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The ascidians of Tossa de Mar (NE Spain) II.- Biological cycles of the colonial species

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Abstract : The ascidians from a locality on the Spanish NE coast were sampled from November 1984 until January 1986, with the aim of studying their biological cycles. Only the results concerning the colonial species will be presented here. The samplings were performed twice a month, and the relative abundance, reproductive state and presence of resistance forms of the compound ascidian species were evaluated.

Many species feature seasonal variations in abundance and even disappear from the samples in the unfavourable season. The reproductive periods are always restricted to a certain part of the year and they are strongly correlated with the biogeographical distribution of the species. The temperature appears as the main factor controlling ascidian reproduction. Notes are made on the significance of the resistance forms in the families Polyclinidae and Didemnidae.

Résumé : Les ascidies présentes dans les fonds rocheux d'une localité de la côte NE espagnole ont été étudiées depuis novembre 1984 jusqu'à janvier 1986, dans le but de préciser leurs cycles biologiques. Dans ce travail, seuls les résultats correspondant à des espèces coloniales seront présentés. L'abondance relative, les périodes de reproduction sexuée et la présence de formes de résistance ont été évaluées deux fois par mois pendant l'étude.

Beaucoup d'espèces montrent des variations saisonnières d'abondance, et quelques-unes même disparaissent pendant la saison défavorable. Les espèces étudiées ne sont en reproduction sexuée qu'à certaines périodes de l'année. Il y a une bonne corrélation entre les périodes de reproduction et la répartition biogéographique des espèces. Le facteur température semble le plus conditionnant. On ajoute quelques remarques sur la signification des formes de résistance dans les familles Polycitoridae et Polyclinidae.

INTRODUCTION

The biological cycles of ascidian species have been dealt with in several works, generally concerning a single species or a small number of species from a particular area on which a number of periodic observations have been made. The papers by Millar (1952, 1954, 1958, 1974), Sabbadin (1957), Dybern (1965) or Brunetti (1976) provide excellent data on the biology of several species.

In the present work the reproductive cycles of all the compound ascidian species found in a locality on the Spanish Mediterranean coast have been studied for an entire annual cycle. The sole precedent of a somewhat similar work is the paper by Medioni (1970), on the ascidians and bryozoans from Banyuls-sur-Mer (France).

MATERIAL AND METHODS

The ascidian specimens for this study were collected during some periodic (approximately twice a month) Scuba diving samplings in a restricted area off the coast near Tossa de Mar (NE Spain) from November, 1984 to January, 1986. Data on the exact position of the area studied, its physical and ecological characteristics, as well as the sampling methodology, have been reported elsewhere (Turon, in press). Fig. 1 represents the water temperature at a level of -10 m throughout the study.

In each sampling event, the number of colonies of each species found was counted, and several (five or more, when possible) specimens were collected and fixed in order to check their reproductive condition in the laboratory.

