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Aspects on the demography of Chub Mackerel (*Scomber japonicus* Houttuyn, 1782) in the Hellenic Seas

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ABSTRACT. The main features of the demography of *Scomber japonicus* in the Hellenic seas were studied during 1996. The dominant size class in the length frequencies of all catches was 190-200 mm, whereas the dominant age class in the samples from the Northern Aegean and Saronikos Gulf was the age class 1, and from the Cretan Sea the age class 3. The formation of the new annulus commences in April for age classes 1 and 2 and in July for the age class 3. The sex ratio in all samples was found to be 1:1. The von Bertalanffy growth functions showed no statistically significant difference between males and females. The growth parameters from the pooled data were estimated as: L_∞ = 475.96 mm, K = 0.154 yr^{-1} and t_0 = -2.177. Scomber japonicus in the Hellenic seas exhibits slower growth rate than that observed in other Mediterranean regions.

KEY WORDS: *Scomber*, Aegean, Saronikos, Cretan Sea.

INTRODUCTION

Chub mackerel (*Scomber japonicus* Houttuyn, 1782), is a cosmopolitan middle-sized pelagic species with a very wide distribution over the continental shelf of the tropical and subtropical regions of the Atlantic, Indian, Pacific Oceans and adjacent seas. It is a primarily coastal species, found from the surface down to 300 m depth (COLLETE & NAUEN, 1983). Along its distribution, the species is found in isolated populations with complex intraspecific structure, which has been studied little (KOTLYAR & ABRAMOV, 1982). Five subspecies have been identified so far, which form discrete populations with different spawning and feeding areas along the species distribution (KOTLYAR & ABRAMOV, 1982; BELYAEV & RYABOV, 1987). In the Mediterranean sea and in the west African waters, the subspecies found is the *Scomber japonicus colias* (MORAITOPOULOU, 1963; KOTLYAR & ABRAMOV, 1982).

Scomber japonicus is a species of high commercial interest in Hellas. According to the bulletins of the Hellenic National Statistical Service, during the last decade the annual production in the Hellenic seas was about 10000 mt. Despite its important commercial value, virtually nothing is known about its stocks in this region and generally in the Eastern Mediterranean. Information regarding the biology and population dynamics of this species comes mainly from studies conducted in other parts of the world, mainly from the Pacific Ocean (SCHAEFER, 1980). The objective of the present work is to elucidate the main features of the demography of this species in the Hellenic seas.

MATERIAL AND METHODS

Fish sampling

Samples were collected monthly during the year 1996 from the landings at the auction sites of Kavala and Thessaloniki (Northern Aegean), Pereus (Saronikos Gulf) and Heraklion and Chania (Cretan Sea). A total of 1138 fish was collected from all sampling regions. From the Cretan Sea samples were found only during June and August because of the seasonal appearance of this species in this area. All fish were caught with purse seines and after landing were refrigerated at -20°C until further work. Sampling was not conducted during January and

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December 1996 because of the prohibition of purse seine fishing during these months. The total weight of each fish was weighed to the nearest centigram and its fork length was measured to the nearest millimeter. Sex was determined through gonadal inspection. The proper interval for the length frequency distribution was estimated by the Goulden method (Cansela da Fonseca, 1965).

Age determination

The age of each fish was assigned by otolith reading. The otoliths (sagittae) of each fish were removed, cleaned and preserved dry. The readings were conducted by a stereoscopic microscope under a 50x magnification, using reflected light against a dark background. Under reflected light the bands formed during periods of fast growth have a white appearance (opaque), while those formed during the periods of slow growth have a dark appearance (translucent). In order to enhance their transparency during reading, the otoliths were kept submerged in 90° alcohol. Each otolith was read twice, with a two month interval between each reading. Whenever there was a discrepancy between the two readings, the age data were excluded from the study. Besides the number of annuli on each otolith, the otolith radius (distance from the focus to the otolith posterior end), the width of each annulus as well as the type of marginal formation were recorded.

Statistical analysis

The time of the annulus formation and the periodicity of the opaque and translucent bands were estimated by the marginal increment ratio (MIR), which gives a measure of the relative width of the otolith edge and is described by the ratio:

$$MIR = (R - r_n) / R$$

where R = the total otolith radius and
r_n = the radius of the most recent annulus.

The MIR was calculated for each month and age separately. Marginal increment analysis was not conducted for ages above 3 due to the lack of sufficient samples.

The sex composition of the samples from all three sampling regions was examined for each age class independently and significant deviations from the expected 1:1 sex ratio were tested using the χ^2 goodness of fit test (Zar, 1996). The correction factor of Yates was applied on the test.

The theoretical rate of growth was obtained by fitting the assigned ages and the corresponding lengths to the von Bertalanffy growth model. The parameters of the model were estimated iteratively using the Simplex minimization algorithm (Wilkinson, 1987). The measure of goodness of fit was the coefficient of determination (r^2). The 1st of June was assigned as the birth date for all fish. During this month, the largest concentration of *S. japonicus* larvae has been detected in the Northern Aegean (Somarakis, personal communication). The growth parameters were estimated for each sex separately and for both sexes combined. The difference in growth of males and females was tested by an analysis of the residual sum of squares using the Ratkowsky method as suggested by Chen et al. (1992).

The length-weight relationship was estimated from the regression of the total weight to the fork length. For this calculation the logarithmic values of weights and lengths were used.

RESULTS

Length frequencies

The dominant size class in the length frequency distribution from the catches of all three regions together was the 190-200 mm size class. For each region seperately, the dominant size classes were: 155-165 and 240-250 mm for the Northern Aegean catches, 190-200 mm for the Saronikos Gulf catches and 275-285 mm for the Cretan Sea catches (Fig. 1). The range of the total lengths and the mean length of the fish caught in each study area were: a) for the Northern Aegean Sea 91-266 mm (mean length=184 mm, sd=28 mm) b) for the Saronikos Gulf 138-293 mm (mean length=206 mm, sd=38 mm) and c) for the Cretan Sea 239-310 mm (mean length=279 mm, sd=15 mm).

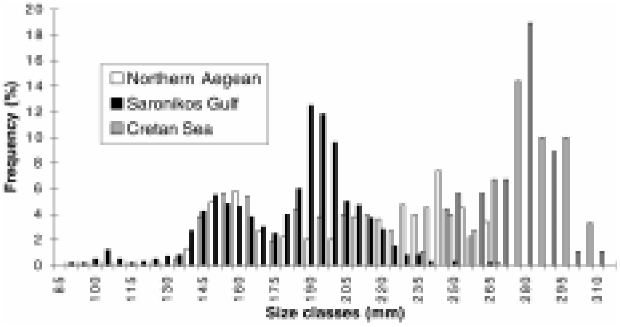

Fig. 1. – Length frequency distributions of chub mackerel caught at the three sampling areas.

Marginal Increment Analysis

Translucent edges on the otoliths were observed in all months, while opaque edges were observed from March

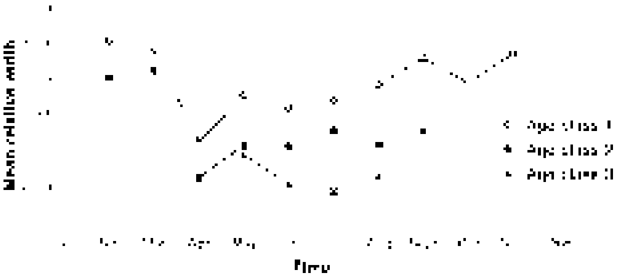

Fig. 2. – Mean monthly relative marginal width on the otoliths of chub mackerel belonging to the age classes 1, 2 and 3.

to September, with highest percentage (59%) on April. Analysis of the Marginal Increment Ratio, showed a distinct fall of the relative marginal width on April for the age classes 1 and 2 and on July for the age class 3 (Fig. 2). During one year period there was only one distinct fall, which proves that one translucent and one opaque band are formed each year constituting one annulus.

Age composition

Conformity in both age readings was found for 1026 otoliths which corresponds to 90,3% of the readings. From the three sampling areas the allocated age classes were: a) for the Northern Aegean the age classes 0 to 5 with dominant the age class 1, b) for the Saronikos Gulf the age classes 0 to 2 with also dominant the age class 1 and c) for the Cretan Sea the age classes 1 to 4 with dominant the age class 3 (Table 1).

TABLE 1

Percentage of each age class of chub mackerel in the three sampling areas

Age class	**Sampling area**			**Total**
	Northern Aegean	***Saronikos Gulf***	***Cretan Sea***	
0	34.5	30.8	0	30.1
1	52.7	43.5	4.4	45.9
2	11.7	25.6	41.2	19.1
3	0.7	0	52.9	3.9
4	0.1	0	0	0.2
5	0.1	0	0	0.1

Sex composition

The χ^2 test for heterogeneity performed for each sample separately in all sampling areas, revealed that the ratio males: females was not significantly different from 1:1 for all cases. The χ^2 test for heterogeneity performed for each age class from each sampling area also showed the ratio males: females to be not significantly different from 1:1 (Table 2).

Finally, the χ^2 test for goodness of fit for all samples combined, verified the former analogy (for all tests performed, it was $p>0,05$).

