manubrium that go out from the mantle cavity of the hosts to quickly spawn in the sea (Palombi, 1935; Kubota, 1996). Their life span is short and all life is spent near the host. No bivalve-inhabiting hydrozoans producing sporosacs have been found, but at low temperature *Eugymnanthea* eumedusoids are not released (cf. Kubota, 1979, 1987).

According to Boero and Bouillon (1993), among the members of the Mediterranean hydromedusae with hydroid stage, the Indo-Pacific element is not abundant, i.e. only 28 species (8.0 %) out of 346. This geographical element is thought to be originated by the migration through the Suez Canal (the Lessepsian migration). However, the genus *Eugymnanthea* can not be included into this zoogeographical element, and the Mediterranean *Eugymnanthea* is considered as endemic by Boero and Bouillon (1993). The origin of the Mediterranean-endemic element is ascribed to essentially three causes: the relict of the Tethys Sea fauna; speciation from species that colonised the basin after the Messinian crisis; 'false endemics' due to lack of knowledge of zoogeographical and/or taxonomic informations.

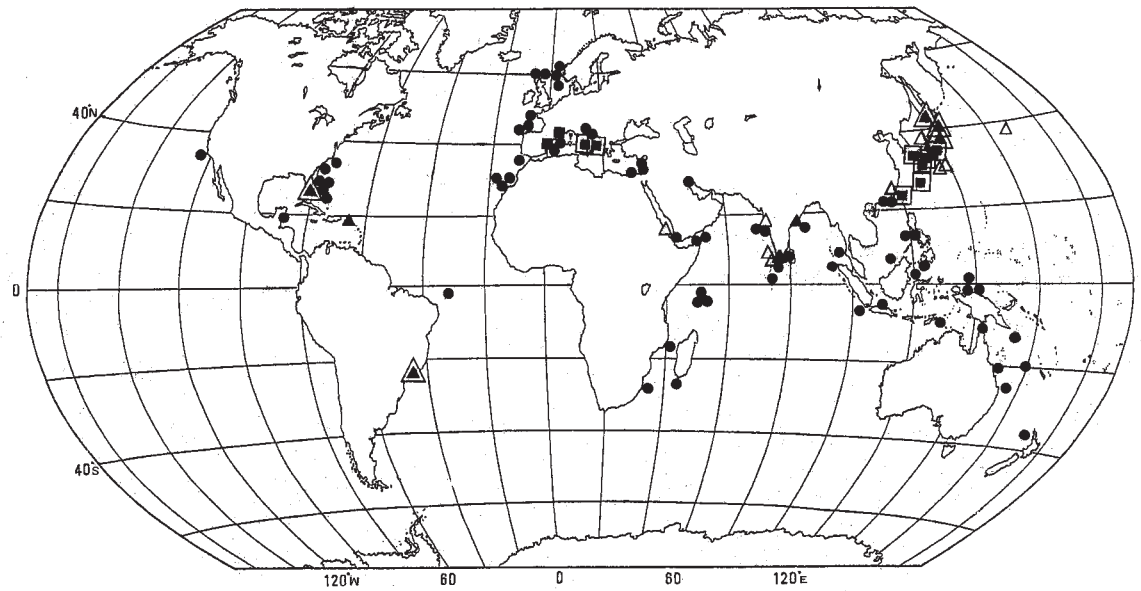

FIG. 1. – Geographical distribution of planktonic medusae of bivalve-inhabiting *Eutima* (*E. japonica* and *E. commensalis*: open triangles), hydroids of bivalve-inhabiting *Eutima* (at least 4 species: closed triangles), hydroids of *Eugymnanthea* (2 species: closed rectangles), and planktonic extant *Eutima* medusae (closed circles: after many references, but not cited everyone and confer van der Spoel, 1996). Doublefold symbols indicate mature medusae of the bivalve-inhabiting hydroids are known by culture.

However, the origin of the Mediterranean *Eugymnanthea inquilina* is entirely unknown. An ancestral type of bivalve-inhabiting hydrozoans, corresponding to the Pacific *Eutima japonica*, has not been discovered in the Mediterranean and also in the east coast of the Atlantic Ocean.

Deduced from the geographical distributions of the bivalve-inhabiting hydrozoans and all the known extant *Eutima* species, the above-mentioned processes of the parallel, paedomorphic evolution occurred in the marginal regions of the distributed area of the ancestral-like *Eutima* species (Fig. 1), although there is a problem that most of the hydroid stages of the extant *Eutima* are still unknown (Table 2). The center of origin of the leptomedusae, the Indo-Malayan region pointed out by van der Spoel (1991, 1996), may not be a suitable location to promote the progenetic evolution leading to the production of *Eugymnanthea* and there are no records of this genus in this region (Kubota, unpublished data; Ho, pers. comm.). Kubota (1987) speculated that this convergent evolution was induced in the Pacific and the Atlantic Oceans and their marginal seas by climatic changes, i.e. cool and/or cold environment in the Pleistocene. If this progenetic evolution produced identical morphological forms of *Eugymnanthea* in two or more different remote places, as the mosaic evolution of the hydrozoans often takes place in different taxonomic groups (Boero and Bouillon, 1987; Petersen, 1990), we deduce the origin of the Pacific *Eugymnanthea* as a mere introduction from the Mediterranean Sea together with its host *Mytilus edulis galloprovincialis*.

Eugymnanthea, together with at least bivalve-inhabiting *Eutima*, could be merged into the one same genus

The genus *Eugymnanthea* is traditionally defined on morphology (Bouillon, 1985, 1994). If the above-mentioned origin of this genus in the Eirenidae is demonstrated, then a taxonomic problem related to phylogeny turns out. General evolutionary processes producing bivalve-inhabiting *Eutima* and *Eugymnanthea* are acceptable, and the phylogenetic tree is depicted as shown in Fig. 2A (Kubota, 1983; Boero, Bouillon and Piraino, 1996). This phylogeny does not conflict to the hypotheses of the Tethys Sea relict and the species introduction, and no taxonomic problems turn out from this tree. However, in the case of parallel evolution a more precise phylogenetic tree is shown in Fig. 2B. In this tree the bivalve-inhabiting *Eugymnanthea* appeared as a polyphyletic genus. Taxonomic treatment of assigning all the bivalve-inhabiting *Eutima* and *Eugymnananthea* into the one genus is, therefore, reasonable. In this paper, however, formal taxonomic changes according to the international nomenclatural rules are not made until more biological studies are made and we confirm the origin of *Eugymnanthea* (Kubota *et al.*, in prep.).

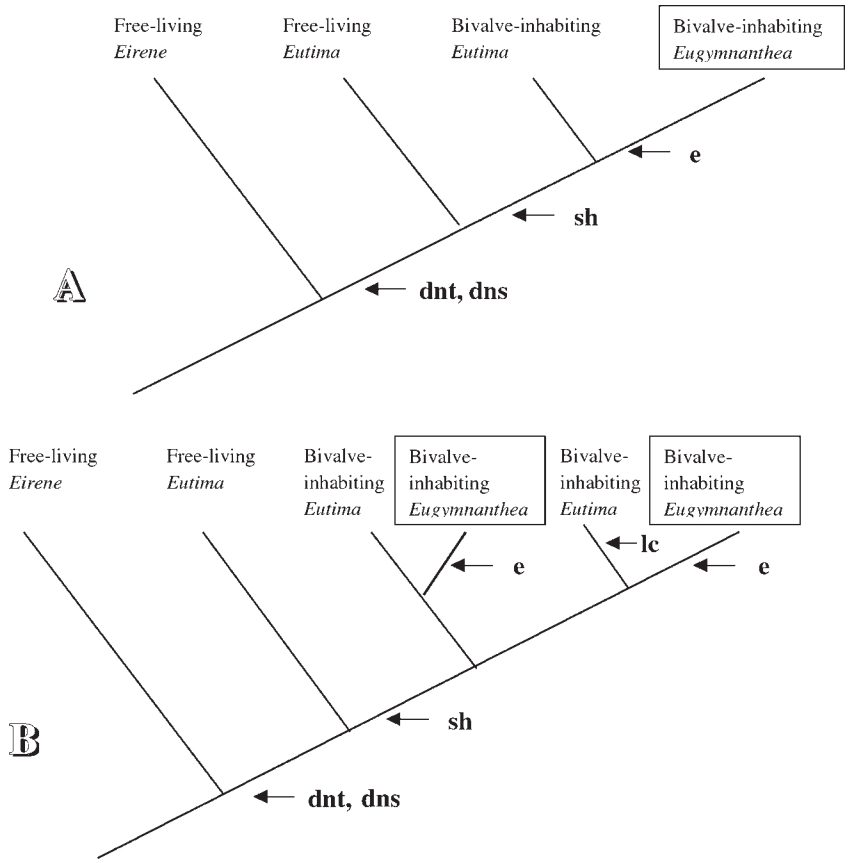

FIG. 2. – A: Phylogenetic tree showing general evolutionary courses of the bivalve-inhabiting hydrozoans in Eirenidae. B: Phylogenetic tree showing bivalve-inhabiting *Eugymnanthea* originated by parallel, progenetic evolution, resulting polyphyly. dns: decrease of the number of statocysts, dnt: decrease of the number of tentacles, e: production of eumedusoid, sh: production of sucker-like hydrorhiza, lc: loss of cirri.

It is generally admitted that the metagenic hydrozoans like bivalve-inhabiting ones are difficult to manage to establish an appropriate classification (Kubota, 1983; Boero and Bouillon, 1987). In the athecate groups producing eumedusoids, Petersen (1990) pointed out that production of either fixed gonophores or of eumedusoids is not a key character to define genera. Convergent evolution producing eumedusoids took place in many branches of diverse taxonomic groups. The same conclusion was obtained by a molecular apporoach on the Hydractiindae by Cunningham and Buss (1993). Therefore, similar studies are needed for bivalve-inhabiting hydrozoans, and they will shed light in the phylogeny and taxonomy of this group.

The phylogeny of Eirenidae, a family comprising 12 genera (Bouillon, 1985, 1994), is not easy mainly due to lack of knowledge of life cycles. Bouillon (1985) considered the related hydrozoans to the Eirenidae are Lovenellidae, Eucheilotidae and Cirrholoveniidae, therefore all these hydrozoans should be taken into account to clarify the phylogeny of the present group. Much care is needed to select outgroups since such choices greatly change the resultin phylogenies, as pointed out by Boero *et al*. (1997).

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Redescription of *Tripoma arboreum* Hirohito, 1995 (Hydrozoa: Campanulinidae) from the Tasman Sea with notes on quasi-parasitism of the species*

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SUMMARY: *Tripoma arboreum* Hirohito, 1995 has been found in deep water in the Tasman Sea south of Australia, the Norfolk Ridge north of New Zealand and Sagami Bay, Japan. The species exhibits unusual quasi-parasitic behaviour towards its host species.

Key words: *Tripoma, Tetrapoma,* Campanulinidae, taxonomy, Tasman Sea, Australia.

INTRODUCTION

A study of the hydroid fauna from deep water seamounts in the Tasman Sea south of Tasmania, Australia, and from the Norfolk Ridge, north of New Zealand, found a species of the family Campanulinidae that has led to critical review of the systematic status of the genus *Tetrapoma* Levinsen, 1893, type *Calycella quadridentatum* Hincks, 1874 and *Tripoma* Hirohito 1995, type *Tripoma arboreum* Hirohito, 1995.

Bouillon (1985) redefined the genus *Tetrapoma* Levinsen, 1893, limiting it to stolonal colonies with nearly cylindrical, pedicellate hydrothecae with four triangular opercular valves distinctly demarcated from the margin. The gonotheca was unknown.

Hirohito (1995) further modified the generic description of *Tetrapoma* to accommodate erect, arborescent colonies from Sagami Bay, Japan. Although his specimens were fertile he did not include the gonosome in the generic description. Nor was his concept of a hydrothecal operculum of four flaps, not demarcated from the hydrothecal body, in accord with the generic diagnosis of *Tetrapoma*.

We have examined a paratype colony of *Tetrapoma fasciculatum* Hirohito 1995 (NSMT-Hy R: 3010, alcohol preserved), a microslide preparation No. 6212 from the same colony, four microslide preparations No. 5139, 5143, 5145, from the holotype colony of *Tetrapoma fasciculatum* (NSMT-Hy R: 3002), and the holotype specimen (NSMT-Hy R: 3009, alcohol preserved) and three microslide preparations (No. 6206, 6207, 6211) of *Tripoma arboreum* Hirohito 1995, loaned by the Showa Memorial Institute, Japan.

The heavily fascicled lower branches of the preserved specimen of *Tripoma arboreum* bear many

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hydrothecae, some pedicellate and others partially immersed in the polysiphonic tubes of the branches as described by Hirohito. The few reasonably well preserved pedicellate hydrothecae have the same range of dimensions as those of *T. fasciculatum* and clearly show four, not the three opercular valves characterised by Hirohito as diagnostic of the genus *Tripoma*. We therefore conclude that Hirohito's genus *Tripoma* cannot possibly be separated from *Tetrapoma* as defined in Hirohito's 1995 paper, *Tetrapoma fasciculatum* Hirohito, 1995 and *Tripoma arboreum* Hirohito, 1995 being evidently conspecific. However, since the colonies are not stolonal but are erect and arborescent and the hydrothecal operculum is not demarcated from the margin, we consider *Tetrapoma quadridentatum* (Hincks, 1874) is so far removed from the arborescent species as to necessitate generic separation. The arborescent species without demarcation of the operculum from the hydrothecal margin is here placed in the genus *Tripoma* Hirohito, 1995, where it should stand as *Tripoma arboreum* Hirohito, 1995. The species name "*arboreum"* is here given preference over "*fasciculatum"* since it represents the type of *Tripoma* Hirohito 1995.

MATERIAL

Specimens from the Tasmanian seamounts are lodged in the Tasmanian Museum, Launceston, Tasmania, Australia (TM K), the Museum of Victoria, Melbourne, Australia (MV F). The New Zealand specimens are in the National Museum of New Zealand, Wellington, New Zealand (NMNZ); some material designated NNM is in the National Museum of Natural History (Nationaal Natuurhistorisch Museum), Leiden, The Netherlands.