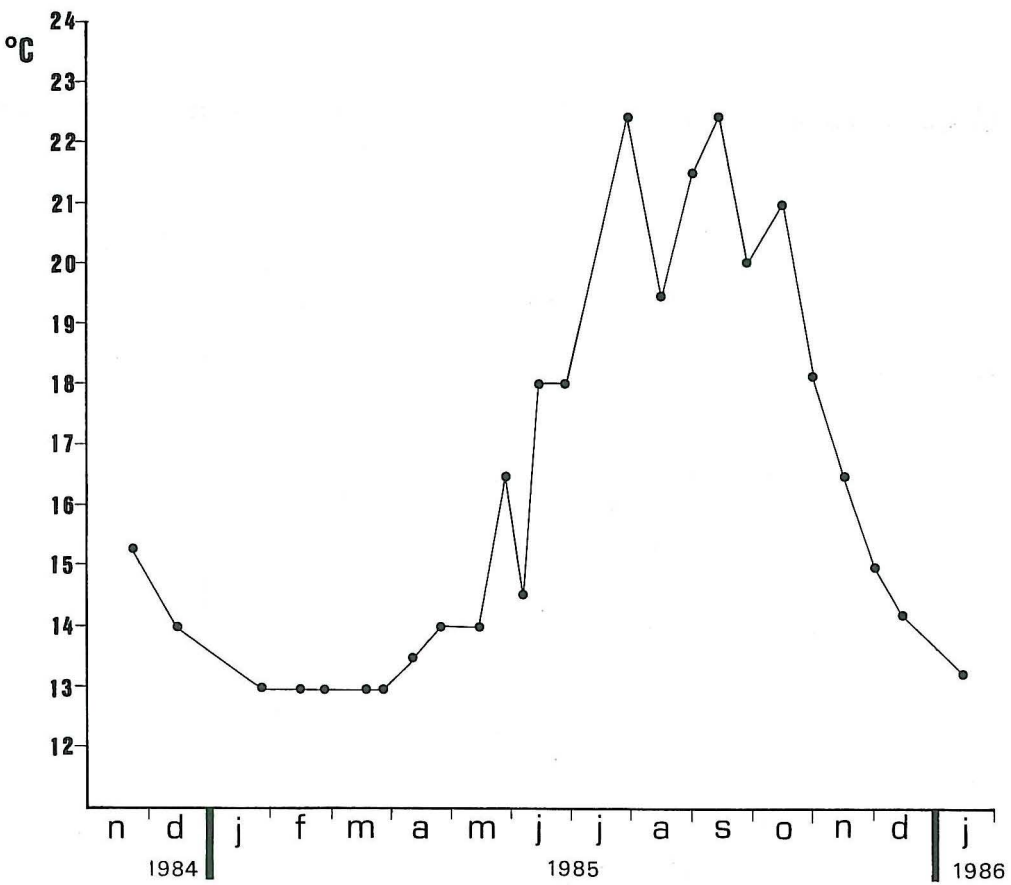


Fig. 1 - Water temperature at a level of -10 m during the study.

RESULTS

A total of 36 compound ascidian species were identified in this work. The systematic list and taxonomic discussion are given in the first part of this study (Turon, in press).

The results will be presented in several graphs, in which the number of specimens of each species found in a sampling event are represented, as well as their reproductive state. Whenever possible, data on the presence of resistance forms have been added.

The abundance is represented in terms of number of colonies found. A species is considered abundant when ten or more specimens have been found; accordingly, the presence of these species is represented by the value ten (= "n").

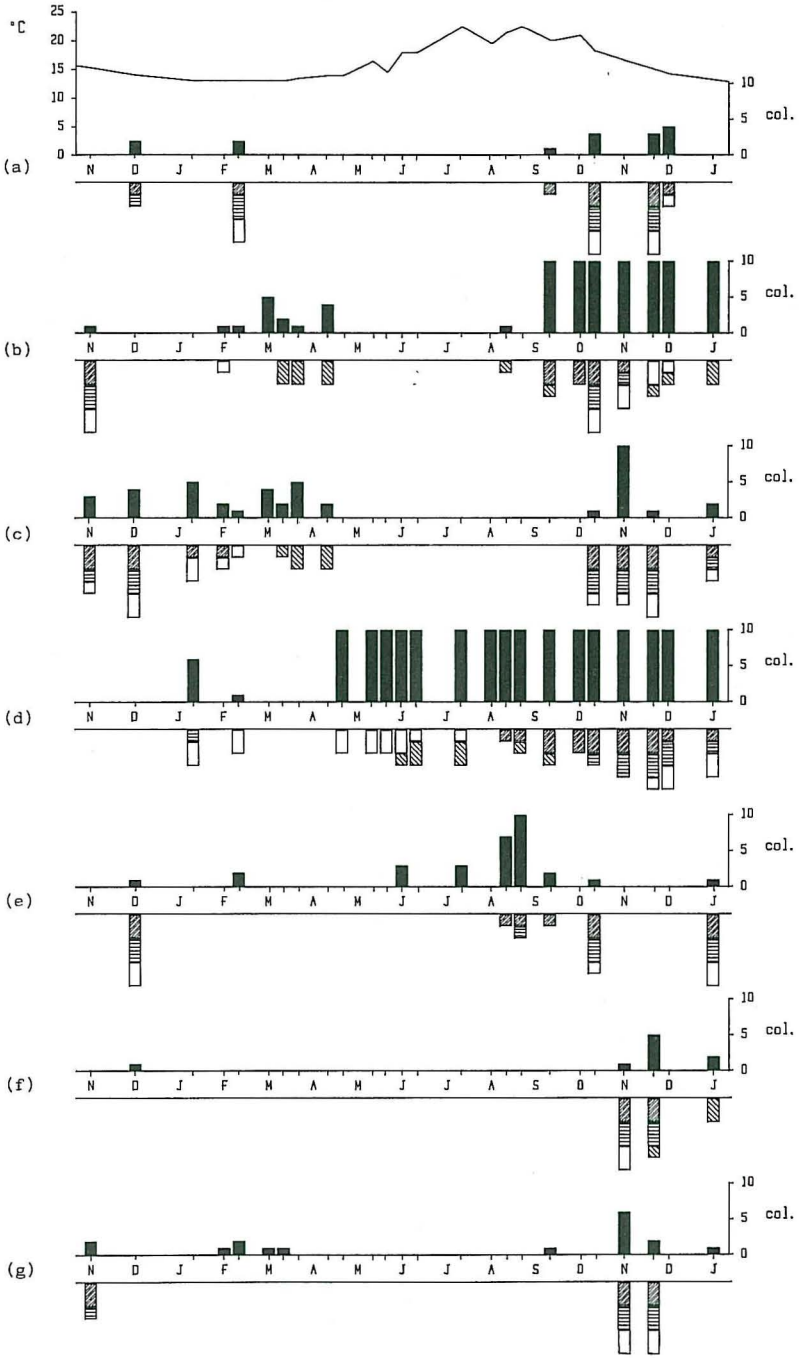
The estimated reproductive condition (obtained by studying not less than 50 zooids per colony) is represented by a column with different patterns for each character considered (testes, ovaries, embryos and resistance forms) which can be either short or long. A short column means that the concerned character is present in less than 50 % of the zooids observed. A long column is used when more than 50 % of zooids feature this character (in some cases, such as the species incubating larvae in the tunic, this representation corresponds to a subjective estimate by the author of the abundance of these larvae). When the character is absent, the corresponding column is not figured. The temperature graph is superimposed in order to make the interpretation of the results easier.