Growth rate

By the iterative method the parameters of the von Bertalanffy equation for males and females were estimated as:

males: $L_\infty = 46.406$ cm, $K = 0.157$ and $t_0 = -1.882$
females: $L_\infty = 34.548$ cm, $K = 0.303$ and $t_0 = -1.531$

with coefficients of determination $r^2 = 0.989$ for the males and $r^2 = 0.987$ for the females respectively. The comparison of the these two growth curves by the Ratkowsky method (Chen et al., 1992), displayed no statistically significant difference between the two curves ($F = 0.7390558$, $F_{(0.05,\ 3,\ 1017)} = 2.61$), thus we cannot reject the null hypothesis of similarity.

The parameters of the Bertalanffy function as they were estimated from the pooled data of both sexes, were: $L_\infty = 47.596$ cm, $K = 0.154\ y^{-1}$ and $t_0 = -2.177$ ($r^2 = 0.988$).

Weight - Length relationship

From the regression of the total weight on the fork length of each fish the relationship derived, was: $\log W = 3.462 \log L - 6.0151 \Rightarrow W = 9.65 * 10^{-7} L^{3.5}$.

DISCUSSION

In natural populations the younger an age class is the more abundant it is expected to be, unless extraordinary environmental conditions have preceded. An unbiased sampling device is expected to show this age abundance sequence in the samples it collects. In the samples coming from the Northern Aegean and Saronikos Gulf (Table 1), the age class 0 is underrepresented, which shows that there is a sampling bias originating either from the preference of the fishermen to the larger fish, or from the ability of smaller fish to escape from the seines. Either way, the 0 age class specimens caught must be the largest ones

TABLE 2

Sex composition of chub mackerel in the three sampling areas, tested for each age class in each area seperarely as well as for all sampling areas combined. The H0 tested was the 1 male : 1 female sex ratio (m = number of males, f = number of females, *÷c2 = ÷2* test value corrected for continuity, * = no fish were caught).

Sampling area	**Age class**											
	0			1			2			3		
	m	f	χ_c^2	m	f	χ_c^2	m	f	χ_c^2	m	f	χ_c^2
Northern Aegean	58	44	1.66	157	149	0.16	19	19	0.00	3	1	0.13
Saronikos Gulf	70	64	0.19	79	68	0.68	4	9	1.23	*	*	*
Cretan Sea	*	*	*	30	22	0.94	12	14	0.04	8	10	0.06
Total	128	108	1.53	266	239	1.34	35	42	0.64	11	11	0.00

of this class, which leads to the conclusion that selective fishing is exerted towards the largest specimens of this class. The remaining age classes in these regions follow the expected pattern which also shows that fishes of the age class 1 and older are fully fished. In the Cretan Sea the complete lack of the age class 0, the very low presence of the age class 1 and the high abundance of larger sexually mature classes (age classes 2 and 3) along with the highly seasonal appearance of this species in this area – the species appears in the area during the summer months (Moraitopoulou, 1963) – lead us to the conclusion that its presence there is related with its spawning migratory routine. The migratory pattern of this fish – whether it comes from the Aegean Sea stock or from other Eastern Mediterranean stocks – is a matter for further investigation.

Due to their annual periodicity opaque and translucent bands are appropriate for assigning age. The time of the onset of the new annulus formation for the age classes 1 and 2 was April, whereas that for the age class 3, was July. This is in agreement with Williams & Bedford (1974) who state that generally, within each stock, younger fish begin to lay down the opaque band before older ones. Analysis of the marginal type has shown that the period of rapid growth (when opaque edges are observed) is from March to August. The same period of fast growth (March to September) has been observed in the Canary Islands, where the same subspecies is found (Lorenzo et al., 1995).

The sex ratio of 1 male: 1 female found in the Hellenic seas was also found for chub mackerel in the Pacific Ocean (Schaefer, 1980). According to Watanabe (1970) during wintering and spawning periods the sex ratio varies among *S. japonicus* schools, but is assumed to be 1:1 for the population as a whole.

Scomber japonicus growth rates are similar in both sexes, as has also been observed in other regions of the species distribution (Gagliardi & Cousseau, 1970; Perrotta & Forciniti, 1989). From studies conducted in most regions of the species distribution, it was shown that *S. japonicus* exhibits a very high growth rate during its first year of life (Schaefer, 1980; Perrotta, 1992). In the Hellenic seas this growth rate reached 38.7% of its asymptotic length ($L_{\bullet}$). During the second and third year of life the growth rate dropped, reaching 47.5% and 55% of its asymptotic length respectively, a phenomenon that can be attributed to sexual maturity (Lorenzo et al., 1995). The growth rate observed in the Hellenic seas was slightly lower than that observed in the Canary Islands, where the species reached 40% of its asymptotic length during the first year of life (Lorenzo et al., 1995). The von Bertalanffy parameters as estimated for the Hellenic seas were very close to those estimated for Mauritania (L_∞ = 48.8 cm, K = 0.20) and Morocco (L_∞ = 51.2 cm, K = 0.32) by Martins & Gordo (1984). We can conclude that the growth and demographic characteristics of *S. japonicus colias* in the Hellenic seas are similar to those in the rest of the subspecies distribution (Mediterranean Sea and NW Africa), yet show a trend towards a slower growth rate. However, differences exist in the growth rates exhibited by the other subspecies. In Argentine waters *S. japonicus marplatensis* reached 50% of its asymptotic length within the first year of life (Perrotta, 1992). Comparative studies have shown that the subspecies of the Pacific Ocean (*S. japonicus peruanus*, *S. japonicus diego and S. japonicus japonicus*) exhibited faster growth rates than *S. japonicus colias* and *S. japonicus marplatensis* (Rodriguez-Roda, 1982; Morales-Nin, 1988). This makes *S. japonicus colias* the slowest growing of all subspecies.

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Peracarida crustacean populations of the artificial hard – substratum in N. Michaniona (N. Aegean)

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ABSTRACT. Quantitative samples were taken during summer and winter in two successive years from artificial substrata at one site (N. Michaniona) in Thermaikos Gulf. Sampling was carried out using a specially designed sampler. Data were analyzed with common biocoenotic methods and non-parametric statistical tests. The examination of approximately 10,500 specimens revealed the presence of 17 peracaridan species, the most dominant of which were *Corophium acutum* (41%), *Leptochelia savignyi* (24%), *Corophium sextonae* (14%) and *Elasmopus rapax* (12%). All the species are very common and have been reported in many sites and assemblages in the N. Aegean and W. Mediterranean Sea.

Shannon index, which was calculated separately for each sample, had similar values in the same year (2.04-2.44 in 1994 and 1.30-2.00 in 1995). Comparison of the samples, on the basis of their abundance and their diversity using Kruskal-Wallis test, showed significant differences among them ($p < 0.05$). Mann-Whitney test was used to find out the differences among the samples. Samples were grouped according to their similarity employing Euclidean distances and Ward' s method.

The total of the examined samples demonstrated that the composition of the peracarida fauna in the region of N. Michaniona, was similar to peracarida fauna of photophyllic algae assemblages. The results of this research must be considered essential to the biomonitoring of Thermaikos Gulf.

INTRODUCTION

Even though our knowledge about the structure and function of hard substratum assemblages has been broadened, this does not apply to the assemblages of the artificial hard substratum in increasingly polluted harbors. This should be attributed to the difficulties involved in quantitative sampling and to the complexity of the abiotic factors, which affect the artificial substratum in ports. Furthermore, the functional and constructive diversity of ports seems to cause added difficulty in reaching reliable conclusions.

According to PÉRÈS & PICARD (1964) assemblages in ports (e.g. Marseilles) should be characterized as assemblages of invertebrates in very polluted waters. Bellan-Santini (1969), who also studied the complexity of these biotopes, noted that the diversity of the assemblages increases significantly proportionally to the distance from the center of the ports (e.g. Vieux Port Marseille). Articles concerning the study of biotic and abiotic conditions in ports (ZAVODNIK & ZAVODNIK, 1978; TURSI et al., 1982; HARGRAVE and THIEL, 1983; Leewis and Waardenburg, 1989; LANTZOUNI et al., 1998) have recently revealed some interesting aspects on this matter.

A review of the relevant bibliography revealed the dependence of the hard substratum assemblages from the port's functionality and form (BELLAN-SANTINI, 1981; DESROSIERS et al., 1982; WENNER, 1987). The characteristics of these assemblages are imminently related to the environmental conditions as well as to the degree of the adaptability of the organisms (e.g. changes in the life cycles or feeding patterns, etc.).

Under these circumstances, the major problem in such a study will be whether it is admissible to determine the degree of pollution in a port on the basis of the composition of the hard substratum organismic assemblages. So far, the use of ecological indicators, such as the composition of the peracarida fauna of these substrata, seems to be a satisfactory approach (BELLAN-SANTINI, 1981).

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The aim of this study is to give the results on the structure of hard substratum peracarida assemblages (Crustacea; Malacostraca; Peracarida) of the port in N. Michaniona (Thermaikos Gulf), setting up the initial base for biomonitoring.