RESULTS AND DISCUSSION

Family CAMPANULINIDAE Hincks, 1868
Genus ***Tripoma*** Hirohito, 1995

Diagnosis. Colony arborescent, stem and branches fascicled. Hydrotheca nearly tubular, pedicellate, operculum of four flaps not demarcated from margin. Gonotheca cocoon-like, sessile, immersed in fascicular tubes of rhizocaulus, orifice terminal, subcircular, operculum membranous.
Type species: *Tripoma arboreum* Hirohito, 1995.

Tripoma arboreum Hirohito, 1995
Figs 1A-D, 2A-J

Tetrapoma fasciculatum Hirohito, 1995: 95, fig. 27a-c, pl. 5, fig. D.
Tripoma arboreum Hirohito, 1995: 98, fig. 28a-e, pl. 6, fig. A.

Material and Records: TM K1708, CSIRO F.R.V. 'Southern Surveyor' Cruise SSO1/97, station 50, 44.21°S 147.04°E to 44.16°S, 147.05°E, 640-700 m, benthic sled, 69.7 km SSE of South East Cape, Tasmania, 29/01/98. Material formalin preserved. Several fertile colonies to 30 mm high on calcareous bryozoan. MV F 83426, 83427, 83428 microslide preparations from these colonies. TM K1709, CSIRO F.R.V. 'Southern Surveyor' Cruise SSO1/97, station 57, 44.18° S, 146.99° E to 44.21°S, 146.95° E, 900-1,100 m, benthic sled, 65.1 km SSE of South East Cape, Tasmania, 29/01/1997. Material formalin preserved. Numerous fertile colonies to 50 mm high on stem of dead bryozoan. Material not well preserved. National Museum of New Zealand, BS 886 (Colln NMNZ 520), 32°35.3'S 167°41.8'E to 32°34.0'S 167°39.39'E, Wanganella bank, Norfolk Ridge, E. Slope, 437-422 m, 29/01/1981, R. V. 'Tangaroa'. Some material as NNM-Coel 27731; two microslide preparations NNM-Coel 2998. Male colony, 60 mm high on sponge. Material alcohol preserved, condition reasonable.

Description

Hydrorhiza a small mass of tubes; hydrocauli erect, stiff, arborescently branched untidily more or less in one plane, branches heavily fascicled to distal region, polysiphonic tubes narrow, parallel to entwining. Distalmost branches monosiphonic, internodes long and slender, nodes a faint constriction in perisarc above apophysis or absent altogether; apophyses of branch distal on internode, moderately long, with a deep transverse distal node. Hydrothecae strictly alternate in monosiphonic branch region, given off in one plane; a hydrotheca in axil of each branch. Hydrotheca borne on a distinct pedicel merging into base of hydrotheca with none to three deep constrictions, hydrotheca deep, perisarc thin (Tasmanian seamount specimens) to moderately thick (Norfolk Ridge specimens), more or less tubular, curved, widening a little from base to about halfway along length, adcauline wall gently convex, abcauline wall slightly concave to straight, a scarcely visible straight or dish-shaped pseudodiaphragm supporting hydranth, often a ring of desmocytes just above. Margin with an operculum of four delicate valves without crease-line.

Gonothecae large, thickly scattered throughout polysiphonic parts of stem and branches, laying along branch or in axil of two branches, cocoon-like, one side adnate to branch. Developing gonotheca flask-shaped, perisarc relatively thin, mature gonotheca cocoon-like, embedded in a meshwork of more or less parallel or entwined fascicular tubes. Empty gonotheca circular to bun-shaped in transverse section with a moderately thick, smooth inter-

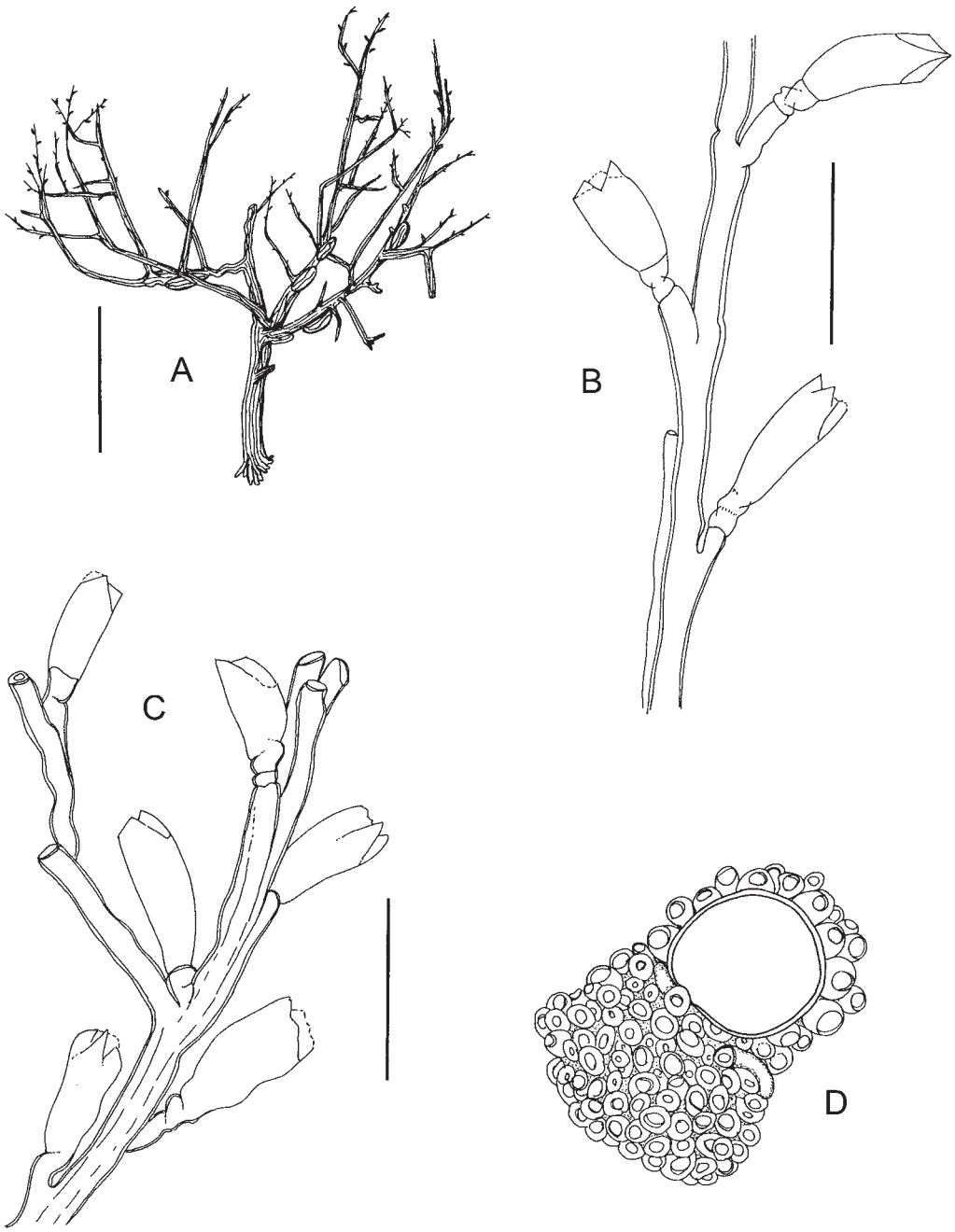

FIG. 1. – A, Colony of *Tripoma arboreum* Hirohito, 1995 (TM K1708) from Tasmanian seamounts. B, Distal end of branch of same colony. C, Distal end of branch of colony from the Norfolk Ridge. D, Transverse section of branch and gonotheca from seamount colony TM K1708. Scale bar:1A, 1 cm; 1B, C, D, 0.5 mm.

nal rind of perisarc surrounded by a tight single or double row fascicular tubes. Orifice terminal, facing outwards or upwards, but frequently obscured, shape variable from subcircular to vaguely quadrangular or scoop-shaped, sometimes surrounded by a short raised collar composed of several perisarcal rings. Operculum a sheet of tissue torn aside at release of contents.

Gonophores eumedusoid but too poorly preserved for detailed description.

Colour

Lower stems buff to yellow-brown, upper stems and monosiphonic branches colourless to transparent, gonophore (preserved) cream coloured.

TABLE 1. – Measurements (μm) of *Tripoma arboreum* Hirohito, 1995.

	Seamount	Norfolk Ridge
Branch, monosiphonic region		
distance between hydrothecal pedicels	530-620	350-450
width at apophysis	80-100	80-120
length of apophysis (adcauline side)	70-180	50-60
width of distal node of apophysis	60-80	80-120
Hydrotheca		
length including pedicel	450-640	580-680
maximum width	180-200	170-220
depth of opercular embayment	140-150	85-110
Gonotheca		
maximum length (estimated)	2500	1430-2060
maximum width	550-900	460-630

Remarks

The opercular valves of the hydrotheca are fragile, most of those of the seamount specimens having collapsed during preservation; the perisarc of the Norfolk Ridge hydrothecae is somewhat thicker and the valves are thus better preserved (Fig. 2C). In some hydrothecae there is an almost imperceptible thickening of the hydrothecal wall at the point of attachment of the very thin, dish-shaped pseudodiaphragm (Fig. 2A). Although Hirohito (1995) reported no diaphragm in his material from Sagami Bay, the same structure together with desmocytes is clearly visible in some hydrothecae in the paratype microslides of *Tetrapoma fasciculatum*.

It is extremely difficult to define the true shape of the gonothecal orifice, most being either damaged in mature specimens, obscured by overgrowth of the fascicular tubes or abraded. The few immature or reasonably intact mature ones suggest a vaguely, subcircular to scoop-shaped rim covered by a very thin operculum fragmented at emergence of the gonophore. There is no evidence in the present material of the four opercular valves described for the gonotheca of *T. fasciculatum* by Hirohito (1995) nor is there any clear evidence of any such structure in Hirohito's specimens.

Quasi-parasitism of Tripoma arboreum

Bale (1915) reported epizootic hydrothecae which he ascribed to the genus *Lafoea* growing on *Acryptolaria arboriformis* (Ritchie, 1911) from Babel Island in Bass Strait, southern Australia. He also (1915) described but did not figure a gonotheca on *Acryptolaria arboriformis* (Ritchie 1911) from deep water off southern Australia, assuming it to be that of *Acryptolaria arboriformis*.

We have examined four microslide preparations labelled "*Cryptolaria arboriformis* Ritchie, off Thouin or Wineglass Bay, near Freycinet Peninsula, Tas. Bale 1915", two microslides labelled "*Cryptolaria arboriformis* Ritchie, Babel Ids, Endeavour, 1915", and one microslide labelled "R.E.Trebilcock, No. 293, *Acryptolaria arboriformis* Ritchie, 7 miles E. of Cape Pillar, Tasmania, 100 fms" in the collection in the Museum of Victoria. Several of the specimens bear Bale's *Lafoea* hydrothecae (Fig. 2I) as well as the cocoon-like gonothecae, typical of *T. arboreum* (Fig. 2H).

We have also examined the holotype specimen of *Acryptolaria arboriformis* (Ritchie 1911) (AM Y257, wet preserved) loaned by the Australian Museum. The much broken colony is about 80 mm high, branching in many directions, both stem and branches being heavily fascicled by thin parallel and entwined tubes. A few of the less heavily fascicled distalmost branches consist of parallel tubes bearing long, tubular *Acryptolaria arboriformis* hydrothecae. The remainder of the colony is almost completely invested in gonothecae of *Tripoma arboreum*. These impart a lumpy appearance to the stems and branches, and together with the surficial meanderings of polysiphonic tubes impart the "frolicsome" appearance of the fasciculations which puzzled Ritchie (1911). The outward-facing, sub-circular orifices of most gonothecae are probably what Ritchie mistook for openings from the polysiphonic tubes to the exterior of the branch. A few small hydrothecae, distinctly those of *T. arboreum,* are also present on the lower regions of several branches. Transverse sections made by us of a branch and gonotheca of the type specimen show that the tubes of *T. arboreum* cannot be distinguished from those of *A. arboriformis* (Fig. 1D). Transverse sections of