Fig. 2 to 4 present the results reflecting the main colonial families found: Polyclinidae, Didemnidae, Polycitoridae and Perophoridae. Only the species which have appeared regularly enough to allow for an interpretation of the results are included.

Fig. 5 shows the relationship between the number of species found and the number of species with incubating embryos for every month. The value for each month is the mean of the data for this month, even if the samplings correspond to two years. In Fig. 6, the same information is given separately for each Aplousobranch family.

The data on abundance show that, in many cases, the species display strong seasonal variations. For instance, almost all of the Polyclinidae species disappear from the samples in summer (only *Pseudodistoma crucigaster* has been found throughout the year). The Didemnidae species are, in general, uniformly distributed among the samples, whereas in the family Polycitoridae there is much variation: *Cystodytes dellechiajei, Eudistoma planum* and *Polycitor cristallinus* are found throughout the year; *Clavelina lepadiformis* and *C. nana* disappear during the summer months.

In most species the periods of sexual activity are restricted to certain seasons. Only in *Pseudodistoma crucigaster, Didemnum maculosum* and *Cystodytes delle-*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chiajei, has the presence of gonads been observed for all seasons (although the incubating period is always more restricted).

Only in *Eudistoma planum* does the maximum for reproductive activity occur during the months in which the temperature reaches its maximum (in this species there seem to be two periods of larval release during the year : in May and August, a condition that has not been detected in any other species). *Polysyncraton canetense* and *Didemnum granulosum* also have larvae in the estival period, although these species only make a sporadic appearance in this study and are not included in the figures.

The rest of the species can be classified into three groups according to the approximate period of sexual activity :

- winter-spring : *Polysyncraton lacazei, Didemnum coriaceum, Didemnum lahillei, Diplosoma spongiforme, Polysyncraton bilobatum, Clavelina lepadiformis, Polycitor cristallinus, Perophora viridis* .
- autumn-winter : *Aplidium albicans, A.* aff. *conicum, A. densum, Sidnyum elegans, S. turbinatum, Synoicum argus, Lissoclinum perforatum, Ecteinascidia herdmanni.*
- autumn-winter-spring : *Clavelina nana.*

In addition, several species, not included in the graphs, have appeared so infrequently that it is impossible to extract any trends from the data available : *Aplidium coeruleum* (gonads in February), *A. conicum* (ovaries and larvae in May), *A. hyalinum* (gonads and larvae in March), *A. pallidum* (gonads and larvae in May), *A. nordmanni* (gonads and larvae in November), *Polyclinella azemai* (testis in February), *Polyclinum aurantium* (gonads and larvae in June), *Didemnum coccineum* (gonads in July), *Didemnum pseudofulgens* (gonads in June), *Distomus variolosus* (gonads and larvae in March), *Synoicum duboscqui, Botryllus schlosseri* and *Botrylloides leachi* (in which neither gonads nor larvae have been observed in the few specimens collected).