MATERIAL AND METHODS

Habitat description

N. Michaniona is situated 30km south-east of Thessaloniki in the east coast of Thermaikos Gulf (Fig. 1). N. Michaniona's port serves the needs of a great number of fish boats (trawls, purse seines, etc.). Until 1994, there was one large pier in N. Michaiona but in 1995 the works for a second, smaller pier started. The works were terminated in 1995. The samples were taken in the area protected by the piers and from the artificial hard substratum at the land side. The sampling area can be characterized as degraded rather than polluted, because it is not directly affected by the industrial area on the western coast of the Bay. This point is reinforced by the presence of *Arbacia lixula, Paracentrotuts lividus, Anemonia viridis, Actinia equina* which are considered as indicators of non-polluted waters (Schmidt, 1972; Harmelin et al., 1981). The surface water temperature varied from 10.5 to 28°C, following a seasonal pattern, with the highest temperatures in July-August and the lowest in January-February (fig. 2). The water salinity was more or less stable (about 37%).

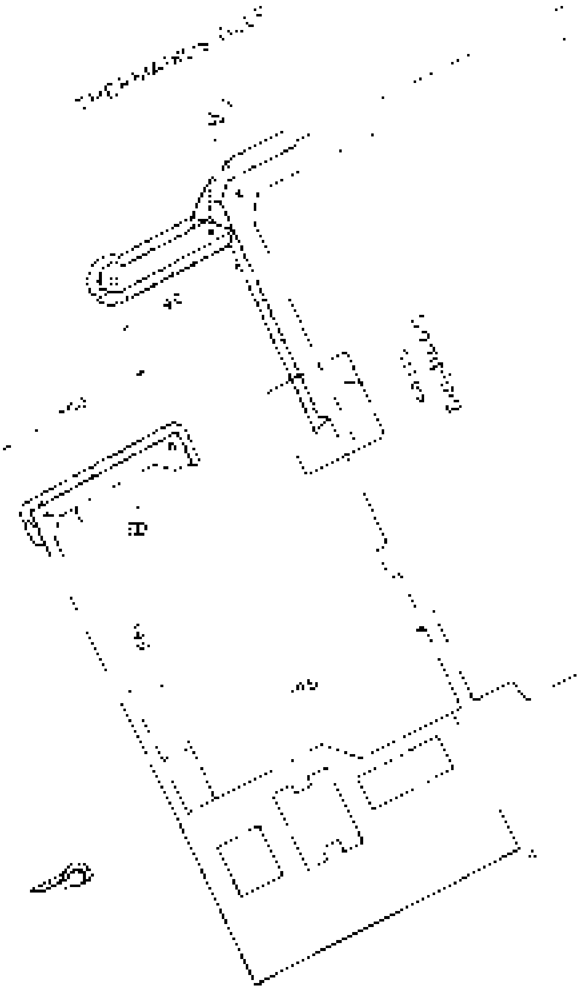

Fig. 1a. – Sampling area in N. Michaniona.

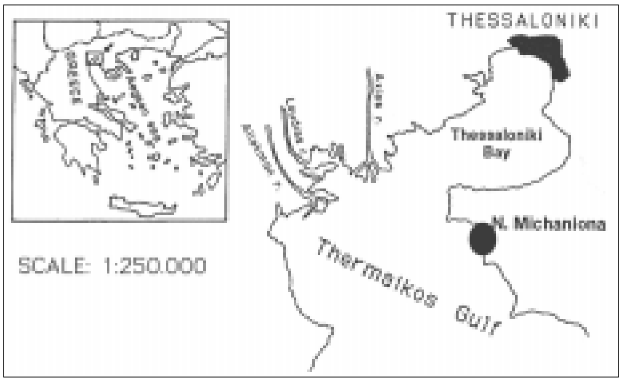

Fig. 1b. – Map of Thermaikos Gulf.

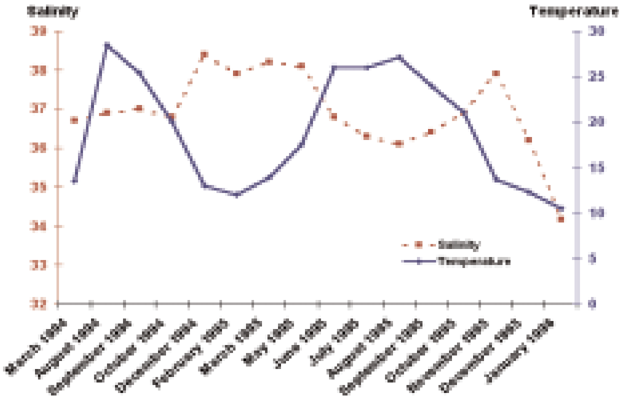

Fig. 2. – Salinity ($^{o}/_{oo}$) and Temperature (°C) values during the sampling period (1994-1995).

Sampling and statistics

Sampling was carried out on the artificial hard substratum of the pier in N. Michaniona, in summer and winter of two successive years (1994 and 1995). Scuba diving was employed and a special hard substratum sampler described by Chintiroglou & Koukouras (1992) was used. The advantage of this particular sampler, which is similar to others (Stirn, 1981) is that it ensures to a large extent the isolation of the sample and it can be well attached and fixed on the substrate. The area covered by this sampler is 400 cm^2 (20 x 20 cm), which is the minimum necessary quadrat area for the investigation of hard substratum assemblages (Bellan-Santini, 1969; Stirn, 1981). Samples were preserved in 10% formaline solution. In total, 12 samples were taken (3 samples per season). In order to quantify the contribution of the various species, mean abundance and partial mean dominance were calculated (Bellan-Santini, 1981). The Shannon-Wiener information function (H') and the Evenness index (J') were used as diversity indices (Daget, 1979). For the comparison of peracarida diversity between seasons and years, non-parametric tests, such as Kruskal-Wallis test and Mann-Whitney test (SIEGEL, 1956) were employed. Ward' s method was used to construct hierarchical classification of peracarida fauna similarities between seasons using Euclidean distances (ALDENDERFER & BLASHFIELD, 1984).

RESULTS

During this study 10,501 peracaridean individuals were identified, which belong to 4 classes (Amphipoda, Isopoda, Tanaidacea and Cumacea) and 17 species (13 Amphipoda, 2 Isopoda, 1 Tanaidacea and 1 Cumacea). Mean abundance and partial mean dominance were calculated (Table 1), as well as the total numbers of species, individuals, Shannon-Wiener index (H') and Evenness index (J'). The most dominant species, by far, was *Corophium acutum* (mA = 360.42, pmD = 41.18); second most was *Leptochelia savignyi* (mA = 207.83, pmD = 23.75) and third *Corophium sextonae* (mA = 125.92, pmD = 14.39). Three other species (*Elasmopus rapax, Ericthonius brasiliensis* and *Jassa marmorata*) showed relatively high abundance in some of the samples.

TABLE 1

Peracarida species in the 4 sampling periods (P: presence, mA: mean abundance, mD: mean dinance)

	Winter 1994			**Summer 1994**			**Winter 1995**			**Summer 1995**		
	P	mA	mD	P	mA	mD	P	mA	mD	P	mA	MD
Corophium acherusicum	3	5.0	0.629	2	2.0	0.185	0	0.0	0.000	0	0.0	0.000
Corophium acutum	3	207.7	26.111	3	267.7	24.780	3	167.0	46.130	3	799.3	57.993
Corophium sextonae	3	107.0	13.453	3	324.3	30.022	3	4.0	1.105	3	68.3	4.958
Elasmopus rapax	3.	91.3	11.484	3	167.3	15.489	3	30.3	8.379	3	142.0	10.302
Ericthonius brasiliensis	3	80	10.059	3	61.31	5,677	1	0.3	0.092	3	22.3	1.623
Jassa marmorata	1	1.3	0.168	1	19.31	1.799	1	0.7	0.184	0	0.0	0.000
Leucothoe serraticarpa	0	0.0	0.000	2	3.01	0.278	0	0.0	0.000	0	0.0	0.000
Leucothoe spinicarpa	1	0.33	0.042	1	0.3	0.031	0	0.0	0.000	0	0.0	0.0001
Liljeborgia dellavallei	0	0.0	0.000	1	0.3	0.031	0	0.0	0.000	0	0.0	0.000
Maera inaequipes	0	0.0	0.000	1	0.7	0.062	0	0.0	0.000	0	0.0	0.000
Stenothoe cavimana	3	8.0	1.006	0	0.0	0.000	3	11.7	3.223	0	0.0	0.000
Stenothoe monoculoides	2	3.0	0.377	1	0.3	0.031	0	0.0	0.000	0	0.0	0.000
Perioculodes aequimanus	0	0.0	0.000	0	0.0	0.000	1	0.3	0.092	1	0.3	0,024
Cymodoce sp.	3	14.0	1.760	3	11.0	1.018	3	7.3	2.026	31	2.0	0.145
Iphinoe sp.	1	0.3	0.042	1	0.3	0.031	2	0.7	0.184	3	9.0	0.653
Leptochelia savignyi	3	277.3	34.870	3	220.0	20.364	3	23.7	6.553	3	310,3	22.512
Paranthura nigropunctata	0	0.0	0.0	1	1.0	0.093	1	1.7	0.460	3	24.7	1.790
Number of individuals	**2386**			**3241**			**743**			**4135**		
Number of species	**12**			**15**			**11**			**9**		
Shannonindex H'	**2.388**			**2.393**			**1.604**			**1.757**		
Evenness index J'	**0.666**			**0.610**			**0.465**			**0.554**		