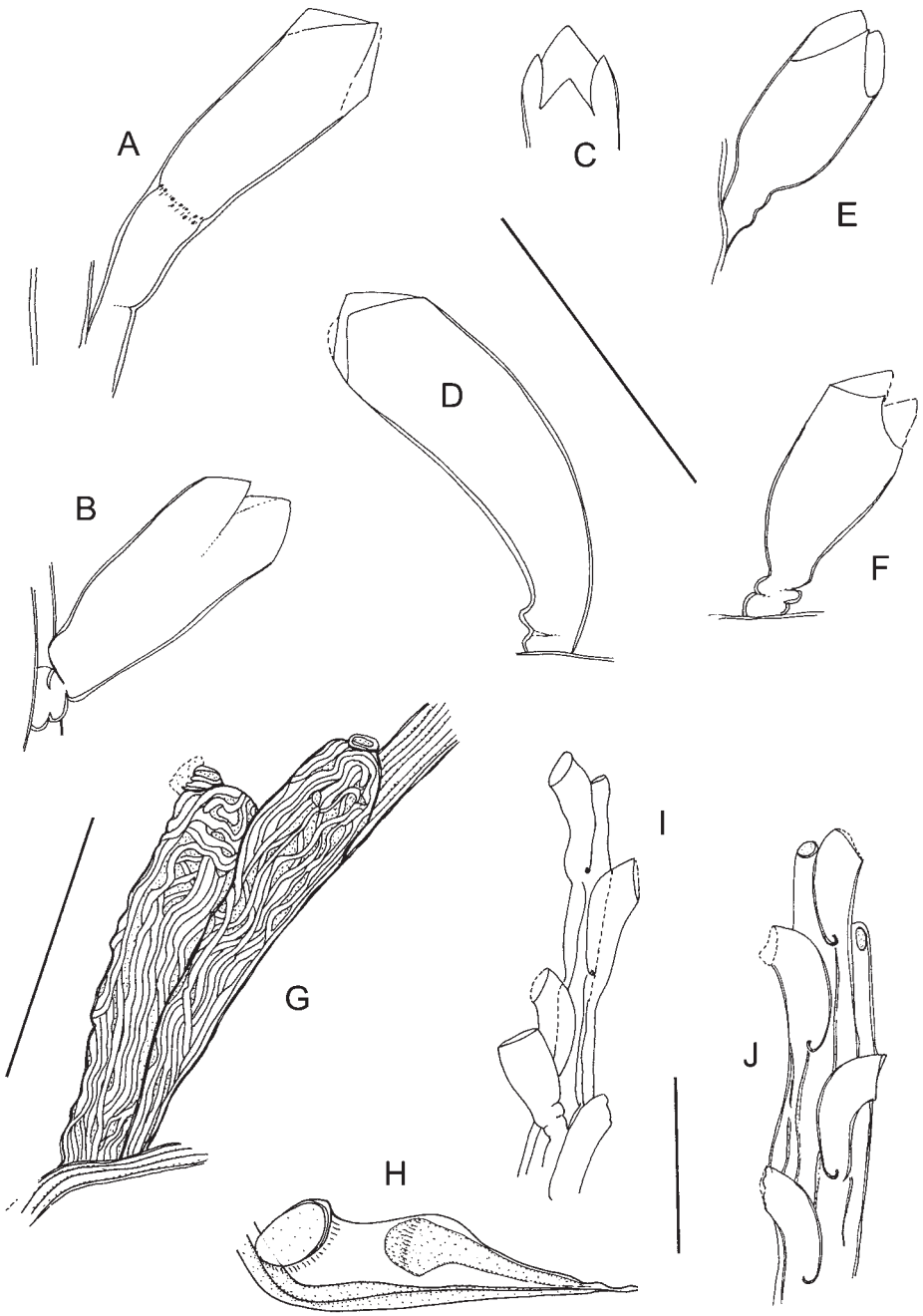

Fig. 2. – A-B, Hydrothecae of *Tripoma arboreum* from Tasmanian seamount colony. C-D, Hydrothecae of *Tripoma arboreum* from colony from Norfolk Ridge. E, Hydrotheca from paratype colony of *Tetrapoma fasciculatum* Hirohito 1995 from Sagami Bay. F, Hydrotheca from holotype colony of *Tripoma arboreum* Hirohito 1995 from Sagami Bay. G, Two adjoined gonothecae from colony of *Tripoma arboreum* from the Norfolk Ridge. H, Young gonotheca of *Tripoma arboreum* without fasciculations from Babel Island, Bass Strait, with *Tripoma* hydrotheca. Slide No 33, Bale hydroid collection, Museum of Victoria. I, Distal part of branch from colony of host colony of *Acrytolaria arboriformis* (Ritchie, 1911) from Babel Island, Bass Strait, with *Tripoma* hydrotheca. Slide No 33, Bale hydroid collection, Museum of Victoria. J, Distal part of branch from holotype colony of *Tripoma arboreum* Hirohito, 1995 from Sagami Bay showing hydrothecae of host colony of *Acrytolaria arboriformis* (Ritchie, 1911). Scale bar: 2A-F, H-J, 0.5 mm; 2G, 2 mm.

branches of the Tasmanian seamount specimens of *T. arboreum* not associated with *A. arboriformis* are indistinguishable from those of the type of *A. arboriformis,* indicating that independent colonies of *T. arboreum* have the same internal structure as the host species of the quasi-parasitic form.

Examination of the less heavily fascicled parts of branches of the preserved holotype colony and microslide specimen of *Tripoma arboreum* Hirohito 1995 reveals biseriate rows of hydrothecae (neither described nor figured by Hirohito) which are clearly those of *Acryptolaria arboriformis* (Ritchie, 1911) (Fig. 2J).

We therefore conclude that *Tripoma arboreum* is capable of adopting two habits of growth: one as independent, arborescent colonies which commence life on invertebrate or inert substrate as in the Tasmanian seamount and Norfolk Ridge colonies. The second, quasi-parasitic habit, seen in the Bass Strait and Sagami Bay *Acryptolaria* colonies is one in which the *Tripoma* stolons become indistinguishably intergrown with the polysiphonic tubes of the host, mimicking its structure and possibly eventually smothering it.

Several examples of epizootism, where juvenile hydroid colonies develop epizootically on colonies of other hydroids then become independently growing, erect colonies are known to us: e.g. colonies of *Halecium delicatulum* Coughtrey, 1876 from the Strait of Gibraltar (Ramil & Vervoort 1992) and the Tasman Sea (W. V. unpub.), and in southern Australia, *Aglaophenia tenuissima* (Bale 1915) on *Gymnangium tubulifer* (Bale, 1915) (see Bale (1915)), *Sertularella pinnata* (Lamouroux, 1816) on *Nemerstesia procumbens* (Spencer, 1891) (see Gordon *et al* 1998) and *Gymnangium longirostre* (Kirchenpauer, 1876) on *Aglaophenia divaricata* (Busk, 1852) (J. E. W. unpub.). Millard (1973) described cases of auto-epizootism in which a species may develop on and within the hydrocaulus of the same species. None of these cases however describe the present situation of quasi-parasitism in which one species invades the hydrocaulus of another species, adopting its structure but without necessarily killing its host.

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

Structure of an epiphytic hydroid community on *Cystoseira* at two sites of different wave exposure*

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SUMMARY: Epiphytism is a strategy by which opportunistic species such as hydroids, escape the intense levels of competition in marine hard bottom communities. Species of the macroalgae *Cystoseira* have a seasonal turnover of the frond, and we hypothesise that epiphytic hydroids colonising such an unstable substrate might show some degree of specialisation. Here the first systematic study on hydroid-*Cystoseira* communities is presented. In particular, the seasonal and spatial distribution of epiphytic hydroids on three species of *Cystoseira* at two sites of different wave exposure at Porto Cesareo (Ionian Sea/Italy) were investigated. Thirty-two hydroid species were recorded which are well known from other substrates and thus are not specific to *Cystoseira*; dominant species were all thecates. The influence of biological factors such as competition and the structure and abundance of the host, seem to have little influence on the hydroid community. The factors of greatest influence were mostly abiotic: sedimentation rate, nutrient levels, temperature and most especially water movement. The importance of water movement was evident in the higher colonisation of algal stems, higher hydroid frequency, larger colonies, reduced colony height, species composition, and distribution on the stems at the wave-exposed site.

Key words: epiphytic hydroids, water movement, *Cystoseira*, Mediterranean.

INTRODUCTION

The environmental factors limiting species distribution in marine benthic communities are temperature, light, water movement and substrate availability, among others. Temperature depends on water currents, depth and season, and influences nutrition, growth, life cycles and reproduction. Water movement supplies the organisms with food, nutrients and dissolved gases, prevents accumulation of sediments, and disperses waste products and larvae, but also acts as a mechanical stress factor. Many organisms have evolved morphological adaptations to withstand such stress. These structural responses are reflected by the different distribution of families, species, or morpho-types due to differences in water movement intensity (Riedl, 1966). Among sessile organisms increasing intensity of water movement induces smaller size, lower colony height, higher population density, and a morphology that withstands high mechanical stress (Riedl, 1971; Boero, 1981b, 1984). The distribution of adults is also the result of competition and settlement behaviour, which is regulated by larval substrate choice (Hayward, 1980).

To avoid competition for space a variety of strategies have evolved. One of the most common of these strategies is epiphytism, which reduces competition because fewer species are encountered and continuous growth of the host produces new substrate for

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less competitive opportunistic species (Seed, 1985). However, compared to living directly on rock, using plants as a substrate limits survival because of the turnover of host tissue and higher mechanical stress due to water movement and the flexibility of the substrate. Therefore an epiphytic life style requires specific adaptations (Seed, 1985), like fast growth to a small body size, early sexual or asexual reproduction, and a short life span (Coma *et al.,* 1992).

Hydroids are among the first colonisers of newly exposed hard substrates and often compete poorly with other sessile organisms (Gili and Hughes, 1995). Most hydroids are substrate generalists, but several species grow on a limited range of surface types. They are especially abundant on seaweeds and their distribution on these hosts is due to different preferences for various aspects of the physical environment, notably water movement and sediment deposition, and substrate choice by larvae, both between and within individual plants (Seed, 1985). The choice appears to be related to both the chemical nature and the structure and texture of the plant surface (Katô *et al.,* 1975; Seed, 1985). Compared to other sessile invertebrates, hydroids grow fast and have a short life span. Together with their, mostly, asexual method of reproduction, these characteristics make them successful epiphytes.

The different hydroid species differ in several features, like reproduction, dispersal and morphology. Thecate hydroids are able to withstand higher mechanical stress than athecates, because of their protective theca (Riedl, 1966). All hydroids have a mainly sessile polyp stage in their life cycle, but many species have a life cycle involving a free-living medusa stage, which can live for several months in the plankton before reproducing sexually. Although reproductive strategy does not seem to influence greatly the colonisation capabilities of a species (Boero, 1984), in epiphytic communities, suppression of the medusa stage and reduction in the frequency of sexual reproduction is common (Boero, 1987).

Among previous studies on epiphytic hydroids the most substantial are those on *Posidonia oceanica* (Boero, 1981a; Garcia Rubies, 1987), *Halimeda tuna* (Llobet *et al*., 1991), *Amphibolis* (Watson, 1992), *Sargassum* (Nishihira, 1966), *Fucus serratus* (Seed *et al*., 1981) and *Laminaria* (Schultze *et al*., 1990). Most of these investigations have been carried out on hydroids that settle on seagrasses with two-dimensional leaves or algae with laminar fronds, which facilitate the quantification and study of the different epiphytes.

Dendroid seaweeds of the genus *Cystoseira* (Fucales, Phaeophyceae) are very abundant in the Mediterranean Sea, growing from the surface down to 100 m depth. Because species distribution depends on wave-exposure, depth and water quality, they are good descriptors of the zonation of the photophilous vegetation on hard bottom substrates (Amico *et al.,* 1985). Species of *Cystoseira* also differ in their morphology, surface characteristics of their stems and in their chemistry (Amico, 1995). As they belong to the hemiphanerophycean brown algae, they have a seasonal turnover of tissue. Their phylloids and branches of higher order are shed in the unfavourable season, usually winter, while the holdfast and primary branches persist (Cormaci, 1995). Therefore this substrate is not always available for epiphytes during the year. Epiphytic algae and other organisms on *Cystoseira* have been studied by Arrontes (1990), Otero-Schmitt and Pérez-Cirera (1996) and Russo (1997), while previous studies of hydroids on *Cystoseira* (e.g. Riedl, 1966) provide only scattered observations.

Here, the first systematic investigation of epiphytic hydroids on *Cystoseira* is presented. In particular, the seasonal and spatial distribution of epiphytic hydroids on three species, *Cystoseira amentacea* Bory*, C. barbata* (Goodenough et Woodward) and *C. compressa* (Esper), at two sites of different wave exposure on the rocky shore of Porto Cesareo (Ionian Sea/Italy) was studied. The following questions were addressed: 1) what are the characteristics of the hydroid community growing on *Cystoseira*? 2) Is there seasonality? 3) Does exposure to wave action influence the *Cystoseira*-hydroid community structure? 4) Do the characteristics of the algal species influence the hydroid communities? 5) Do differences in morphology, such as the presence or absence of a theca influence the distribution of hydroid species epiphytic on *Cystoseira*?

METHODS

The study was carried out on the rocky shore of the Ionian Sea at Porto Cesareo/Italy between July 1996 and July 1997. Algal stems were collected by SCUBA at 0.5, 2 and 3 m depth along four transects at two sites of different wave-exposure. For depth and transect, 5 stems of each of the three species of *Cystoseira* (*C. barbata, C. compressa, C. amentacea*) were collected within an area of 50x50 cm. Samples were taken monthly, weather conditions

permitting, and on each sampling date water temperature was measured. Samples were examined under a binocular.

The following data were taken for each algal stem: a) maximal stem length (cm), as a measure for the amount of substrate available and the seasonality of the algae; b) the hydroid species present; c) estimated hydroid colony size, i.e. abundance of the hydroid colonies on the stem (1: present, 2: abundant, 3: very abundant (Boero and Fresi, 1986)) where all polyps of the same species on the stem were assumed to belong to the same colony; d) distribution of the hydroid colonies on the stem (1: stipe and holdfast, 2: inner or intermediate part of the stem, 3: outer part of the stem (Arrontes, 1990)). The following diversity indices were calculated for the hydroid communities epiphytic on the stems of the three species of *Cystoseira* and from the two sites. Community diversity was measured by species number, Shannon-Wiener index of diversity (H'),

$$H' = - \sum_{i=1}^{s} p_i \ln p_i,$$

where S = number of species and p_i = proportion of total sample belonging to ith species of total algal stems, and Pielou's index of evenness (J), J = H' / ln S , where H' = Shannon-Wiener index and S = number of species (Krebs, 1989). These two indices were tested for significance by a randomisation test (Solow, 1993). Species dominance was measured by Simpson's dominance index (I_{dom}) (Watson, 1992).

Ordination by Canonical Correspondence Analysis (CCA) (Ter Braak, 1986) was used to summarise the variation in species composition and frequency related to site, including their environmental variables, and season. The environmental variables were water depth (m), water temperature (°C), algal stem length (cm), number of algal stems collected, percentage of algal stems colonised, degree of algal structure (1: low structured, smooth surface; 2: high structured, smooth surface; 3: high structured, rugose surface), and dummy variables for exposed/sheltered and the three species of *Cystoseira*. As a measure of goodness of the ordination, species-environment correlations for the first two ordination axes were used. Significance levels of the ordinations were computed by a Monte Carlo permutation test. Ordination analysis and Monte Carlo permutation test were computed using the CANOCO program package developed by Ter Braak (1988).

RESULTS

Thirty-two hydroid species, twelve athecate and twenty thecate species, were recorded on the three species of *Cystoseira* (Table 1). Nine species have a free-living medusa, three have liberable eumedusoids, and twenty have fixed gonophores.

Hydroid abundance and distribution

The number of hydroid species varied from zero to seven per algal stem. Most stems from the wave-exposed site were colonised by one to three species, whereas stems from the sheltered site were mostly colonised by zero or one species. With a maximum of four species per stem, *C. compressa* was colonised by fewer species per stem than the other species of *Cystoseira*, both with maximal seven species per stem.

The percentage of stems colonised by at least one hydroid ranged during the year from 15% to 100% and was typically higher at the exposed site than at the sheltered one (Fig. 1). Colonisation was highest in autumn, but declined in winter/spring at the sheltered site, especially on *C. compressa*. This decline in colonisation correlates with the decline of algal stem length. *C. compressa* was on average less colonised than *C. barbata* and *C. amentacea*.