The presence of resistance forms is generally difficult to detect, since they are usually hardly visible in the water and unidentifiable in the laboratory. However, in some species, like *Pseudodistoma crucigaster* and *Polysyncraton lacazei,* this fact is readily observable because the colonies in resistance form feature a bright glassy surface pellicule. Consequently, in these species, the relative abundance of resistance forms has been evaluated (Fig. 7).

Fig. 2 - Number of colonies collected and biological condition of the members of the family Polyclinidae. a : *Aplidium albicans ;* b : *Aplidium* aff. *conicum ;* c : *Aplidium densum ;* d : *Pseudodistoma crucigaster ;* e : *Sidnyum elegans ;* f : *Sidnyum turbinatum ;* g : *Synoicum argus.*

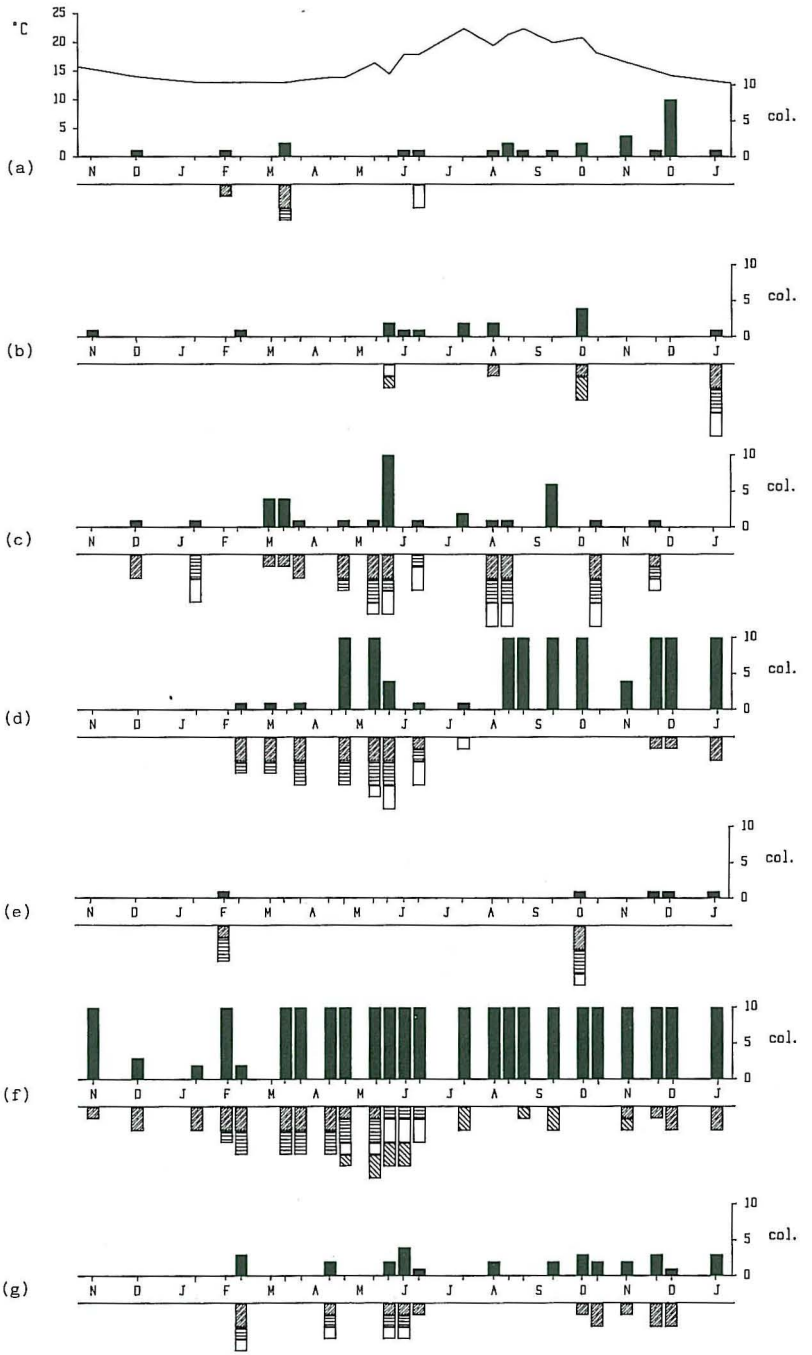


Fig. 3 - Number of colonies collected and biological condition of the members of the family Didemnidae. a : *Didemnum coriaceum* ; b : *Didemnum lahillei* ; c : *Didemnum maculosum* ; d : *Diplosoma spongiforme* ; e : *Lissoclinum perforatum* ; f : *Polysyncraton lacazei* ; g : *Polysyncraton bilobatum*.