Shannon index (H'), separately calculated for each season, showed higher values in 1994 (2.388 in winter and 2.393 in summer) than in 1995 (1.604 and 1.757, respectively). The highest value of the evenness index (J') appeared in the winter of 1994 (J' = 0.666) and the lowest in the winter of 1995 (J' = 0.465) (Fig. 3). The value of number of species reached its highest level in the summer of 1994 (16) whereas the lowest value of number of species was in the summer of 1995 (9). The number of species decreased between the two years (Fig. 4). Comparison of the four seasons' samples on the basis of their peracarida fauna diversity by Kruskal-Wallis test, showed significant differences among them (H = 7.9, p = 0.044). Nevertheless, the samples that belong to the same year grouped together. The comparison of the 1994's samples with those of 1995 using the Mann-Whitney test, revealed significant differences between the

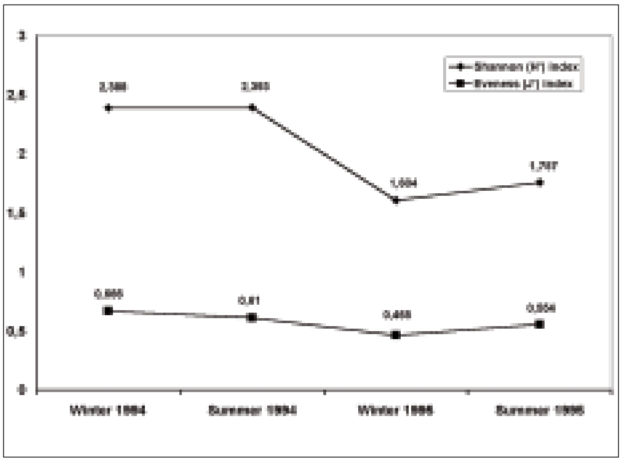

Fig. 3. – Shannon (H') and Evenness (J') indices for the four sampling periods.

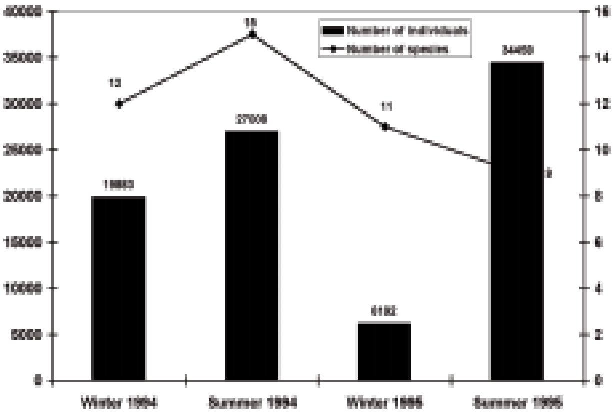

Fig. 4. – Number of individuals and number of species for each sampling period.

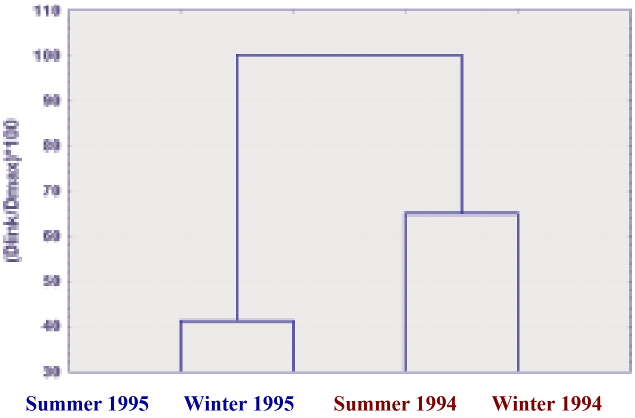

Fig. 6. – Tree diagram for the four sampling periods (Euclidean distances, Ward's method)

two years (Z = 2.9, p = 0.003). As far the differences among the samples are concerned, there were significant differences between the samples of the winter of 1994 and 1995 (Z = 1.96, p = 0.495). Differences were also found between the samples of winter 1994 and summer 1995 and the samples of summer 1994 and winter 1995, as expected. On the contrary, the application of Mann-Whitney test showed that there were no significant differences regarding the samples of winter and summer of 1994 (Z = 0.218, p = 0.827) and the samples of winter and summer of 1995 (Z = 0.218, p = 0.827). In addition, there were no significant differences among the samples of the two summer periods in 1944 and in 1995 (Z = 1.528, p = 0.127).

Samples were grouped according to their similarities (Fig. 5). They are divided into three major clusters; the first cluster includes 2 samples of the summer of 1995 and the second includes the remaining summer samples (1994 and 1995) – plus one 1994 winter sample, which is similar to the summer samples, as the number of individuals and species indicates. The third cluster contains the winter samples (1994 and 1995). On the whole, the samples of the three clusters do not show great differences among them. When the four periods of sampling were grouped with reference to presence/absence data (Fig. 6), the samples of 1995 were grouped together, as were the samples of 1994. According to this tree diagram, the samples were grouped in relation to the sampling year and not to the sampling period (winter or summer)

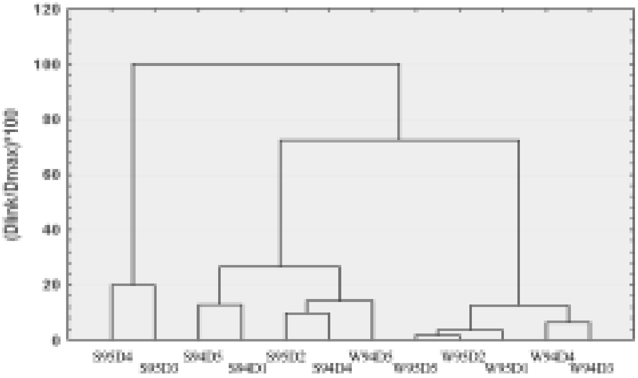

Fig. 5. – Tree diagram for the 12 samples (Euclidean distances, Ward's method)
(W: Winter, S: Summer, 94: 1994, 95: 1995, D1: Number of sample)

DISCUSSION

A review of the relevant bibliography showed that the peracarida genera found were almost the same as normally appear in the corresponding hard substrata of other Mediterranean areas (Bellan-Santini, 1981; Tsuchiya and Bellan-Santini, 1989). Furthermore, genera like *Elasmopus, Corophium, Jassa, Stenothoe* are very common in almost all the facies of the photophilic algae biocoenosis (Bellan-Santini, 1969; Bellan-Santini, 1981; Pérès, 1982; Tsuchiya & Bellan-Santini, 1989). Additionally, the genera that were recorded in this study have already been reported in the *Mytilus galoprovincialis* beds from the same area (Lantzouni et al., 1998) and they are very common in the N. Aegean Sea (Stefanidou & Voultsiadou-Koukoura, 1995).

The most dominant species in the studied area were *Corophium acutum*, *Leptochelia savignyi*, *Corophium sextonae* and *Elasmopus rapax*. These species were always present in winter and summer samples. The species of the genus *Corophium* showed a greater abundance when compared with other hard substratum assemblages of the Mediterranean (Bellan-Santini, 1969; Kocatas, 1978; Bellan-Santini, 1981). Besides, *Corophium acutum* is reported for the first time in hard substratum assemblages, while other species of the genus *Corophium* have rarely been found by other authors and only in small proportion (e. g. *Corophium acherusicum* by Kocatas, 1978; Bellan-Santini, 1981). In addition, *Leptochelia savignyi* was reported in hard substratum assemblages and it is considered as an indicator of organic rich waters (Tsuchiya & Bellan-Santini, 1989).

As mentioned above, N. Michaniona is located far from the major industrial area of Thessaloniki. This is the reason N. Michaniona is not under an imminent pollution impact. River runoffs (especially from the river Axios) are considered as pollution sources in the NW of Thermaikos Gulf. The surface currents transfer organic matter and wastes towards N. Michaniona coast (Anagnostou et al., 1997; Krestenitis et al., 1997).

Most of the samples that were collected in the same sampling period (summer or winter) showed a great affinity. Nevertheless, there is a fluctuation that is due to the diversity of the hard substratum microhabitats (Bellan-Santini, 1969). When the samples were examined as a total and their clustering was based on presence/absence data, greater affinity among the samples of the same year (1994 or 1995) was discovered rather than in the samples of the same sampling period (summer or winter). This fact implies that there was no seasonal alteration in the synthesis of the peracaridean community at the examined time and place. However, such seasonal alterations have been observed in amphipod assemblages associated with *M. galloprovinciallis* beds from Thermaikos Gulf (Lantzouni et al., 1998).