The abundance of the hydroid colonies on a single algal stem (Table 2a) belonged mostly to the categories 1 (present) and 2 (abundant). The difference of colony-abundance on the three species of *Cystoseira* was significant between the hydroids from the exposed site (Chi-square Test: p=0.004) and the thecates from both sites (p=0.008, p=0.003), but not between hydroids from the sheltered site or between athecates (p-value between 0.05 and 0.16). The differences between sites were always significant (Chi-square Test: p<0.0001). At the wave-exposed site more colonies belonged to category 3 (very abundant) than at the sheltered site.

The general distribution of the hydroids on the algal stem is shown in Table 2b. The central parts (category 2) of the algal stems were generally the most evenly colonised ones. The outer parts (category 3) showed the lowest colonisation, especially at the wave-exposed site. At the sheltered site the athecate hydroids were primarily distributed between the central parts of the stem and its base, whereas the thecate species were more often distributed on the central and outer parts. The differences between the sites and athecates/thecates were significant (Chi-square Test: p<0.0001).

TABLE 1. – Hydroid species recorded on *Cystoseira*. Specimens of *Aglaophenia* indet. were not identifed at species level mostly because of their small size; specimens of *Clytia* sp. belong either to *Clytia hemisphaerica* or perhaps a new species of *Clytia*. x: present, f: fertile, p: asexual propagules; g: fixed gonophores, m: medusae, mg: liberable eumedusoids or swimming gonophores; E: endemic, MA: Mediterranean Atlantic, B: boreal, TA: tropical Atlantic, IP: Indo-Pacific, CT: circumtropical, C: cosmopolitan, nc: non classifiable. (after Boero and Bouillon, 1993)

Nº	species	life cycle	zoogeogr. distrib.	exposed site							sheltered site						
				July '96	September	October	February	April	June	July '97	July '96	September	December	January	February	April	May	July '97
1	*Clava multicornis* (Forskal, 1775)	g	B				x						x	x	x			
2	*Eudendrium glomeratum* Picard, 1952	g	CT?												x	x		
3	*Eudendrium motzkossowskae* Picard, 1952	g	TA		x	x	x	x	x		x	x	x	x	x	x	x	x
4	*Eudendrium ramosum* (L., 1758)	g	C										x					x
5	*Hydractinia fucicola* (M. Sars, 1857)	g	MA		x	x	x	x	x			x	x		x	x	f	x
6	*Cladonema radiatum* Dujardin, 1843	m	CT												x			
7	*Coryne muscoides* (L., 1761)	g	MA		f	f	x	f			x	f	x	x	f	x	x	x
8	*Coryne pusilla* Gaertner, 1774	g	B								x				x			
9	*Sarsia producta* (Wright, 1858)	m	TA				x											
10	*Ectopleura wrighti* Petersen, 1979	m	E										x			x		
11	*Cladocoryne floccosa* Rotch, 1871	g	CT		x							x	x	x	x	x	x	x
12	*Zanclea* sp. see Gravili *et al*., 1996	m					x						x	x	x			
13	*Eirene viridula* (Péron and Lesueur, 1810)	m	IP				x	x								x		
14	*Mitrocoma annae* Haeckel, 1864	m	E												x			
15	*Anthohebella parasitica* (Ciamician, 1880)	mg	nc				x	x										x
16	*Halecium nanum* Alder, 1859	g	TA				x	x	x					x	x	x		x
17	*Halecium pusillum* (M. Sars, 1857)	g	TA	p	f/p	x	p	p	p	x	p	f	x	x	p		x	x
18	*Hydrodendron mirabile* (Hincks, 1868)	g	MA				x	x					x		x	x		
19	*Aglaophenia octodonta (Heller, 1868)*	g	MA		x	f	f	f	x	x	x	f	x		x	f	f	x
20	*Aglaophenia pluma* (L., 1758)	g	C				f	f	x				x			x	x	
21	*Aglaophenia tubiformis* (Marktanner-Turneretscher, 1890)	g	MA				f	f	x	x		x	x	x	x	x	x	x
22	*Aglaophenia indet.*	g		x	f	x	x	f	x	x	x	x		x	x	x	x	x
23	*Kirchenpaueria* sp.	g																x
24	*Ventromma halecioides* (Alder, 1859)	g	C	x	x	x	x	x	x	x	x	x	x	x	x	x		x
25	*Plumularia setacea* (L., 1758)	g	C	x			x	x	x	x	x				x		x	
26	*Dynamena disticha* (Bosc, 1802)	g	C						x			f					x	
27	*Sertularella gaudichaudi* (Lamouroux, 1824)	g	TA		x		x	x	x	x		f	x	x	x	x	x	
28	*Clytia noliformis* (McCrady, 1859)	m	TA			x	x											
29	*Clytia* sp.	m		f		x	f	f	f	x	f		x	x	f	f	f	x
30	*Obelia dichotoma* (L., 1758)	m	C					f	f						x	f	x	
31	*Orthopyxis crenata* (Hartlaub, 1901)	mg	CT							x								
32	*Orthopyxis integra* (Macgillivray, 1842)	mg	C	x	f	f	x	x	x	x	x	x	x	x	x	x		x

TABLE 2. – Abundance and distribution of hydroid colonies on the stems of *Cystoseira*.

a) abundance on stem/colony size (% per category*)

site	*C. compressa* exp.			*C. compressa* shelt.			*C. barbata* exp.			*C. barbata* shelt.			*C. amentacea* exp.			*Cystoseira tot* exp.			*Cystoseira tot* shelt.		
category	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
athecata	56	44	0	87	13	0	59	18	23	61	32	7	54	46	0	**58**	**27**	**15**	**64**	**30**	**6**
thecata	46	37	17	74	22	4	32	39	29	57	35	8	37	42	21	**38**	**39**	**23**	**62**	**31**	**7**
total %	**47**	**38**	**16**	**76**	**20**	**4**	**36**	**36**	**28**	**59**	**34**	**8**	**38**	**42**	**20**	**40**	**38**	**22**	**62**	**31**	**7**

b) distribution on stem (% per category**)

site	*C. compressa* exp.			*C. compressa* shelt.			*C. barbata* exp.			*C. barbata* shelt.			*C. amentacea* exp.			*Cystoseira tot* exp.			*Cystoseira tot* shelt.		
category	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
athecata	80	20	0	69	19	12	70	27	3	37	49	14	70	15	15	**73**	**23**	**4**	**40**	**46**	**14**
thecata	34	50	16	15	42	43	39	56	5	17	38	45	16	70	14	**31**	**58**	**11**	**16**	**39**	**44**
total %	**37**	**48**	**15**	**22**	**39**	**39**	**42**	**53**	**5**	**23**	**42**	**35**	**19**	**67**	**14**	**35**	**55**	**10**	**23**	**41**	**36**

*1 = present, 2 = abundant, 3 = very abundant
**1 = stipe and holdfast, 2 = inner or intermediate part of the stem, 3 = outer part of the stem

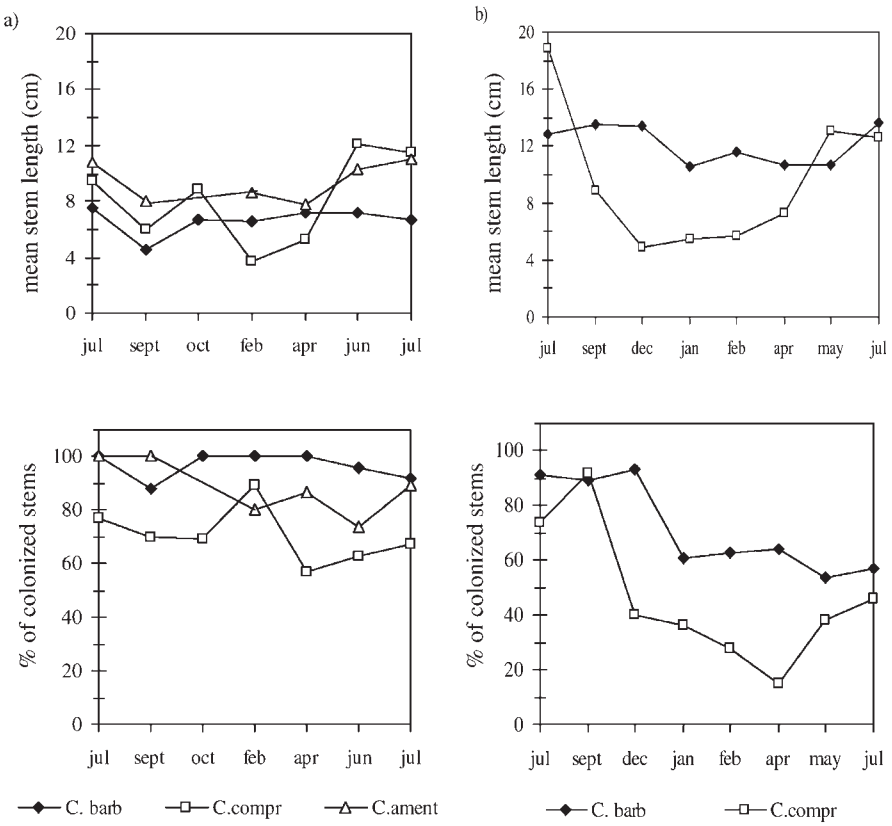

FIG. 1. – Mean algal stem length and percentage of colonised stems of *Cystoseira* between July 1996 and July 1997 for a) sheltered and b) exposed site.

Decrease in colony height on *Cystoseira* could be observed in the arborescent species *Halecium pusillum, Ventromma halecioides, Aglaophenia* spp and *Sertularella gaudichaudi*. Colonies of the genus *Aglaophenia* had a much higher frequency at the wave-exposed than at the sheltered site.

Hydroid diversity and dominance

Of the 32 species of hydroids (Table 1) sixteen were found at both sites and on all three species of *Cystoseira*. With 27 species, the hydroids on stems of *C. barbata* from the sheltered site showed the highest species richness (Table 3). No host-specificity was found amongst the hydroids on *Cystoseira*.

The Shannon's diversity index (H') and Pielou's evenness index (J) for the hydroid communities are shown in Table 2. Differences in H' and J on the three algal species at the two sites were not significant (randomisation test: p-value between 0.074 and 0.709).

Simpson's index of dominance (I_{dom}) is shown in Table 4. The five dominant (I_{dom}>1.0) species were *Halecium pusillum*, *Aglaophenia tubiformis*, *Ventromma halecioides*, *Clytia* sp and *Orthopyxis integra*, all of which were thecate. They comprised 34-73% of the hydroid colonies found. The subdominant species with an index of dominance between 1.0 and 0.6 were *Eudendrium motzkossowskae, Hydractinia fucicola, Coryne muscoides, Cladoco-*

TABLE 3. – Percentage of algal stems colonized by at least one hydroid, total number of hydroid species recorded, Shannon-Wiener diversity index and Pielou's evenness index for the hydroid communities on the three species of *Cystoseira* at the two sites of different wave exposure.

algal substrate	*C. compressa*		*C. barbata*		*C. amentacea*
site (exposed/sheltered)	exp	shelt	exp	shelt	exp
% of colonized stems	69	43	96	67	86
mean n° of colonies/stem	1.15	0.63	2.41	1.17	1.9
total n° of hydroid species	17	19	19	27	21
H' (Diversity Index)	2.34	2.33	2.64	2.81	2.59
J (Eveness Index)	0.77	0.7	0.87	0.84	0.82

TABLE 4. – Species and order of dominance of hydroids on *Cystoseira*. Dominance index Idom = S (n/N)*10, where: n = number of colonies of species 'x', N = total colonies of all species in sample. Order = order of dominance.

No.	species	*Cystoseira compressa* exposed order	exposed I(dom)	sheltered order	sheltered I(dom)	*Cystoseira barbata* exposed order	exposed I(dom)	sheltered order	sheltered I(dom)	*C. amentacea* exposed order	exposed I(dom)
	ATHECATA										
1	*Clava multicornis*	-	-	11.5	0.28		<0.01	13	0.1	16	0.01
2	*Eudendrium glomeratum*	-	-	-	-	-	-	22	0.01	-	-
3	*Eudendrium motzkossowskae*	10	0.1	13	0.16	15	0.06	7	0.75	-	-
4	*Eudendrium ramosum*	-	-	-	-	-	-	16	0.03	-	-
5	*Hydractinia fucicola*	8	0.4	7	0.71	10	0.34	10	0.23	4	0.96
6	*Cladonema radiatum*	-	-	-	-	-	-		<0.01	-	-
7	*Coryne muscoides*	15	0.03	16	0.07	12	0.21	6	0.8	11	0.11
8	*Coryne pusilla*	-	-	-	-	-	-		<0.01	-	-
9	*Sarsia producta*		<0.01	-	-	-	-	-	-	-	-
10	*Ectopleura wrighti*	-	-	-	-	-	-		<0.01	-	-
11	*Cladocoryne floccosa*	-	-	8	0.63	14	0.08	11	0.2	-	-
12	*Zanclea* sp.	-	-	11.5	0.28	-	-	14	0.09	16	0.01
	THECATA										
13	*Mitrocoma annae*	-	-	-	-	-	-		<0.01	-	-
14	*Eirene viridula*	-	-	-	-			-	-	-	-
15	*Anthohebella parasitica*	11.5	0.04	-	-	16	0.04		<0.01	16	0.01
16	*Halecium pusillum*	2	3	3	2.83	1	4.53	5	1.34	1	5.17
17	*Halecium nanum*	16	0.02	10	0.29		<0.01	21	0.01	12	0.07
18	*Hydrodendron mirabile*	-	-	-	-		<0.01	17	0.03	0	<0.01
19	*Aglaophenia octodonta*	7	0.41	6	0.8	4	0.91	8	0.47	9	0.18
20	*Aglaophenia pluma*	13	0.04	-	-	13	0.14	20	0.02	14	0.01
21	*Aglaophenia tubiformis*	1	4.01	2	6.92	3	1.38	1	3.61	3	1.04
22	*Aglaophenia indet.*	3	2.69	5	1.16	2	1.96	3	1.9	2	2.35
23	*Kirchenpaueria* sp.	-	-	-	-	-	-		<0.01		<0.01
24	*Ventromma halecioides*	6	0.41	4	2.75	6	0.49	4	1.45	10	0.15
25	*Plumularia setacea*	9	0.15	15	0.1	9	0.41	18.5	0.02	8	0.27
26	*Dynamena disticha*	-	-	-	-	8	0.42	18.5	0.02		<0.01
27	*Sertularella gaudichaudi*	17	0.02	-	-	5	0.64	12	0.11	6	0.42
28	*Clytia noliformis*	14	0.03	-	-		<0.01	-	-	-	-
29	*Clytia* sp.	5	0.98	1	8.02	7	0.48	2	2.5	5	0.44
30	*Obelia dichotoma*	11.5	0.04	14	0.12		<0.01	15	0.07	13	0.03
31	*Orthopyxis crenata*	-	-	-	-	-	-	-	-		<0.01
32	*Orthopyxis integra*	4	1.5	9	0.33	11	0.32	9	0.43	7	0.39

ryne floccosa, *Aglaophenia octodonta* and *Sertularella gaudichaudi,* which together with the dominant species comprised 55-84% of the hydroid communities.