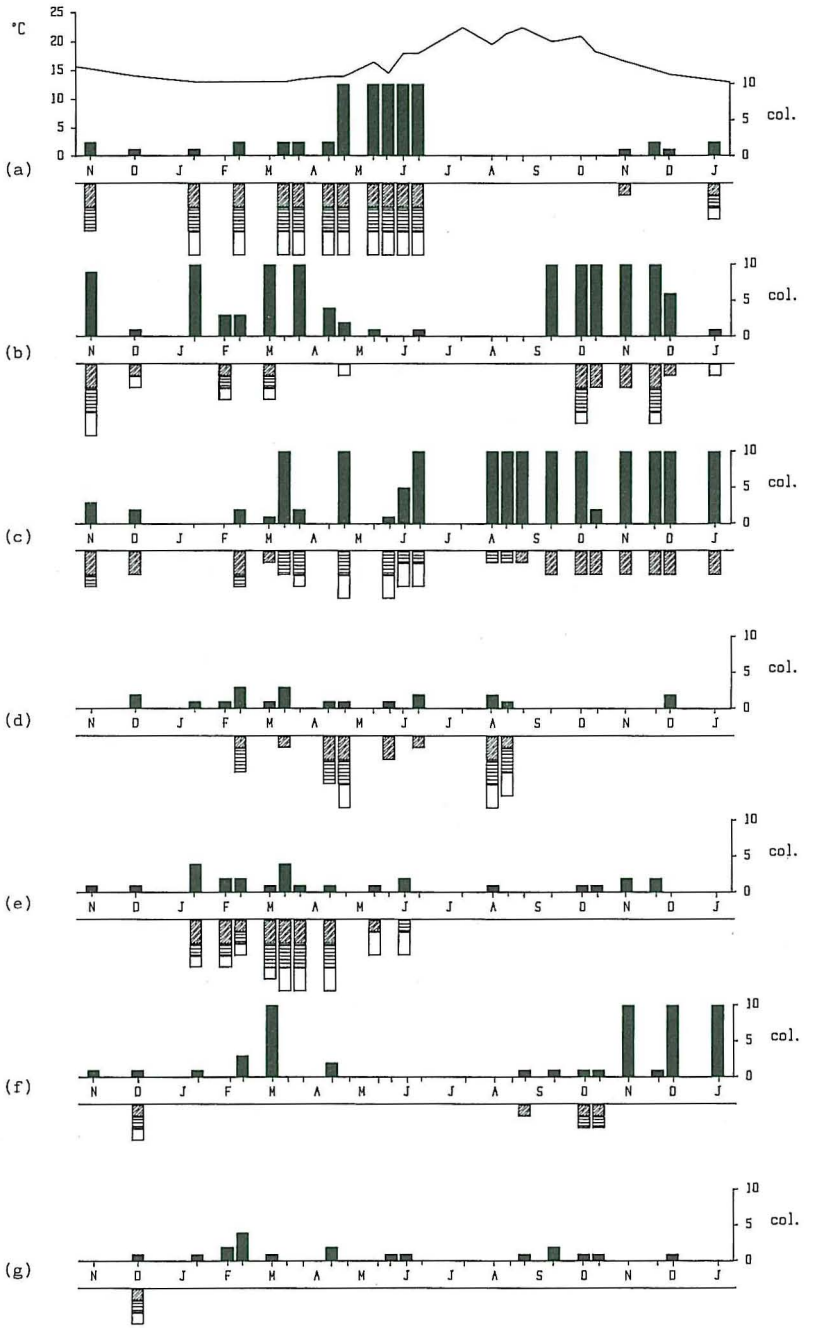


Fig. 4 - Number of colonies collected and biological condition of the members of the families Polycitoridae and Perophoridae. a: *Clavelina lepadiformis*; b: *Clavelina nana*; c: *Cystodytes dellechiajei*; d: *Eudistoma planum*; e: *Polycitor cristallinus*; f: *Ecteinascidia herdmanni*; g: *Perophora viridis*.