Shannon indices for winter and summer of 1994 were found to be similar to those of other photophilic algae assemblages, such as in Marseille region (Bellan-Santini, 1981). In this study, the highest value of H' index was observed in the summer of 1994 (2.393) and the lowest value in winter of 1995 (1.604). The values of the H' index for non-polluted areas ranged from 2.29 to 2.89 and for polluted ones from 0.87 to 1.70 (Bellan-Santini 1981). There was a sharp decrease of Shannon H' index between the summer of 1994 and the winter of 1995 (Fig. 3). In addition, the number of species was smaller in 1994 than the number of species in 1995 (Fig. 4). Furthermore, the great abundance of certain species in 1995, such as *Corophium acutum*, *Leptochelia savignyi* and *Elasmopus rapax*, indicated a disturbed area (Gray & Mirza, 1979). Besides, *Corophium acutum* and *Leptochelia savignyi* are tube-dwellers. Particularly *Leptochelia savignyi* is a characteristic species of organic-rich environments (Tsuchiya & Bellan-Santini, 1989).

The former changes should be attributed to the construction of the pier, marked as B in Fig. 1b, which took place in 1995. The pier, which is part of a major port, comprises an artificial barrier, which changes the physiognomic aspect of the sampling area and also influences the structure of the peracarida assemblages in hard substratum. This pier affected the hydrodynamic of the region by preventing the movement of currents in the port and allowed the accumulation of organic material sediment. Additionally, changes in the composition of the fauna and the flora due to the influence of human disturbances can be detected in a small period of time (Hargrave and Thiel, 1983; Desrosier et al., 1986).

In conclusion, the sampling before, during and after the construction of man-made installations is highly recommended, so that the evolution of the assemblages can be observed. The biomonitoring of the hard substratum fauna, especially the peracaridean one, can eventually produce remarkable results as far as the impacts of the human activities are concerned, since Crustacea are one of the most sensitive groups to environmental changes.

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THE SCORPIONS (ARACHNIDA : SCORPIONES) OF THE AEGEAN AREA : CURRENT PROBLEMS IN TAXONOMY AND BIOGEOGRAPHY

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ABSTRACT. The fauna and zoogeography of the scorpions in the Aegean area are not well researched and based upon specimens randomly collected and described by various authors in the past 150 years. A first revision ot the scorpions in this region was provided by Kinzelbach in 1975 (completed with maps in 1985). However, the taxonomical validity of some species and most of the subspecies, first of all those in the genus *Euscorpius* (Euscorpiidae) is still unclear and their geographic ranges remain rather uncertain. Current comparative studies on mitochondrial DNA and nuclear gene (allozymes) variation (Gantenbein et al., 1999a, b) of these scorpions, revealed such promising first results that, as a consequence, a longer-termed research project is now introduced to analyze the scorpion fauna of the Aegean area on a larger scale and with requested close collaboration of zoologists from universities in Greece, Turkey and Cyprus.

INTRODUCTION

The first revision of the scorpion fauna of the circum-Aegean area in Europe and the adjacent parts of Asian Turkey was published by KINZELBACH (1975). It was not only a compilation of most noteworthy data that had been published by previous authors, but also the introduction of a new theory of the phylogeny of the subfamilies Euscorpiinae (now family Euscorpiidae) and Calchinae (now Iurinae, family Iuridae) included by Kinzelbach in the family Chactidae. This theory was based upon alleged mechanisms of hybridization, and the coincidence of distributional patterns of morphological characteristics with the results of the reconstruction of distribution during the Tertiary period. Earlier contributions and often contradictory data relevant to this subject have been published by BIRULA (1917), HADZI (1931), VACHON (1947a, 1947b, 1951, 1953) and CAPORIACCO (1950). Kinzelbach's phylogenetic and zoogeographical theories have since not been discussed or confirmed except by himself (KINZELBACH, 1982, 1985; VACHON & KINZELBACH, 1987), although a few more recent papers deal with the taxonomy and zoogeography of the scorpions in general or particular species in this area (VOULALAS & MICHALIS, 1977; BONACINA, 1980, 1982; FRANCKE, 1981; MICHALIS & KATTOULAS, 1981; SISSOM, 1987; MICHALIS & DOLKERAS, 1989; KRITSCHER, 1993; CRUCITTI, 1993, 1995a, b; LACROIX, 1995; GANTENBEIN et al., 1999b).

PROBLEMS IN THE TAXONOMY AND BIOGEOGRAPHY OF SCORPION TAXA IN GREECE, TURKEY AND CYPRUS

Below, we describe the current problems in taxonomy and biogeography for all scorpion taxa occurring in Greece, Turkey and Cyprus.

Family BUTHIDAE C. L. Koch, 1837
Genus *MESOBUTHUS* Vachon, 1950

Mesobuthus gibbosus (Brullé, 1832)

This common species is recorded from Albania, Yugoslavia (Montenegro), Greece, Cyprus, Turkey (except north), Syria, and Lebanon. Recent studies of distribution and ecology in Greece include CRUCITTI & MARINI (1987), CRUCITTI (1993), and CRUCITTI et al. (1998). The species is well-defined and separated from

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Corresponding author : V. Fet, e-mail : fet@marshall.edu other species of *Mesobuthus* which inhabit exclusively Asia (from eastern Turkey to China). Some variation exists across the range but its extent is poorly studied. A subspecies *M. g. anatolicus* Schenkel, 1947 was described from Kayseri, Anatolia (types in Basel Museum) and later confirmed by Kinzelbach (1975). However, Kritscher (1993) doubted the existence of a clear separation between the European and Asian populations of *M. gibbosus*. Further, detailed investigation combined with genetic methods is necessary; and first genetic data on this species are being currently obtained (B. Gantenbein et al., pers. comm). The type locality of *M. gibbosus* is unclear and was referred only to "Morea" (=Peloponnesos). Type(s?) probably are lost. Designation of the neotype is planned from Greece. In addition, the status of Eastern Mediterranean populations (east Turkey, Syria, Lebanon) is unclear. If confirmed as a separate taxon, there is an available name for these populations since Ehrenberg in 1829 described his *"Androctonus" nigrocinctus* from the mountains near Beirut (juvenile type in Berlin Museum). This name was for a long time considered a synonym of *M. gibbosus* (in fact being a senior synonym) (Braunwalder & Fet, 1998).

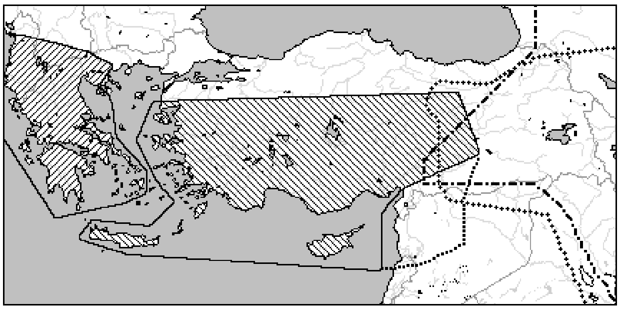

Fig. 1. – Distribution of *Mesobuthus* (Buthidae) according to Kinzelbach (1985): / / / *M. gibbosus* (Brullé), \ \ \ *M. g. anatolicus* (Schenkel), + + + *M. eupeus* (C.L. Koch), - . - . - *M. caucasicus* (Nordmann), ... *M. gibbosus ssp.* (maybe identical with „*Androctonus*"*nigrocinctus* Ehrenberg 1829).

No other buthids are known from Greece and the Balkans. The record of *Androctonus bicolor* (Ehrenberg, 1829) from Thessaly in Greece (Michalis & Dolkeras, 1989) is erroneous and is based on *Mesobuthus gibbosus* (V. F., observations of the original material in the Zool. Museum Hamburg). From eastern Anatolia, there are confirmed records of some characteristically Asian species of buthids: *Mesobuthus eupeus* (C.L.Koch, 1838), *M. caucasicus* (Nordmann, 1840), *Androctonus crassicauda* (Olivier, 1807), *Leiurus quinquestriatus* (Ehrenberg, 1829), and *Compsobuthus matthiesseni* (Birula, 1905) (Kinzelbach, 1985; Kovárik, 1996). Their study could be beneficial for further understanding of the taxonomy and zoogeography of Asian buthid genera. Any records of other species of Buthidae from Turkey should be confirmed. The occurrence of *B. occitanus* on Cyprus also also requires verification.

Family IURIDAE Pocock, 1893
Subfamily IURINAE Pocock, 1893
(= CALCHINAE Birula, 1917)

Genus *Calchas* Birula, 1899
(=*Paraiurus* Francke, 1985)

The new generic name *Paraiurus* was proposed for this genus by Francke (1985), but Fet & Madge (1987) demonstrated that Birula's name is valid. The single species of this monotypic genus, *C. nordmanni* Birula, 1899, was described from the northeastern Anatolia (Russian territory before 1918; ?oruh River drainage; type in the Zoological Institute, St. Petersburg, Russia) but later found also in the southeast and south of Turkey (Kinzelbach, 1982, 1985) and off the Aegean coast on the Greek islands of Samos (Sissom, 1987) and Megisti (Kastellorizo) (collected by the staff of the Natural History Museum of Crete). The most southern occurrence of *C. nordmanni* from Antakya (Gulf of Iskendurun) has recently been communicated by Kovárik (collection Kovárik, pers. comm.) The full range of this rare species is still unknown as its current distribution appears to be in six disjunct populations. Also its ecology requires further detailed observations.