Canonical Correspondence Analysis

The CCA ordinations were all statistically significant (P=0.01 or P<0.001), as assessed by unrestricted Monte Carlo permutation tests (overall test for the first four axes for 1000 permutations). The species-environmental correlations for the first two axes of ordination ranged from 0.72 to 0.89 and the eigenvalues for the first two axes from 0.12 to 0.2. Figure 2 summarises across both algal and hydroid species. The sites of different wave-exposure are separated by the first CCA axis in all seasons. The samples of the exposed site lie below the first axis of the plot, where colonisation is high. Most variation at the wave-exposed site is due to stem length and number of stems. Samples suggest a seasonal cycle. Samples with the lowest colonisation levels and

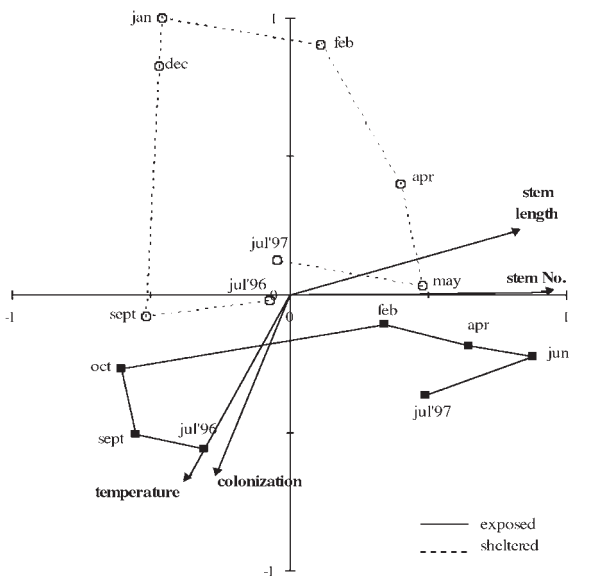

FIG. 2. – Ordination of faunal samples for exposed and sheltered sites using Analysis of Canonical Correspondence with environmental variables. x and y are the first two CCA axes. Arrows show direction and rates of variation among month in water temperature, % of colonised algal stems, algal stem length and number of sampled stems (P = 0.01).

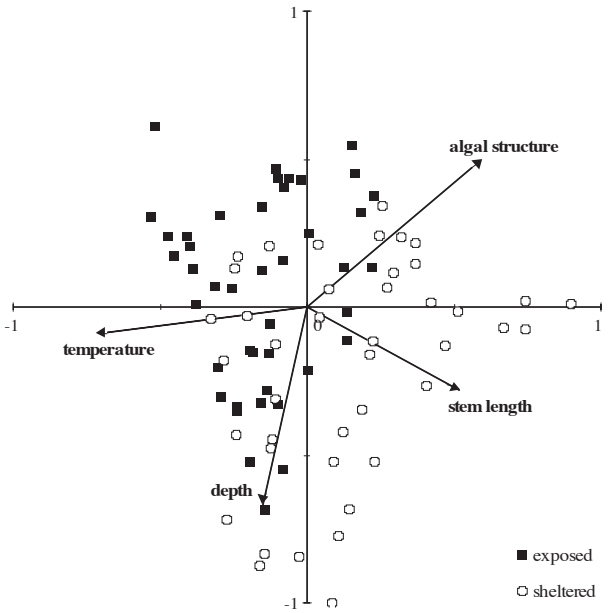

FIG. 3. – Ordination of faunal samples for exposed and sheltered sites using Analysis of Canonical Correspondence with environmental variables. x and y are the first two CCA axes. Arrows show direction and rates of variation among sites in water temperature, water depth, algal stem length and degree of algal structure (P = 0.001).

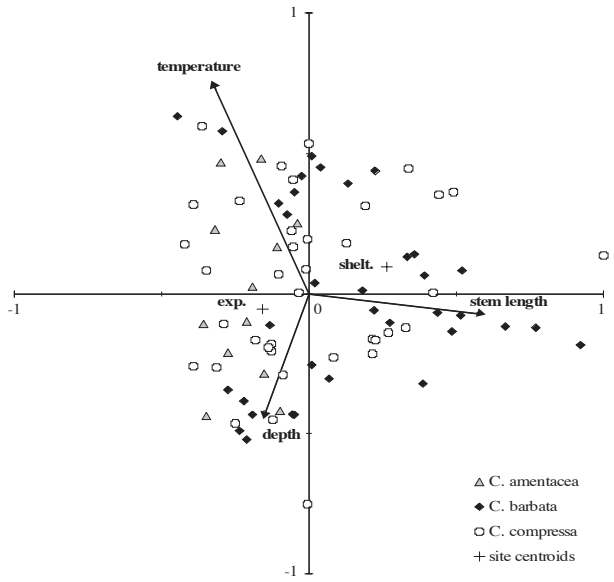

FIG. 4. – Ordination of faunal samples for *Cystoseira amentacea*, *C. barbata* and *C. compressa* using Analysis of Canonical Correspondence with environmental variables. x and y are the first two CCA axes. Arrows show direction and rates of variation among samples in water temperature, water depth and algal stem length. The sheltered and exposed sites are shown by their centroids (P = 0.001).

temperature are the ones at the sheltered site during the cold season.

Figure 3, in which each point is the species composition at a particular site indicates that there is a tendency for samples at the sheltered site to lie

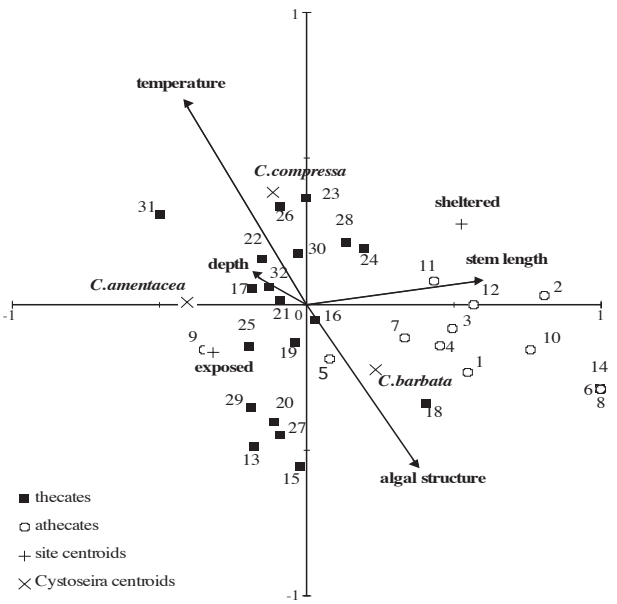

FIG. 5. – Ordination of hydroid species for athecates and thecates using Analysis of Canonical Correspondence with environmental variables. x and y are the first two CCA axes. Arrows show direction and rates of variation among samples in water temperature, water depth, algal stem length and degree of algal structure. The sheltered and exposed sites, and the three species of *Cystoseira* are shown by their centroids. Numbers indicate the hydroid species (P = 0.001).

towards the lower right of the plot. This region also represents sites where algae had long stems with high structure and where the water was colder and deeper.

Figure 4, which displays differences among the three algal species, shows that samples from *C. amentacea*, which was only found at the exposed site, lie to the left of the second CCA axis, where the centroid of the exposed site is represented. Samples for *C. barbata* show a tendency to lie along the positive region of the first CCA axis, representing sites with high stem length and shelter. Variation between the samples are influenced by water temperature, stem length and depth.

The species points in Figure 5 summarise the preferences of species for different types of sites. The athecate hydroids are separated from the thecate hydroids and lie on the plot where stem length, algal structure and shelterness is high.

DISCUSSION

This study examined the seasonal and spatial distribution of epiphytic hydroids on three species of the brown algae belonging to the genus *Cystoseira*. Of the 32 epiphytic species of hydroids (Table 1) the

most abundant species are all widespread (Boero and Bouillon, 1993). The two species endemic to the Mediterranean Sea were only found occasionally.
The dominant hydroid species present on *Cystoseira* are well known from habitats with a high level of stress factors (Boero, 1981b; Boero and Fresi, 1986). There are no differences in diversity and evenness among the communities of the two sites and on the three species of *Cystoseira*, but species compositions differ.

No host-specificity was found, although host-specific hydroid species are well known from other seaweeds and seagrasses (Boero, 1981a; Hughes *et al.*, 1991; Watson, 1992). All hydroid species epiphytic on *Cystoseira*, except perhaps a new species of *Clytia*, are well known from other biotic or hard bottom substrates. Twenty-three of the hydroid species found (74%) are well known from other epiphytic systems in the Mediterranean Sea (Boero 1981a; Llobet *et al.*, 1986). Substrate availability, i.e. abundance of *Cystoseira*, does not seem to strongly influence the epiphytic hydroid community. Algal substrate is generally more abundant at the sheltered site, but colonisation and number of species is higher, and colony size bigger at the wave-exposed site. Only on *C. compressa* does colonisation correlate with stem length. The high variance of stem length of *C. compressa* from the sheltered site, and therefore the high instability of substrate could have led to its low colonisation. However, the lowest monthly mean in algal stem length of *C. compressa* at the sheltered site is not lower than any mean in algal stem length at the wave-exposed site. Assuming therefore that maximal stem length is a good measure of the available space on the stems of *Cystoseira*, in the present study the amount of available substrate does not seem to be a limiting factor for the hydroid community.

This study provides evidence that hydroid communities on *Cystoseira* have a seasonal cycle. This seasonality could be dependent on substrate availability, but in this study, this factor did not seem to influence the hydroid community. As the abundance of the hydroids on *Cystoseira* coincides with those of the hydroids in the Mediterranean Sea in the cold season in general, their seasonality does not seem to be influenced by their special habitat. The occurrence of the most fertile colonies, the highest species richness and the period of highest hydroid abundance in the cold season, also supports this.

Hydroid abundance differed between the two sites experiencing different levels of wave-exposure. The stems of *Cystoseira* differ between sites in their length and colonisation by hydroids. At the wave-exposed site the stems of *Cystoseira* were probably shorter because of the higher mechanical stress. The higher levels of water movement could result in a higher frequency of stem breakage and so in a higher turnover rate, or in a limitation of growth to a certain length. Water movement supplies the organisms with food, nutrients and dissolved gases, prevents accumulation of sediments, and disperses waste products and larvae (Riedl, 1969). Therefore the stems were probably more frequently colonised, had a higher species frequency, a higher number of species per stem, and larger colonies. However, the tendency for hydroid colonies, especially of the fragile athecate species, to be distributed more towards the protected parts of the stems at the exposed site is likely to be a response to higher mechanical stress.

Another characteristic of epiphytic species is the reduced size or colony height, especially of arborescent species. This was observed among the hydroid species growing on *Cystoseira*. The change in morphology is also likely to be a structural response to water movement. With increasing intensity of water movement colony height decreases and population density and number of hydranths per colony increases. Because growth forms with greater hydranth density require a higher influx of nutrient particles, they tend to occupy more wave-exposed habitats (Riedl, 1971). This can be seen by examining the distribution and fertility of species with different hydranth densities. Colonies of the genus *Aglaophenia*, which have the highest hydranth density per colony, have a higher frequency and abundance at the exposed site than at the sheltered site. Species of the family Campanulariidae have the lowest hydranth density per colony. Among them, *Clytia* sp, the most frequent species colonising *Cystoseira* was clearly more abundant at the sheltered site. This is underlined by the distribution of fertile colonies among the hydroids: *Aglaophenia* spp contribute 44% to the fertile colonies at the wave-exposed site, while campanulariid species contribute only 28%. At the sheltered site the campanulariids contribute 66% and the *Aglaophenia* species only 11%.

The hydroid communities on the three species of *Cystoseira* do not differ greatly. There is, however, a trend towards decreasing colonisation, hydroid abundance and colony size from *C. barbata* to *C. amentacea* to *C. compressa*. These differences could be due to host preferences by larvae which select on

the basis of algal architecture and surface, its age or chemical substances (Nishihira, 1966; Katô *et al.,* 1975; Amico, 1995). The influence of chemical exudates seems only to be important on the same stem where the antibacterial effect prevents colonisation of young branches. This was observed mainly on *C. amentacea*, which has more complex metabolites with a much higher antibacterial action (Amico, 1995) than *C. barbata* and *C. compressa*. The most probable factor responsible for the differences in colonisation among the three species of *Cystoseira* is their morphological nature. *C. barbata* and *C. amentacea*, which are both highly branched with many micro-habitats facilitating hydroid settlement and persistence, are also more heavily colonised than *C. compressa*, which shows a simpler morphology with its flattened and smoother branches. It can be concluded, therefore, that the highest abundance of hydroids on *C. barbata* is mainly due to its highly structured morphology.

Thirty-nine percent of the epiphytic hydroid community on *Cystoseira* comprises athecate species. This is greater than that found on *Posidonia oceanica* (Boero, 1981a) or *Halimeda tuna* (Llobet *et al*., 1991), where athecates comprise 23% and 14% of the hydroid community, respectively. In the Mediterranean Sea athecates generally comprise 48% of all hydroids (Boero and Bouillon, 1993). One reason why athecates are less common in epiphytic communities is the higher level of mechanical forces acting on them. Athecates are presumably more sensitive to these forces because they have no rigid thecae for support or for the polyp to retract into. This may also be the reason why they are more distributed on the more protected lower and inner parts of the stem. The higher frequency of athecate species on *Cystoseira* compared to other seaweeds could be due to its dendroid growth form, which offers more protected places for fragile species to withstand high mechanical stress. The dominance of thecate species among epiphytes underlines the idea that habitats with high mechanical stress are less favourable to the more fragile athecate species.