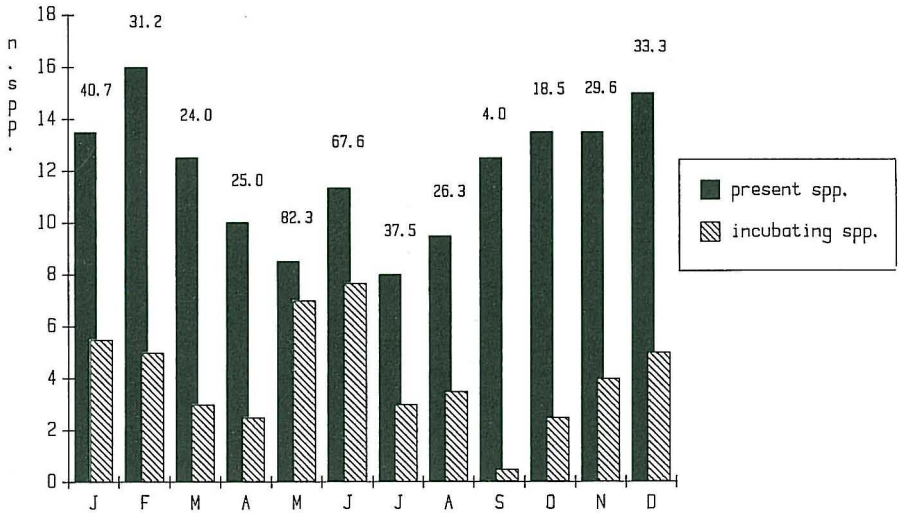
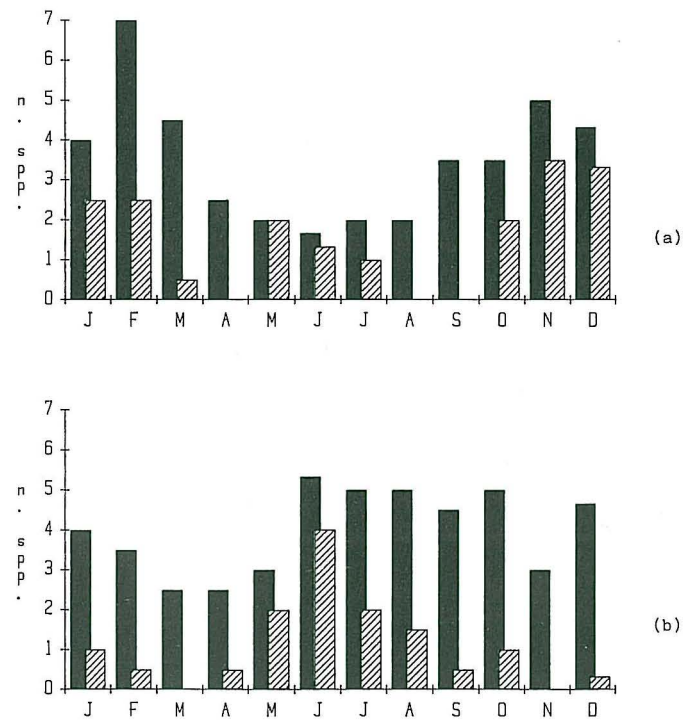


Fig. 5 - Relationship between the number of species found and the number of species with incubating embryos (mean for every month). The value above each column represents the percentage of incubating species.



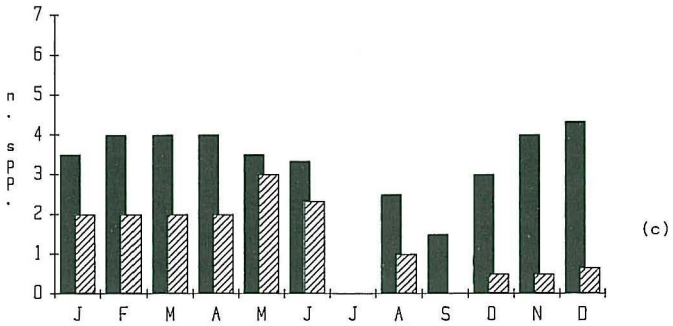


Fig. 6 - Relationship between the number of species found and the number of species with incubating embryos (mean for every month) in the families Polyclinidae (a), Didemnidae (b), and Polycitoridae (c). Same symbology as in Fig. 5.