Genus *Iurus* Thorell, 1876

The only species currently recognized is *Iurus dufoureius* (Brullé, 1832), common in the southern Greece, the Aegean islands and southern Turkey. Its ecology in Peloponnesos was recently studied by Crucitti (1995a, b). The type locality is unclear and was referred only to "Morea". Type(s?) probably is lost. Designation of the neotype is planned from Greece. The status of subspecies *I. d. asiaticus* Birula, 1903 (type in St. Petersburg) from Anatolia is problematic. Francke (1981) considered it a separate species. Kritscher (1993) analyzed a larger series of specimens and concluded that this form has only the status of a subspecies. Variation of this species from

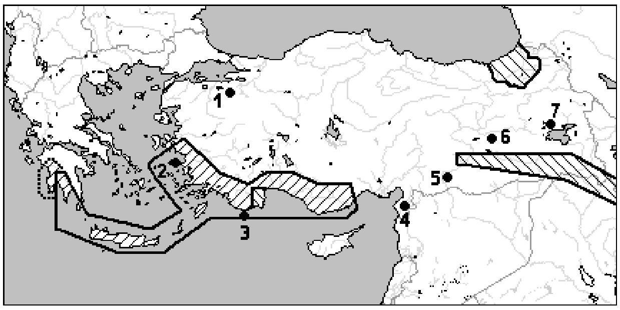

Fig. 2. – Distribution of Iuridae: *Iurus dufoureius* (Brullé) according to Kinzelbach (1985) / / / ; Crucitti (1995b) ..... *Calchas nordmanni* (Birula) according to Kinzelbach (1985) \ \ \ and recent new findings: 1 = Vachon & Kinzelbach (1987); 2 = Island of Samos (Sissom, 1987); 3 = Mengisti Island (collection Braunwalder); 4 = Antakya; 5 = Halfeti, 6 = Malatya, 7 = Nemrut Dagi (collection Kovarik, pers.comm.).

Greece, Turkey and the island populations should be studied in detail using genetic techniques, thus adding new data for the study of its origin and distribution initiated by VACHON (1947a, b; 1953).

Family SCORPIONIDAE Latreille, 1802

Genus *Scorpio* Linnaeus, 1758

Scorpio maurus Linnaeus, 1758. This is an extremely polymorphic, arid Asian-African species (or possibly a species complex) (BIRULA, 1910; LEVY & AMITAI, 1980). In the southeastern Anatolia, the endemic Middle Eastern subspecies (or maybe a species) *S. m. fuscus* (Ehrenberg, 1829) is found. This population represents the northernmost extreme of its range and should be important for a comparative study with populations from Syria, Iraq, Lebanon, Jordan and Israel in morphological, genetic and ecological aspects. The neotype specimen of *S. m. fuscus* should be designated from Lebanon.

Family EUSCORPIIDAE Laury, 1896

Genus *Euscorpius* Thorell, 1876

This is the most widespread genus of scorpions in the discussed region, where it includes at least three, and possibly five or more, species. Multiple subspecific forms are described, but their validity is not clear. A wealth of information is scattered in the literature (published in Italian, German, French, Serbo-Croatian, Russian etc.) but a comprehensive modern revision of the entire genus has never been done and is long overdue.

Euscorpius carpathicus (Linnaeus, 1758)

Species complex [including "E. mesotrichus Hadzi 1929"= E. tergestinus (C.L. Koch, 1837)].

Traditionally treated as one species (DI CAPORIACCO, 1950; VACHON, 1981; FET, 1986, 1989, 1997a), *E. carpathicus* is the most widespread scorpion species in Europe (from Baleares to Crimea; see Fet 1997a), with a large number of valid subspecies (over 20!). A number of those forms is described from Greece (including the islands of the Ionian and Aegean Seas), but their validity is unclear. KINZELBACH (1975) divided *E. carpathicus* into two species, designating the second one as "*E. mesotrichus* Hadzi, 1929". This name, however, is not available since it is a junior homonym of *E. italicus mesotrichus* Hadzi, 1929 (Fet 1997b). According to the International Code of Zoological Nomenclature, the correct name for such species should be *E. tergestinus* (C.L. Koch, 1837) (which was listed by KINZELBACH (1975) as a synonym). KINZELBACH (1975) classified all variation of the described subspecific forms of *E. carpathicus* into two species, without providing sufficient justification. His observation of sympatry for *E. carpathicus* and "*E. mesotrichus*" in Greece (Ossa, Pindos, Pilion, and Olympus) led to the conclusion of their sympatry over a wide area of the Mediterranean, and to restriction of the range of true *E. carpathicus* to the Eastern Mediterranean and Southeast Europe. The name "*E. mesotrichus* Hadzi 1929" was used afterwards by some authors (VACHON & KINZELBACH, 1987, MICHALIS & DOLKERAS, 1989; KRITSCHER, 1993), while others (BONACINA, 1983; FET, 1986, 1989, 1997a) did not accept Kinzelbach's division, but no detailed, critical analysis has yet been published. The type locality for *E. carpathicus* is Romania (type in the Linnean Society, London), and that for *E. tergestinus* is Trieste, Italy (type lost; neotype designation is planned). DI CAPORIACCO (1950) indicate that Koch's form inhabits also part of Italy, the Dalmatian coast of ex-Yugoslavia (now Croatia) and possibly goes as far eastward as Taigetos in Greece. No study has been done (but is much needed) of all these forms all over their range; the first results of genetic analysis (Gantenbein, Fet et al., in progress) reveal high variation and the possible existence of more than two species in this complex.

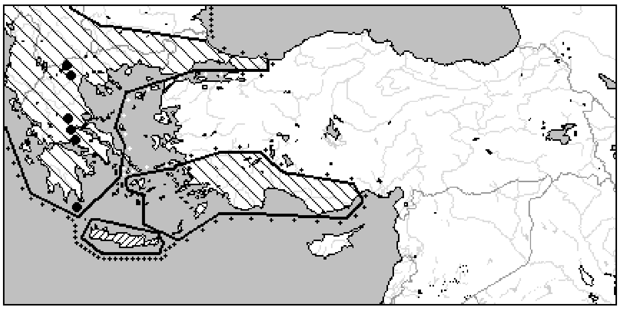
Fig. 3. – Distribution of *E. carpathicus* (L.) \\\ and *E. c. candiota* (Birula) / / / (and black dots) according to Kinzelbach (1985). + + + distribution range of *E. carpathicus* (Fet & Braunwalder, present study).

KINZELBACH (1975) also promoted a species origin theory, which, in fact, advocated a purely hybridogenic origin for all *Euscorpius* species. The only substantiation for this theory was an ordered characterization of meristic morphological characters (number of trichobothria on pedipalp). In particular, KINZELBACH (1975) maintained that all forms of the *E. carpathicus* complex that had an intermediate number of trichobothria are in fact hybrids between *E. carpathicus* and *"E. mesotrichus*"; one of the conclusions in this theory was that the entire Crete population, described by BIRULA (1903) as *E. candiota* (syntypes in St. Petersburg), is in fact a hybrid. Further analysis is warranted, including detailed genetic comparisons (allozyme and DNA techniques) of this crucial Crete population with other "intermediate" morphological forms from the Balkans as well as with two alleged ancestral species.

An additional problem in *E. carpathicus* complex concerns some localized, probably disjunct mountain populations in the Balkans, first of all "*E. germanus croaticus*" Caporiacco, 1950 (Croatia, Bosnia-Herzegovina; type from Velebit Mts in Zoological Museum, Florence, seen) and a similar (if not identical) form from the Western Rhodopi Mts in Bulgaria (Smolyan District, our data, unpublished); the latter form most likely will be found within the territory of Greece. These forms have morphological characters indicating their affinity to *E. carpathicus* rather than to *E. germanus*, although its reduced trichobothrial formulae match those of *E. germanus*. Further studies should clarify the status of these populations, which could constitute glacial relicts of *E. carpathicus* complex.

Euscorpius italicus (Herbst, 1800)

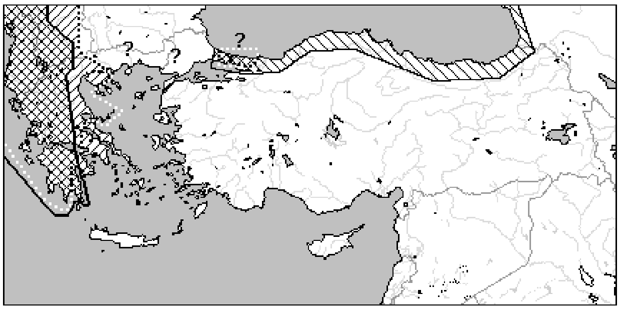

Fig. 4. – Distribution of *Euscorpius italicus* (Herbst) \ \ \ and *„Euscorpius mesotrichus* Hadzi, 1929" = ?*Euscorpius tergestinus* (C.L. Koch) / / / according to Kinzelbach (1985). ? = estimated but not yet verified occurrence of *E. tergestinus* (Fet & Braunwalder, present study).