CONCLUSIONS

The hydroid species colonising *Cystoseira* may avoid the higher levels of competition found on other substrates, but only those species with features enabling them to persist in an environment of high mechanical stress survive as epiphytes. This is probably why the dominant epiphytic hydroids on stems of the brown algae *Cystoseira* from the sites of this study are thecates. The factors with the greatest influence on hydroid communities occupying sites exposed to wave action rather than more sheltered sites appear to be mostly abiotic: sedimentation rate, nutrient levels, temperature and, especially water movement. Biological factors such as the structure and surface of the host and competition seem to be secondary. The importance of water movement, which in this study seemed to have the greatest influence on the hydroid community, could be due to the shallow water depth (0.5-3 m) and high exposure to wave action of the study site.

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Note added in proof: *Clytia* sp. has been identified as *Clytia viridicans* (Leuckart, 1856) (see Pagliara *et al*., this volume)

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TRENDS IN HYDROZOAN BIOLOGY - IV. *C.E. MILLS, F. BOERO, A. MIGOTTO and J.M. GILI (eds.)*

A 'semi-closed' recirculating system for the *in situ* study of feeding and respiration of benthic suspension feeders*

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SUMMARY: Suspension feeding is one of the most widespread feeding strategies among benthic organisms. However, natural feeding ecology and energetics of benthic suspension feeders are poorly known. The scarcity of field methods, apparatus and protocols that facilitate obtention of reliable *in situ* data has contributed to this lack of knowledge. A detailed description of an improved semi-closed recirculating system as well as the experimental set up is provided for the study of energetics in benthic suspension feeders. The system, completely submersible and surface-independent, allows us to assess oxygen concentration changes and feeding rates under natural conditions. Methodological examinations are conducted to investigate: a) the circulation of the water within the chamber; b) the time required for the flushing pump to entirely renew the volume of water of the incubation chambers; c) the behavior of the species within the chambers; d) the time of acclimation to the chamber conditions for the different species; e) the maximum decrease in oxygen concentration without affecting respiration rate; f) the time required to detect changes in concentration of the natural food sources. The system and experimental protocol is tested with species from three representative phyla, Porifera, Cnidaria and Tunicata.

Key words: suspension feeding, methods, recirculating system, respiration, natural feeding.

INTRODUCTION

Suspension feeding is one of the most widespread feeding strategies among benthic organisms. It can be observed in organisms from several different taxa such as Porifera, Cnidaria, Bryozoa, Brachiopoda, Annelida (Polychaeta), Mollusca (Bivalvia), Echinodermata, Crustacea and Tunicata. The main advantage of suspension feeding strategy is an energetic one. No energy is spent in searching for food in passive suspension feeders, and, even in active filter feeders, the cost of pumping has been estimated to be very low (0.3-4% of the total metabolic expenditure for different taxa, Riisgård and Larsen, in press). The main handicap is that suspension feeders can not choose the amount and quality of food they have access to. Therefore, it has long been postulated that most suspension feeders are non-selective (Jørgensen, 1966), and that their diet is primarily controlled by structural constraints (Rubenstein and Koehl, 1977). If this is so, then, within morphological limitations, the composition of the ingested material should be similar to that of suspended material in the surrounding water. How-

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ever, most feeding studies have been carried out with restricted or artificial diets (e.g., Leversee, 1976; Lasker, 1981) or through gut contents analysis which underestimates small soft-bodied organisms that leave no recognizable remains (e.g., Coma *et al.,* 1994). These studies do not reveal which organic fractions are being used as food sources and in what proportions.

Microbial communities are the main contributors to planktonic communities in terms of biomass (Stoeckner and Antia, 1986) and production (Platt *et al.,* 1983; Burkill *et al.,* 1993). As a consequence, much research has been conducted on the dynamics of these planktonic communities in the water column and their trophic interactions with other groups of plankters (Azam *et al.*, 1983; Sherr and Sherr, 1991). In littoral ecosystems, little work has been completed on the trophic interactions between microbial communities and benthic macroinvertebrates. However, recent studies have shown that the role of bacteria, protozoa and phytoplankton in the diet of benthic suspension feeders other than bivalves appears to be higher than previously thought (e.g., Petersen and Riisgård, 1992; Pile *et al.*, 1996, 1997; Ribes *et al.*, 1998, 1999a,b). These studies reveal that one of the less known aspects of the biology of benthic suspension feeders is their natural diets. Despite the little knowledge about feeding of benthic suspension feeders under natural condition, several recent studies suggest an important impact of benthic suspension feeders on planktonic populations (Ayukai, 1995; Gili and Coma, 1998) and, in some special environments, benthic suspension feeders have been suggested to be able to control the dynamics of planktonic populations (e.g., Cloern, 1982). These studies indicate that the feeding ecology of benthic suspension feeders may represent an important step to understanding functioning of littoral ecosystems.

Energetic studies contribute to the comprehend of the dynamics of benthic organisms especially in areas with important seasonal variations of the main environmental factors (Coma *et al.*, 1998). Within the framework of an energetic approach to the study of the dynamics of benthic organisms, respiration constitutes one of the largest fractions of the energy demand of benthic organisms. Respiration can be defined as an energy producing process in living systems that degrade organic matter. The energy released during this degradation is used by the living system to achieve the goals of its survival strategy (Lucas, 1996). Physical factors, including temperature, salinity, ambient oxygen concentration, water flow, and trophic effects, including particle size, filtration activity and food concentration, have been suggested as important factors affecting respiration (Shumway, 1982; Jørgensen, 1966; Sebens, 1987; Patterson *et al.*, 1991; Lucas, 1996; Riisgård and Larsen, in press). Most respiration studies have been performed under laboratory conditions allowing isolation of the effects of the different parameters that affect respiration. Although laboratory studies have made an important contribution to understanding the physical and biological factors that affect respiration, it is difficult to extrapolate laboratory estimates of respiration rates to the field. The main constraint of *in situ* studies is that they cannot isolate the effects of the different parameters that are affecting respiration, but they do provide realistic estimates of the processes because they are conducted with organisms under natural conditions.

One of the most important limitations in order to progress in the knowledge of the natural feeding and energetics of benthic suspension feeders is the scarcity of field methods, apparatus and protocols that facilitate obtention of reliable *in situ* data. Some methods devoted to examine the metabolism of invertebrates (mainly respiration) in the field have undergone significant development and modification during the past years (e.g., Svoboda and Ott, 1983; Tengberg *et al.*, 1995). However, advance in the study of natural diet has been mainly constrained by the large volume required for sample analysis and by the length of time required to evaluate potential food sources. The improvement of flow cytometry and related techniques to examine natural microbial populations has occurred during the last decade, providing an exponential rise in this field due to the substantial reduction in the volume of sample required and in the effort of examination (e.g., Gasol and del Giorgio, 2000). This technical improvement has allowed the scientific community to realize the importance of pico- (0.2-2 μm) and nanoplankton (2-20 μm) in marine ecosystems, now considered to be the main contributors to planktonic communities in terms of biomass and production (Tremblay and Legendre, 1994).

Recent field studies on the natural diet of several sessile benthic suspension feeders suggest that these organisms feed on a wider spectrum of prey type and size than previously recognized by laboratory experiments (Gili and Coma, 1998). A field methodology to study the energetics of benthic suspension feeders should account for the assessment of the

entire range of potential prey, which includes dissolved organic carbon and all sources of particulate organic carbon, both live (i.e. planktonic communities) and detrital. The main goal of this paper was to develop a field technique to obtain reliable samples and to provide an analytical procedure to examine energetics of sessile suspension feeders. Using a combination of oxygen electrodes, pumps and a data logger, we designed a system that solved most of the problems related with closed systems. We report here a detailed description of the system and of the experimental protocol. The system, completely submersible and surface-independent, allows us to assess oxygen concentration variation and feeding rates under natural conditions. The technique was tested with several benthic suspension feeding species from different taxa.

DESCRIPTION OF THE SYSTEM

A general view of the arrangement of the monitoring unit in the field is shown in Fig. 1A and B. A diagrammatic representation of the system (Fig. 1C)

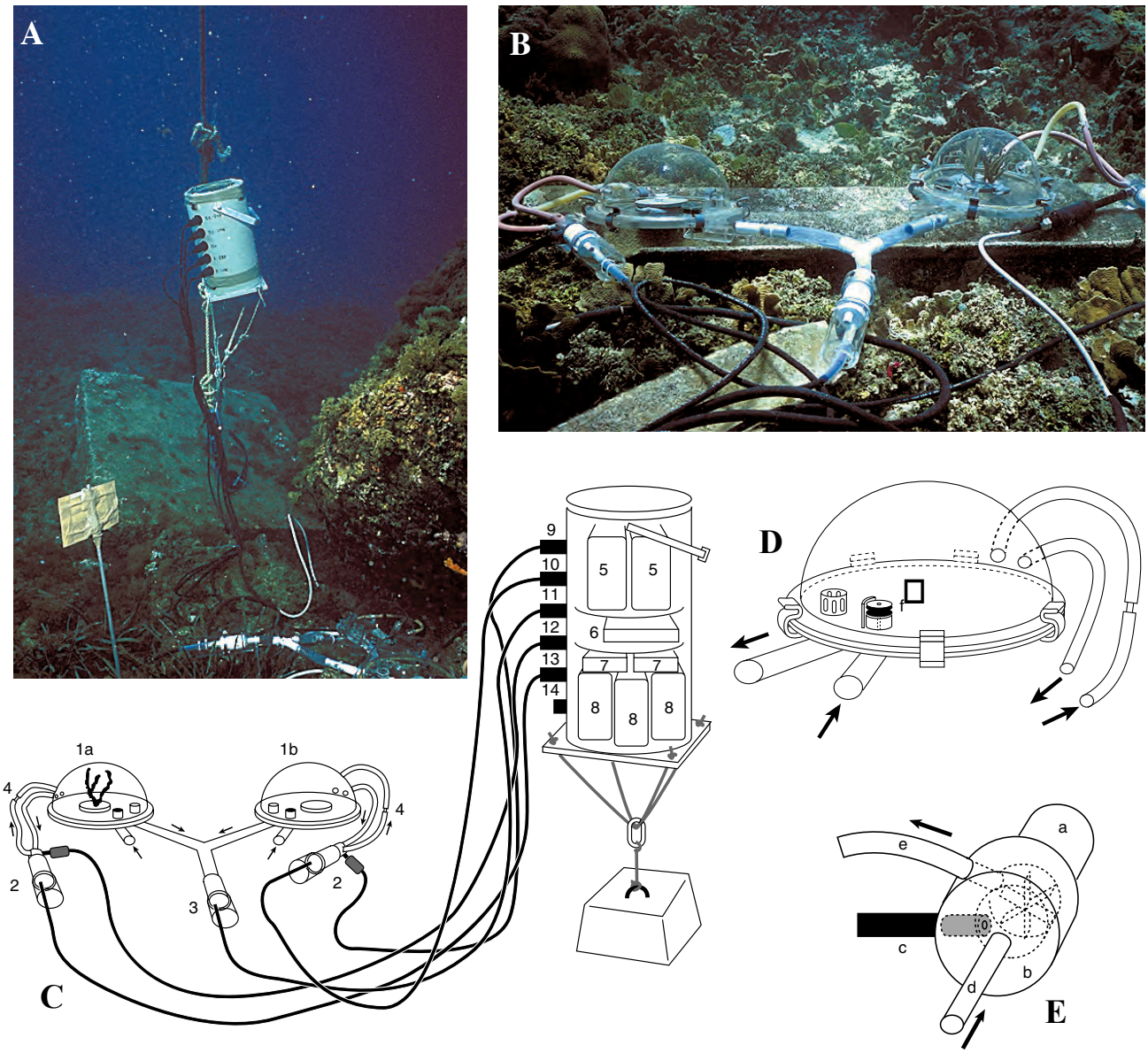

FIG. 1. – Images showing the arrangement of the monitoring units in the field: A.- set-up of the underwater housing containing the electronics. B.- set-up of the chambers, pumps and oxygen probes; C.- Diagrammatic illustration of the whole system. 1a: experimental chamber, 1b: control chamber, 2: flow pumps (3 volts), 3: flushing pump (12 volts), 4: PVC tube connecting inlet and outlet apertures of the chambers, 5: electronics of the oxymeters, 6: data logger, 7: voltage-converter, 8: batteries, 9-13 waterproof electrical connectors, 14: ON/OFF switch. D.- Illustration of an incubation chamber, f: valve. E.- Illustration of the connection between the pump and the oxygen probe, a: flow pump, b: plexiglas pump head piece, c: oxygen probe, d: chamber outlet connection, e: chamber inlet connection.

provides a detailed view of the experimental set up and its components. The unit has two major components, the incubation chambers and the underwater housing. Both chambers (1a and 1b in Fig. 1C) are made from hemispherical pieces of transparent Plexiglas (acrylic glass bei Röhn and Hass, Germany; Svoboda, 1978; Svoboda and Ott, 1983), approximately 3 liters in volume and sealed to a flat transparent plexiglas base with a soft O-ring of foam rubber, glued firmly to the rim of the hemisphere. Plastic fasteners with hooks securely anchor the chambers (Fig. 1D). Inlet and an outlet apertures are connected, via PVC and rubber tubing 0.8 cm in inner diameter (silicone rubber tubing was not used because it is highly permeable to oxygen), to create a closed system. A small submersible electric pump (flow pump designed for caravan cars and boats, 12V DC, with modified head; 2 in Fig. 1C) is placed at the outlet aperture of each chamber. These two pumps operate at 3.3 V. During normal operation, sea water recirculates through the chamber at a flow of 2.4 cm^3/s (this flow becomes turbulent inside the chamber). Homogeneous mixing takes place within a few minutes. The submersible polarographic oxygen and temperature probe (Clark-type oxygen probe, WTW EO-196) is pressure and temperature-compensated (Kanwisher, 1959). It is located close to the flow pump propeller to avoid the formation of microgradients. The probe and the pump, joined through a piece of Plexiglas (Fig. 1E), are connected to the underwater housing by 2 meters of underwater cable and through waterproof electrical connectors. At the base of each chamber there is an outlet for the flushing (3 in Fig. 1C) that allows the water inside the chamber to be automatically changed. The flushing pump, controlled through the data-logger software, removed the water simultaneously from both chambers. An inlet, with a check valve, at the base of each chamber allows water to come in while the flushing pump is working (f in Fig. 1D).