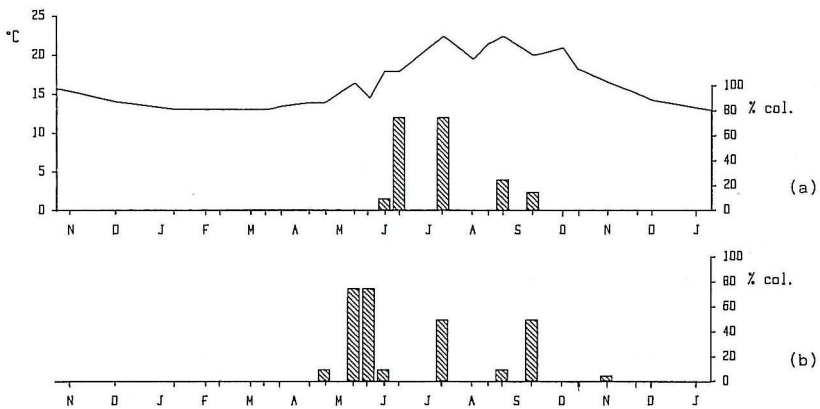


Fig. 7 - Percentage of colonies in resistance form of *Pseudodistoma crucigaster* (a) and *Polysyncraton lacazei* (b) throughout the study.

DISCUSSION

The abundance data reveal a strong seasonal variability in some of the colonial species, as already pointed out by Medioni (1970) for Banyuls-sur-Mer. Several species disappear for certain months, and since no continuous studies on marked specimens have been performed, it is impossible at present to state, for these species, whether there is one generation per year (so what is observed is the full cycle of recruitment, growth and disappearance of each generation), or whether the species is pluriannual, in which case several generations are seen superimposed and the season in which no specimens are found corresponds to the resistance period in which the species is unidentifiable.

The reproductive period is very variable both in its duration and in the season in which it occurs. There is a clear correlation between geographical distribution and reproduction, as already noted by other authors (Millar, 1958) : Mediterranean and temperate species feature gonads over a long period, whereas those species far away from their centre of distribution are active for only 4-5 months.

The three families of Aplousobranches, on the other hand, display some uniformity in their reproductive cycles, thus reflecting the biogeographical origin of their members : the family Polyclinidae is made up, in general, of species with Atlanto-mediterranean affinities (Pérès, 1958) with a boreal distribution, and they are here in the southern part of their range of distribution. Consequently, their reproductive periods, as seen by the number of species with incubating embryos (Fig. 6 a) are concentrated in the cold season, and many of the species disappear during the hotter months. As an exception, *Pseudodistoma crucigaster,* an endemic Mediterranean species, displays a long reproductive period with larval release in summer.

The family Didemnidae, on the other hand, has a strong endemic element, being other species common with the Senegalian fauna (Lafargue & Wahl, 1987) ; accordingly, these species feature, in general, long reproductive periods, with larval release in spring-early summer (Fig. 6 b).

The family Polycitoridae displays a greater variability in its reproductive period, although with a maximum of incubating forms in spring (Fig. 6 c). In any case, the reproductive periods can be easily correlated with the biogeographical origin of the species : *Cystodytes dellechiajei* and *Eudistoma planum,* circumtropical ; *Polycitor cristallinum,* Western Africa and the Mediterranean sea ; *Clavelina lepadiformis* and *Clavelina nana,* Atlantomediterranean with European distribution.

These results seem to confirm that the temperature is the main factor controlling the reproduction of ascidians, as generally acknowledged for all marine invertebrates (Orton, 1920 ; Millar, 1971). However, the possible influence of other factors (light, availability of food, etc.) must be kept in mind. Other reasons may be ecological ; in particular, winter reproduction when the algal populations grow at a slow rate in the Mediterranean may be a temporal competitive strategy for boreal species unable to compete during spring-summer with native populations.

In general, the months with highest temperatures appear as the unfavourable season for the ascidian populations in this area. Certainly, the number of species present is low, as well as their percentages in sexual activity (the minimum, 4 %, occurs in September) (Fig. 5). In addition the number of resistance forms increased. The water stratification in summer, with a decrease in nutrients in this area (Ballesteros, 1984) may also contribute as an indirect effect of temperature on this general pattern. This is not the condition found in other Mediterranean ambients, such as the Venice Lagoon, where temperatures in winter fall much below those of Tossa due to the shallowness of the water. In this area, the unfavourable season is the winter, with the appearance of hibernation phenomena in some species (Sabbadin, 1958 ; Brunetti, 1976).