This species is found from France to the Caucasus (Caporiacco, 1950; Kinzelbach, 1975). No subspecies are currently recognized (Vachon, 1981; Bonacina, 1982). Caporiacco (1950) and Vachon (1981) characterized an unusual "oligotrichous" form from Taigetos, Greece. Crucitti (1995a) observed ecology, probably of the same form as "*Euscorpius* cf. *italicus*". From new DNA-based information available (analysis of the 16S rRNA mitochondrial DNA gene sequences by Gantenbein et al., 1999b) it appears that *E. italicus* holds a derived place in the phylogenetic tree of the genus *Euscorpius* and is very closely related to *E. carpathicus* complex (or maybe even to a certain part of it). Since the major diagnostic character set in *E. italicus* appears to be a dramatic increase in trichobothrial numbers (Vachon, 1975, 1981), it is very important that "oligotrichous" *E. italicus* forms from Greece be studied in their relation to "polytrichous" forms of *E. carpathicus* known from the Balkans (Caporiacco, 1950), to further clarify phylogeny of these species. In addition, the range and status of the Black Sea coast populations (all Anatolia to Russia) has to be reanalysed. Designation of the neotype for *E. italicus* is planned from Italy.

Euscorpius mingrelicus (Kessler, 1874) (and related taxa)

Kinzelbach (1975) treated this taxon as a subspecies of *E. germanus* (C.L. Koch, 1837). Bonacina (1980) demonstrated that this species is separate, and that in fact the major part of the range formerly recognized for *E. germanus*, belongs to *E. mingrelicus*. *E. germanus* (C.L. Koch, 1837) was described from the historical Tyrol (now Trentino-Alto Adige in northeast Italy) (type lost; neotype designation is planned) (Fet & Braunwalder, 1997). Since Bonacina (1980) separated *E. mingrelicus*, this species appears to be the most geographically restricted of all *Euscorpius*, occupying the southern part of the Alpine belt in Italy, Switzerland, Austria, and Slovenia; there is no evidence that true *E. germanus* is found in Greece.

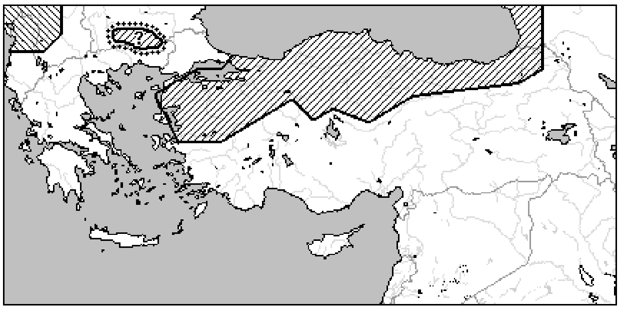

Fig. 5. – Distribution of *Euscorpius germanus* (C.L. Koch) \ \ \ and *Euscorpius mingrelicus* (Kessler) / / / (Euscorpiidae) according to Kinzelbach (1985). + + + and ? probably a form of *E. carpathicus („E. germanus croaticus")* (Fet & Braunwalder, present study).

E. mingrelicus was originally described from Georgia (Caucasus) (type lost; neotype designation is in press). However, it is recorded from northeast Italy to Russia; its presence in Greece requires confirmation, and its distribution in the Balkans and Turkey is poorly documented.

An additional species, *E. gamma* Caporiacco, 1950, has been now separated from this complex (Scherabon et al., in press). Subspecific structure requires revision; recently, Lacroix (1995) described three new subspecies from Anatolia in addition to three already existing (*E. m. mingrelicus, E. m. phrygius* and *E. m. ciliciensis*; see Birula, 1898; Bonacina, 1980; Fet, 1986, 1993). It appears that the species is found over the entire Anatolian Peninsula, including high mountain ranges (Bulghar Dagh, Taurus Mts). It is not clear which form is found on the Aegean islands (Tinos and Ikaria; Kinzelbach 1975); we suggest that these records might refer to *E. carpathicus*. Designation of the neotype for *E. mingrelicus* is planned from Georgia.

CONCLUSIONS

Application of modern molecular phylogenetic techniques, especially DNA-based (Gantenbein et al., 1999a, 1999b) opens unprecedented opportunities for the study and interpretation of ancient and diverse scorpion fauna of one of the most complicated biogeographic regions of the world, the Eastern Mediterranean (Oosterbroek & Arntzen, 1992). The molecular data analysis should be combined with detailed morphological investigation and exhaustive geographic sampling to re-analyse and reveal the importance of diagnostic characters in order to understand real taxonomic relationships and reconstruct the history of taxa. It is thus our goal to reanalyze extensive collections accumulated in all major European museums and to establish international cooperation, which is necessary to further facilitate and support these studies with new material and ecological data. For this purpose, we are initiating a long-term comprehensive project for which we invite regional zoologists, university professors and students, and amateurs to actively participate. This project will be coordinated by the non-profit Swiss agency ARACHNODATA, which currently runs a number of international cooperative projects (Switzerland, USA, Middle East) on the taxonomy, biology, ecology and zoogeography of scorpions.

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Ecological quality of the River Axios (N. Greece) during spring and summer, 1997

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ABSTRACT. This study examines the applicability of five European biotic indices combined with the measurements of physicochemical parameters in order to determine the water quality at ten sites along the Greek part of the river Axios during the high flow (spring) and low flow season (summer) in 1997. The river Axios is situated in northern Greece, west of the city of Thessaloniki. Its source is located in the Former Yugoslavian Republic of Macedonia close to the city of Skopia. The Greek part of the river Axios receives mainly agricultural runoff but also urban sewage and industrial wastes. The following physicochemical parameters were measured in situ: substrate, flow, water temperature, pH, concentration and percentage saturation of dissolved oxygen, BOD_5, conductivity and total dissolved solids. Total suspended solids, orthophosphates, nitrate, nitrite and ammonia were measured in vitro. The benthic macroinvertebrates, sampled with the 3 min kick/sweep method, were identified to the lowest possible taxonomic level. Three British and two Iberian biotic indices were applied. The data were analyzed with Twinspan, Fuzzy, Canonical Correspondence Analysis and Discriminant Analysis. The faunal composition was typical of a lowland river, i.e. the zoobenthos was not very diverse. The quality of the water was influenced by human activities during both seasons. The deterioration in water quality during spring was due to excess suspended solids of diffuse agricultural origin, and during summer was due to organic pollution. Lower discharge and impoundment accentuated this deterioration. Overall, changes in physicochemical parameters were in agreement with the results of the biological data, despite their instant nature. However, some problems arose with regard to the applicability of the European biotic indices, thus emphasizing the need to develop a Greek Biotic Index.

KEY WORDS: biomonitoring, macroinvertebrates, Axios.

INTRODUCTION

The European Union has obliged member states to establish national freshwater monitoring networks [EU Proposal Directives C184/20/17.6.97 and COM(98)76 final (17.02.1998)]. In Greece there is as yet no such national or regional monitoring network. The combination of chemical and biological methods constitutes the best approach for an integrated assessment of freshwater quality. Benthic macroinvertebrates are considered the best biological indicators of water quality in EU countries (METCALFE, 1989), including Greece (ANAGNOSTOPOULOU et al., 1994) and their responses to organic pollutants have been used to develop contemporary biotic indices (METCALFE, 1989).

The river Axios originates in the Former Yugoslavian Republic of Macedonia (FYROM). It discharges into the Thermaikos Gulf in northern Greece. Only the last 80km of its total 320km are within Greek territory (ARGIROPOULOS, 1991). At 49 km from the border, there is an irrigation dam (Fragma Ellis), which remains closed from May to September. Due to the closure of the dam, discharge falls to 1 m^3/s during the dry season (ARGIROPOULOS, 1991). Catchment land use is predominantly agricultural. Urban and industrial effluents are discharged into the river mainly in the vicinity of Axioupoli-Polykastro and Koufalia. It should also be kept in mind that there is an unknown pollution load coming in from FYROM. Additionally, significant sand extraction is carried out at several points. An integrated water quality assessment of the Axios was carried out at 4 sites in February, April and May 1997 (LANGRICK et al., 1998) and at 10 sites during the low flow season in 1998 (DROUIN et al., 1999).

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This paper examines the relationship between the physicochemical characteristics, water quality and macroinvertebrate benthos of the river Axios during high and low flow conditions (May and July 1997). The use of the biota to predict Axios water quality is also evaluated.

MATERIAL AND METHODS

Ten sampling sites along the Greek part of the river course were chosen for their proximity to known sources of pollution (Fig.1). In July, sites Gorgopis and Anthofito were dry and, therefore, were not sampled. Three samples of benthic macroinvertebrates were taken at each site with the 3-min kick-sweep method (ARMITAGE & HOGGER, 1994). Substrate type and 13 physicochemical parameters were measured; five European biotic indices and four methods of statistical analyses were applied. For details of the materials and methods used in this study, see LAZARIDOU-DIMITRIADOU et al. (2000). In addition to the statistical methods CANOCO and FUZZY applied as described in LAZARIDOU-DIMITRIADOU et al. (2000), Twinspan and Discriminant Analysis were used. Twinspan classification arranges site groups into a hierarchy on the basis of their taxonomic composition (MASON, 1991). Discriminant analysis was used to identify the discriminating environmental variables among the given Fuzzy groups and to state whether the actual groups coincide with the predicted ones.