The second main component, a cylindrical underwater housing (70x35 cm), contains the power supply and converters, a data logger, and the electronics of the probes. The power supply is located at the base of the underwater housing and consists of three batteries (Hitachi Sealed lead-acid, 12 V, 6.5Ah, Shin-kobe Electric Machinery Co., Ltd) that provide the power for the three pumps (8 in Fig. 1C). The flushing pump is powered at 12 volts and is activated when there is a preset change in percentage of the initial oxygen concentration in the chamber were the organism is located. The percentage of change in oxygen concentration can be selected through the data logger software.

A voltage-converter system (DC/DC converter) reduce voltage from the general power supply (12 V) to the flow pumps (3.3 V) (7 in Fig. 1C). The data logger (Tattletale Model 4A, Onset Computer Corporation) with its own battery (9 V) is located above the batteries and separated from them by a PVC platform (6 in Fig. 1C). Two oxymeters electronics (WTW microprocessor Oxymeter Oxi 196), which process the signal from the oxygen sensors,

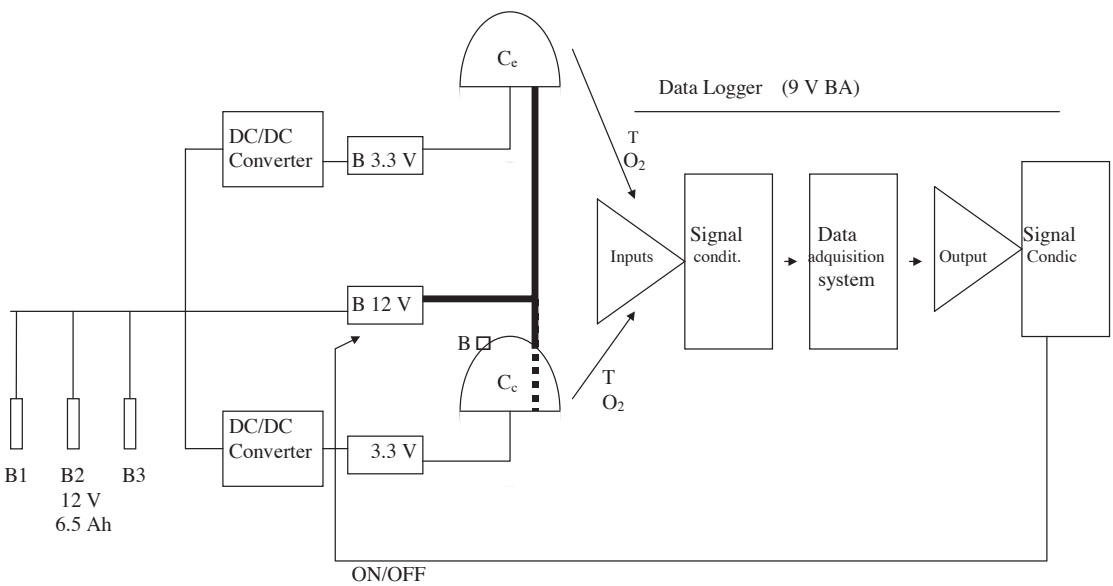

FIG. 2. – Schematics of the electronic parts of the system with detail of the data logger. B1, B2, B3: batteries, DC/DC converter: voltage converter system, B 3.3 V: flow pumps, B12V: flushing pump. Ce: experimental chamber, Cc: control chamber. Signal condit: conditioner of the analogue outputs.

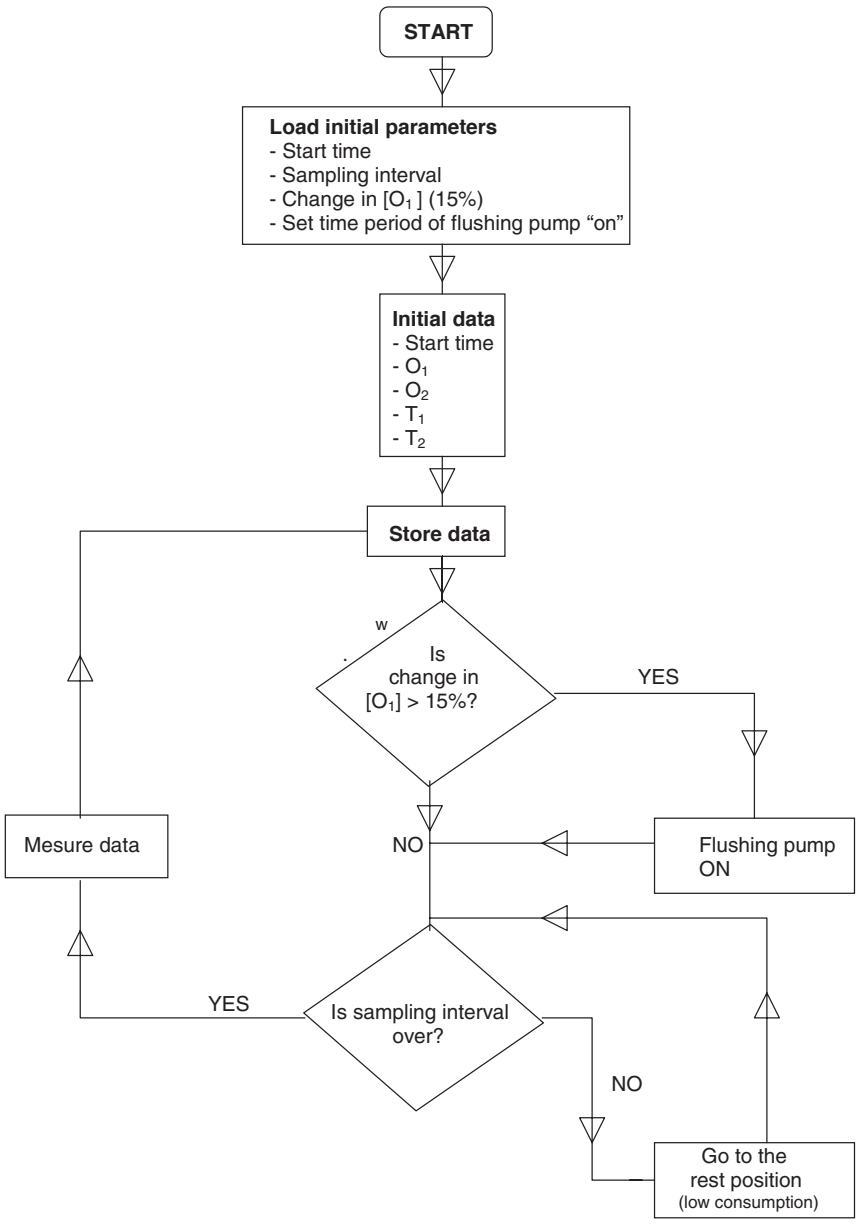

FIG. 3. – Flow diagram of the data logger logics. Subscript 1 refers to change in oxygen (O) and temperature (T) data from the experimental chamber. Subscript 2 refers to data from the control chamber. The program, written in Visual Basic, is provided in Appendix I.

are located on top of the data logger. They are separated from the data logger by another PVC platform (5 in Fig. 1C). The data logger records the measurements of both oxymeters. The software automatically activates the flushing pump when the percentage change in oxygen concentration reaches the preset value. The measurement produced after a 36 seconds flushing period becomes the next oxygen concentration of reference.

The data logger, who has 8 analog input and 16 digital output channels, is connected to the electronics of both oxymeters and controls the flushing pump (Fig. 2). Through the data logger software it is possible to determine the start time of measurement collection, the time interval between measurements, the oxygen concentration from the initial concentration that determined the activation of the flushing pump, as well as the time period of flushing. The analogue outputs of the oxygen and temperature meters are fed through a signal conditioner to the data logger where the signal was recorded (Fig. 2). The data logger records the oxygen and temperature measurements of both chambers every 128 seconds. In Appendix I we provide a flow diagram of the data logger functioning is exhibited in Figure 3. The program, written in Visual Basic.

The upper side of the underwater housing is made of clear acrylic glass. The digital displays of both oxymeter units can be observed. This allows detection of any problem in the functioning of the instrument during the experiments. The cover is sealed with silicone greased O-ring. The chambers and the underwater housing were designed to be handled by SCUBA divers. The underwater housing with the sensors and pumps has a positive buoyancy of about 12 kg. A weight belt was used to provide an slightly negative buoyancy. Then, a diver easily carries it to the experimental site where it was attached to a 50-kg cement flat, allowing us to study the organisms *in situ*. The device could be left in place for 24 h to allow us to examine the potential variation in the respiratory activity along the daily cycle.

APPLICATION AND METHODOLOGICAL CONSIDERATIONS

The system was used to study feeding and respiration in three common Mediterranean benthic invertebrates species: the sponge *Dysidea avara*, the ascidian *Halocynthia papillosa*, and the asymbiotic gorgonian *Paramuricea clavata*. The study was conducted at the Medes Islands Marine Reserve (NW Mediterranean Sea, 42º03'N, 3º13'E). Specimens of the sponge *D. avara*, the ascidian *H. papillosa*, and the gorgonian *P. clavata* were selected to have a similar size (*D. avara*: 0.20 ± 0.003 SD g ash free dry weight (AFDW); *H. papillosa*: 0.51 ± 0.14 SD g AFDW; *P. clavata*: 0.95 ± 0.19 SD g AFDW) to reduce a size effect on the study of both respiration and feeding rates.

D. avara dry weight (DW) was determined by drying at 100ºC for 24 h, and ash free dry weight (AFDW) was determined by combustion at 500ºC for 6 h. *H. papillosa* and *P. clavata* dry weight was determined by drying at 90ºC for 24 h and ash free dry weight by combustion at 450ºC for 5 hours.

About a month preceding the experiments, several specimens from the three species were removed with a piece of substrate, cleaned from macro-epibionts, and placed on artificial supports using inert cement. These specimens were returned to their natural environment close to conspecifics.

Methodological examinations were conducted to investigate: a) the circulation of the water within the chamber; b) the time required for the flushing pump to entirely renew the volume of water of both chambers; c) the behavior of the species within the chambers; d) the time of acclimation to the chamber condition for the different species; e) the maximum decrease in oxygen concentration without affecting respiration rate; f) the time required to detect changes in concentration of food sources.

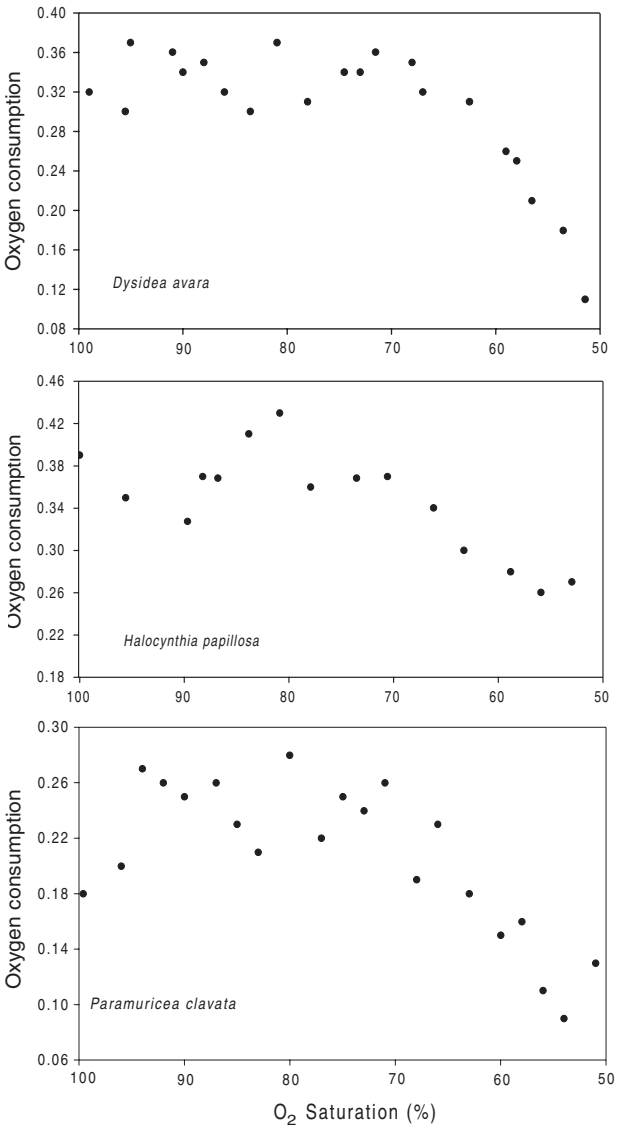

FIG. 4. – Oxygen consumption (in mg O_2 g DW^{-1} h^{-1}) for the 3 species as a function of oxygen saturation percentage: the sponge *Dysidea avara*, the ascidian *Halocynthia papillosa* and the gorgonian *Paramuricea clavata*.

By means of injecting a dye (rhodamine b solution) and monitoring the oxygen values within the chambers, a series of examinations were conducted to verify the homogeneity of the water circulation within the chambers. The flushing pump required about 22 seconds for the complete exchange of the water of both chambers. Therefore, to ensure a good renovation of all the water within the chambers, the duration of the flushing (conducted by the

flushing pump) was programmed to last for 36 seconds. This value could be modified through the software of the data logger.

The system and the chambers (one experimental - with organism-, and one control) were placed at 15 m depth by SCUBA divers. At the beginning of each experiment, one specimen was placed on the base of the experimental chamber. We observed that almost all specimens of the three species fully expand within a few minutes. In the few cases that the incubated specimen did not expand fully within a few minutes, it was eliminated from the experiment. The behavior of the incubated specimens, as well as that of conspecifics outside the chambers, was monitored by direct observation at time intervals ranging between 15 min and 1 hour. The oscula in the sponge species and the syphons in the ascidian species were always open throughout the experiments. The gorgonian species exhibited an expansion and contraction of the polyps similar to that observed in conspecifics and to that previously described from natural populations in the field (Coma *et al*., 1994; and unpubl. data).

Respiration rates of an individual or colony (mg O_2 biomass^{-1} h^{-1}) were estimated from each 0.4 mg O_2 l^{-1} decrease in oxygen concentration in the organism chamber during each experiment. The control chamber was used to compensate for ambient oxygen variations such as respiration or oxygen output of planktonic organisms or respiration of bacteria attached to the internal surfaces of the chambers.