As for the resistance forms in *Pseudodistoma crucigaster* and *Polysyncraton lacazei,* both species display a high percentage of such forms in summer, but the significance of this phenomenon seems to be different. In *Pseudodistoma crucigaster* the resistance forms appear at the end of the reproductive period, after larval release. The thorax becomes inactive and regresses, whereas the abdomen and post-abdomen are filled with reserve substances, thus enabling the species to survive during the unfavourable period (this fact being called "survival budding" by Nakauchi, 1982). In *Polysyncraton lacazei,* on the other hand, the appearance of resistance forms takes place in a period when the colonies are active. These colonies acquire a bright surface pellicule and the siphonal apertures become inactive, but the colonies have gonads or larvae exactly the same as the active colonies from the same period. The only morphological feature of the zooids of these colonies is the regression of the thoraxes and the appearance of new ones formed by budding. This resting period seems to be short, since great differences in the percentage of these forms have been found from one sampling to the next (Fig. 7). Perhaps the term resistance form, as used for *Pseudodistoma crucigaster,* is unsuitable in this case, and the whole phenomenon may be only a thoracic renewal, somehow associated with high temperatures. Observations on other members of the family Didemnidae (*Didemnum lahillei*) and the family Polyclinidae (*Aplidium densum, A.* aff. *conicum* and *Sidnyum turbinatum*) concur, respectively, with the model for the two above-mentioned species, strongly suggesting that the significance of the resistance forms in both families is different. Further research on this point is necessary to clarify the exact physiological significance of these forms.

REFERENCES

BALLESTEROS, E., 1984. Els vegetals i la zonació litoral : espècies, factors i comunitats que influeixen en la seva distribució. Ph. D. Thesis, University of Barcelona. 380 pp.

BRUNETTI, R.H., 1976. Biological cycle of *Botrylloides leachi* (Savigny) (Ascidiacea) in the Venetian Lagoon. *Vie Milieu,* 26 (1A) : 105-122.

DYBERN, B.I., 1965. The life cycle of *Ciona intestinalis* (L) f. *typica* in relation to the environmental temperature. *Oikos,* 16 : 109-131.

LAFARGUE, F. & M. WAHL, 1987. Faune de France des ascidies Didemnidae. *Ann. Inst. océanogr.,* Paris, 63 : 1-46.

MEDIONI, A., 1970. Les peuplements sessiles des fonds rocheux de la région de Banyuls-sur-Mer. Ascidies-Bryozoaires (1e partie). *Vie milieu,* 21 (3B) : 591-656.

MILLAR, R.H., 1952. The annual growth and reproductive cycle in four ascidians. *J. mar. biol. Ass. U.K.,* 31 : 41-61.

MILLAR, R.H., 1954. The annual growth and reproductive cycle of the ascidian *Dendrodoa grossularia* (Van Beneden). *J. mar. biol. Ass. U.K.,* 33 : 33-48.

MILLAR, R.H., 1958. The breeding season of some littoral ascidians in Scottish waters. *J. mar. biol. Ass. U.K.,* 37 : 649-652.

MILLAR, R.H., 1971. The biology of ascidians. *Adv. mar. biol.,* 9 : 1-100.

MILLAR, R.H., 1974. A note on the breeding season of three ascidians on coral reefs at Galeta in the Caribbean Sea. *Mar. Biol.,* 23 : 127-129.

NAKAUCHI, M., 1982. Asexual development of ascidians : its biological significance, diversity, and morphogenesis. *Amer. Zool.,* 22 : 753-763.

ORTON, J.H., 1920. Sea-temperature, breeding and distribution in marine animals. *J. mar. biol. Ass. U.K.*, 12 : 339-366.

PÈRÈS, J.M., 1958. Origine et affinités du peuplement en ascidies de la Méditerranée. *Rapp. p.v. Réun. Comm. Int. Expl. Scien. Mer. Médit.*, 14 : 493-592.

SABBADIN, A., 1957. Il ciclo biologico di *Ciona intestinalis* (L.), *Molgula manhattensis* (De Kay) e *Styela plicata* (Lesueur) nella Laguna Veneta. *Archivio oceanogr. Limnol.*, 12 : 97-107.

SABBADIN, A., 1958. Sur les caractéristiques du cycle biologique de quelques ascidies dans la Lagune de Venise, en rapport avec le régime thermique. *Rapp. p.v. réun. Comm. Int. Expl. Scient. Mer Médit.*, 14 : 577-581.

TURON, X., in press. Las ascidias de Tossa de Mar (Girona). I. Generalidades, faunística y taxonomía *Misc. Zool.*