Initial oxygen concentration was always saturated or slightly supersaturated. To determine the decrease in oxygen concentration that does not affect respiration rate, several experiments were conducted without the use of the flushing pump and allowing oxygen concentration to decrease to about 50% from the initial values. These experiments showed two important points: a) that respiration rate estimates during the acclimation period exhibited high variation and, b) that respiration rate estimates were significantly affected by the decrease in oxygen concentration at about 70% oxygen saturation (Fig. 4a,b,c). Therefore, to avoid the initial variation in respiration rate measurements, most probably due to the period of progressive expansion and acclimatization of the specimens, an acclimation period of 1 hour was chosen. During the period that the incubated specimens were allowed to expand fully, the inlet and oulet apertures of the both experimental and control chambers were not connected, so that the system worked was open-flow. After an acclimation period of 1 hour, inlet and outlet apertures were connected, and the system started to run as closed-flow. In both chambers, oxygen concentration and temperature were recorded continuosly (every 2 minutes by the data logger). The renewal of the water inside both chambers was set to occur at an oxygen concentration drop of 15%. This is a conservative value for the three species that does not affect the estimation of respiration rate.

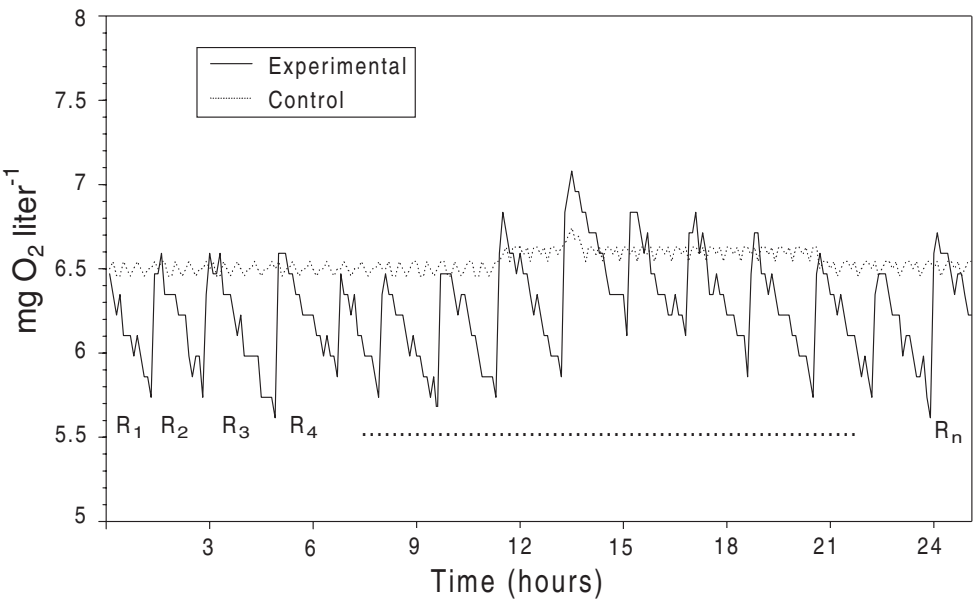

FIG. 5. – *Dysidea avara*. Example of the oxygen concentration (mg O_2 l^{-1}) inside the chambers during a daily cycle. Oxygen values were recorded every 2 minutes in both chambers and water inside both chamber were renewed when oxygen concentration changed 15% from its initial value. R_1..R_n refers to each complete renewal of the water inside the chamber. Experimental: chamber with organism. Control: chamber without organisms. Respiration was determined from decrease in oxygen concentration over time.

The autonomy of the system was tested by allowing it to work over a 24 hour period because one of the goals was to examine respiration rate over daily cycles. The system was set up with the threshold for the activation of the flushing pump at a 15% decrease from the initial oxygen concentration and the time period of the flushing programmed to last for 36 seconds and run for a 24 hour cycle. Typically the system flushed the chambers several times per cycle (see Fig. 5 as an example).

Respiration rate values along with the decrease in oxygen concentration within each renewal were calculated (Fig. 5). This allowed a test of whether or not a 15% drop in initial oxygen concentration affected respiration rates. The test was carried out by comparing, for each renewal within a daily cycle, the first respiration rate estimate (when the organism was subjected to the initial oxygen concentration) with the last one (when the organism was subjected to an almost 15% drop in oxygen concentration) by means of a student t-test for dependent samples (Sokal and Rohlf, 1995).

The time required to detect changes in concentration of food sources was determined by examining food concentration within both chambers over a 5-6 hour period at 1 hour intervals. The experimental set up followed the previously determined protocol (i.e., placing the specimens within the chamber and allowing an acclimation period of 1 hour during which the inlet and outlet apertures of both the experimental and the control chambers were not connected, so that the system worked as an open-flow one). After the acclimation period and before closing the system, three replicate water samples of 50 ml were collected from the outlet apertures of both the experimental and the control chambers (initial water samples). At this point, inlet and outlet apertures were inlet and outlet apertures were connected and the system worked as a closed flow. Water samples were collected at 1 hour intervals.

The water sample was preserved with formaldehyde (0.5% final solution). Subsamples of 15 ml were stained with DAPI and filtered onto 0.2 μm filters. The same subsamples were used to count heterotrophic bacteria, *Synechoccocus sp.,* pico- and nanoeukaryotes and heterotrophic nanoflagellates with an epifluoresce microscope (Porter and Feig, 1980). Grazing was calculated from the decrease in prey concentration in the experimental chamber relative to the control chamber. Changes in abundance of food sources over time for the different species

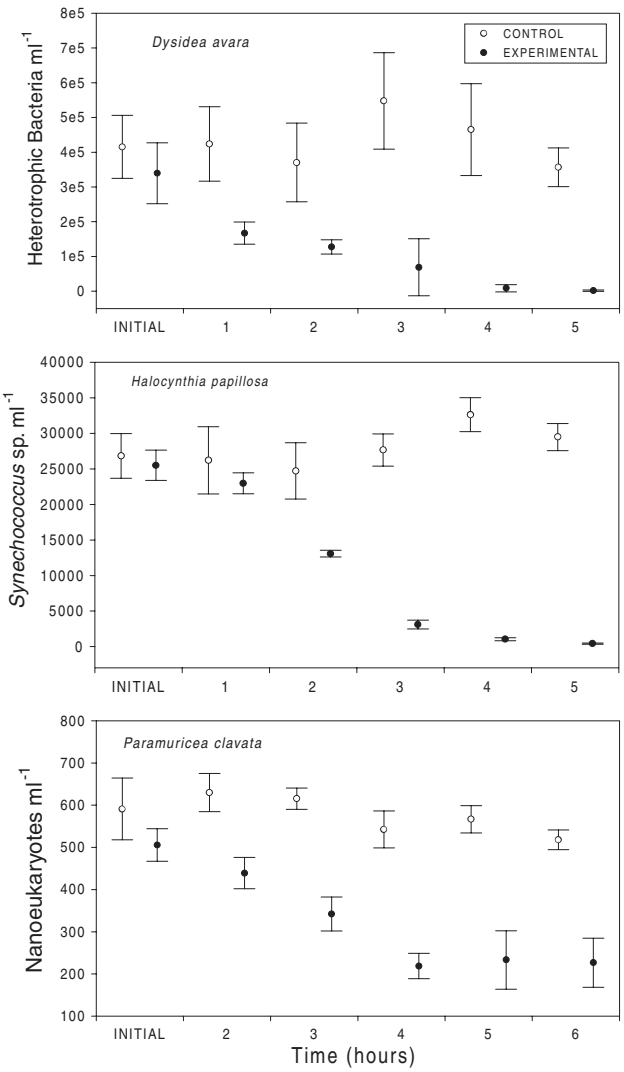

FIG. 6. – Examples of cell concentration (cells ml^{-1}) over time for the 3 species. From the top to bottom: a) capture of heterotrophic bacteria by the sponge *Dysidea avara*, b) capture of *Synechococcus* sp. by the ascidian *Halocynthia papillosa* and, c) capture of nanoflagellates by the gorgonian *Paramuricea clavata*.

within both the experimental and the control chamber were tested using a one-way ANOVA followed by Scheffé´s contrast test (Sokal and Rohlf, 1995). Significant differences were observed in abundance of food sources over time for the 3 species (the sponge *Dysidea avara*, 1-way ANOVA, $F_{5,24}$ = 29.88, p<0.0001; the ascidian *Halocynthia papillosa,* 1-way ANOVA, $F_{5,24}$ = 102.84, p<0.0001 and, the gorgonian *Paramuricea clavata,* 1-way ANOVA, $F_{5,30}$ = 39.92, p<0.0001; Fig. 6a,b,c). A significant decrease occurred after the first one hour of incubation for the sponge species (Scheffé's contrast test, p=0.0015), after the second hour of incubation for the ascidian species (Scheffé's contrast test, p=0.005) and, after the third hour of incubation

for the gorgonian species (Scheffé´s contrast test, p=0.005) (Fig. 6a,b,c). Significant differences in abundance of food sources over time in the control chambers were only observed for the gorgonian and only after 6 hours of incubation (1-way ANOVA, $F_{5,30}$ = 5.78, p=0.0008, Scheffé's contrast test, p=0.005; the sponge *Dysidea avara*, 1-way ANOVA, $F_{5,24}$ = 2.01, p=0.1134, and the ascidian *Halocynthia papillosa,* 1-way ANOVA, $F_{5,24}$ = 0.75, p=0.5955; Fig. 6a,b,c). These experiments allowed us to determine the minimum incubation time to detect significant decrease in prey concentration within the experimental chamber. Minimum incubation time varied among the three species and was 1 hour for the sponge, 2 hours for the ascidian and 3 hours for the gorgonian.

One of the main problems of closed systems is the duration of the experiment (Kamler, 1969). Then, minimization of the residence time of the water inside the chambers is a prioritary objective of closed systems. The residence time of the water inside the chamber during feeding experiments was 1 h, 2 h and 3 h respectively for the sponge, the ascidian and the gorgonian, because this was the minimum time required to detect significant changes in food sources. At the end of this period animals were subjected to an almost 20% drop in oxygen concentration. The above described respiration experiments showed that a drop 20% drop in oxygen concentration did not affect respiration rate and animal behavior which is in accordance with the results observed with other species (Fiala-Medioni, 1979, Crisp, 1984, Fabricius and Klumpp, 1995). For the respiration experiments, organims were subjeted to a maximum of a 15% drop in oxygen concentration because this drop in oxygen concentration already provides several respiration measurements.

This setup allowed us to also examine later the whole spectrum of potential food sources for suspension feeders, including DOC and POM, both live (pico-, nano- and microplankton) and detrital. Sampling, preserving and analysis procedures for all potential food sources as well as calculation procedures and for estimating feeding rates of the three species over a annual cycle are carefully described in Ribes *et al*., 1998, 1999a,b).

DISCUSSION

There are several problems related with the use of closed systems to conduct studies of metabolism, such as the decline in oxygen concentration and the accumulation of metabolites, the formation of oxygen gradients as a result of water stagnation, and inconsistent respiration readings obtained in relation to the duration of the experiment (Kamler, 1969). During the last years, several studies have optimizated the use of closed systems and have shown that they represent an important tool to examine *in situ* change in oxygen concentration related to benthic communities (Smith *et al*., 1972; Zeitzschel and Davies, 1978; Hall *et al*., 1979; Svoboda and Ott, 1983; Patterson *et al*., 1991; Glud *et al., 1*995). As in the mentioned works, we have described a system which solves the potential problems of a closed system and allows us to perform feeding studies. The formation of oxygen gradients has been solved by the continuous flow forced by both flow pumps. Oxygen concentration decrease which can affect the behavior of the organism (Herreid, 1980) and the accumulation of metabolites were avoided by the automatic flushing system which periodically renewed the water of both chambers and that can be modified depending on the studied species. Therefore, this autonomous operating system is a useful tool to study metabolic activity of benthic organisms (both production and consumption of oxygen) and feeding rates under, as close as possible, natural conditions.

We are aware that an important parameter, water movement, which is known to affect the energetic of aquatic organisms (Boynton *et al*., 1981; Patterson and Sebens, 1989; Patterson *et al*., 1991) has not been taken into account. This study was limited to a single flow speed which is rather low (1.2 cm/s). However, the described apparatus may allow the user to have different flow speeds by modifying the voltage converter. This modification could easily provide a range of voltage to the pump from 3 to 12 V that would permit a wide range of flow speeds.

Field techniques to analyze the natural range of potential food sources have not received much attention other than for phytoplankton and zooplankton assessment. This is due to the difficulties involved in the elaboration of specific protocols for each of the different components of the seston. Some of these protocols are of current use by other fields such as microbiology. However, the compilation and standardization of these methods for a relatively small water samples (such as those required to analyse the water whithin the chambers, Ribes *et al*., 1998, 1999a,b) may facilitate the performance of feeding experiments to examine natural diets and feeding rates.

The development of both experimental sampling devices and analytical protocols is a necessary component of field research, even though funding is notoriously difficult to obtain for it and the time consumption for the development of technical preparations is discouraging. However, without the development of new methodological field approaches, our progress in understanding the functioning and dynamics of littoral marine ecosystems is constrained because laboratory experiences can not simulate the extremely diversified biological processes that work in natural ecosystems.

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APPENDIX I. Program to control the functioning the autonomous system by means of the data logger (Tattletale Model 4A, Onset Computer Corporation). Written in Visual Basic.

```
ASM &H9A, DW 8,0
  1 IF F>=1 GOTO 70
 20 RATE 1
 30 PCLR 0
 40 W=65500
 50 SLEEP 0
 60 SLEEP (H*100)
 70 IF PIN(0)=1 GOTO 340
 80 IF F>=G GOTO 340
 90 ONERR 340
 94 RTIME
 95 STIME A
 96 IF A<5 A=128
 97 STORE X,#2,A
 99 PRINT A
100 BURST X,5,2
101 FOR E=0 TO 4
102 PRINT CHAN(E)
103 NEXT E
110 F=F+1
120 M=X-8
130 K=GET(M,#2)
132 IF N=1 W=K
133 N=0
135 PRINT "OXYGEN MEASUREMENT=",K
136 PRINT "COMPARATION=",W
140 B=X-12
150 FOR E=1 TO 6
160 C=GET(B,#1)
170 D=GET(B,#1)
180 B=B-2
190 STORE B,#1,D
200 STORE B,#1,C
210 NEXT E
220 IF (100*K)>(Z*W) GOTO 250
230 IF (100*K)<(Y*W) GOTO 250
235 PRINT "*———————-*"
240 DONE
250 PSET 10
255 PRINT "PUMP ON"
260 SLEEP (T*100)
270 PCLR 10
280 N=1
330 GOTO 70
340 F=0:A=X:X=10
350 STOP
```

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