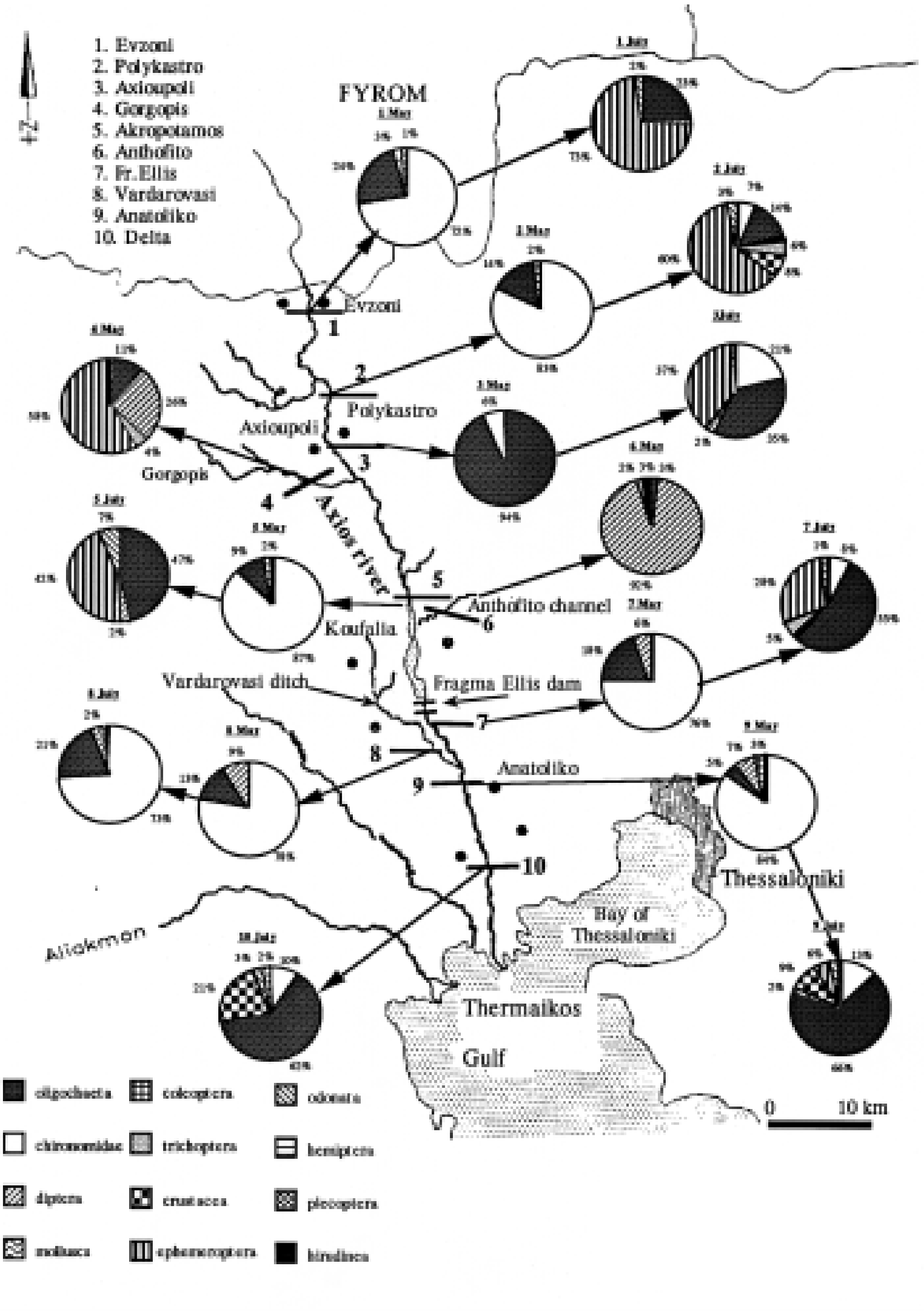

Fig 1. – Study area, sampling sites along the Axios river and their benthic macrofauna in May and July 1997.

RESULTS

Physicochemical parameters

In May, all sites except for Gorgopis and Anthofito were characterized by silty substrate. In July, the substrate was more differentiated than in May. Physicochemical data are presented in Table 1. Flow velocity appeared lower in May than in July because, during the flood, flow was measured, at most sites, close to the bank side as the middle of the river was not easily accessible. Temperature was higher in July and increased downstream on each sampling occasion, reflecting diurnal fluctuations. pH was quite alkaline and in July exceeded the E.U. suggested values for drinking water (80/778/EU) at many sites. BOD$_5$ was very elevated at all sites in May and mainly at three sites (Fragma Ellis, Anatoliko and Delta) in July. Dissolved oxygen values were slightly higher in July than in May. On both sampling occasions, dissolved oxygen was significantly lower at site Vardarovasi. Conductivity values exceeded the E.U. suggested value (80/778/EU) at Anthofito in May and at all sites in July. Total suspended solids were significantly higher in May while in July they were elevated only at site Fragma Ellis because of sand extraction carried out upstream of the site. All nutrients appeared quite elevated in July compared to May values. Nitrites were present in high concentrations in July at Vardarovasi and Anatoliko and nitrates at Axioupoli, Anatoliko and Delta. Ammonia exceeded the E.U. permitted value (80/778/EU) at Vardarovasi during both seasons. In comparison with the results of this study, the concentrations of nutrients were lower in July 1998 (Drouin et al., 1999).

TABLE 1

Physicochemical parameters at ten sites on the river Axios in May and July 1997. Where: DO= dissolved oxygen, TDS= total dissolved solids, TSS= total suspended solids, ND= not detectable

Sites	BOD$_5$ mg/l	DO %sat	DO mg/l	Flow m/s	T °C	pH	Cond. µS	TDS mg/l	TSS mg/l	N-NO$_3$ mg/l	N-NO$_2$ mg/l	N-NH$_3$ mg/l	P-PO$_4$ mg/l
1 May	6,12	99	10,4	0,172	13	8,05	287	143	166,3	0,79	0,01	ND	0,047
2 May	4,51	91	9,3	0,126	14	8,07	292	146	194,1	0,34	ND	ND	ND
3 May	5,94	103	10,5	0,299	14	8,01	290	145	186,9	0,76	0,02	0,01	0,05
4 May	3,36	93	8,6	0,541	19	8,36	338	169	6,93	0,49	ND	ND	ND
5 May	7,67	79	7,9	0,133	15	8,07	298	149	189,7	0,71	ND	ND	0,3
6 May	4,54	90	8,2	0,352	20	8,51	914	458	3,67	0,1	ND	ND	ND
7 May	3,62	73	7,1	0,21	15	8,12	299	150	53,07	0,77	0,01	0,01	0,039
8 May	15,4	5	0,1	0	17	7,7	2,56	1,26	193,8	ND	ND	1,73	0,19
9 May	3,62	103	9,6	0,082	16	8,09	303	152	70,17	0,75	0,01	0,02	0,048
10 May	4,68	92	9,6	0,167	16	8,27	308	154	102,3	1,45	ND	ND	ND
1 July	1,7	107	9,7	0,667	20	8,57	543	272	19,33	2,61	ND	ND	0,57
2 July	2,6	124	10,8	0,635	21	8,82	598	298	21,33	2,84	ND	ND	0,7
3 July	1,75	120	10,3	0,383	22	8,95	610	306	21,66	3,78	0,03	ND	0,01
5 July	2,7	133	11,4	0,515	23	9	597	297	43,9	2,54	0,03	ND	0,011
7 July	3,3	139	12,1	0,58	22	9,16	616	289	125,1	1,46	0,04	ND	1,01
8 July	2,77	82	7	0,216	24	7,81	820	410	13,17	2,26	0,14	1,28	0,02
9 July	6,25	148	12,1	0,355	24	9,6	563	284	52,63	5,58	0,15	ND	0,94
10 July	6,27	154	12,5	0,344	25	9,65	783	394	64,9	5,98	0,04	ND	0,56

Biotic Indices and scores

Biotic scores and indices are presented in Table 2. Water quality was characterized as better in July than in May by all scoring systems. In May, Gorgopis was notable for its high score and characterized as of "excellent" water quality by the LQI (Class I in the Iberian system). All remaining sites in May had "very poor" to "moderate" water quality (Class IV and V). Sites in July were of "moderate/good" water quality (class II, III, IV and V) while site Fragma Ellis had "excellent" water quality (Class II).

Benthic macroinvertebrates

Over 11500 individuals were sampled at the ten sites. In May, the benthic community consisted mostly of Oligochaeta, the dipteran family Chironomidae, while other families of Diptera, Coleoptera, Odonata and Hemiptera were present in smaller numbers (Fig. 1). No pollution-sensitive taxa (BMWP score 7-10) were collected. The benthic community at Gorgopis consisted of 3290 individuals belonging mainly to the Ephemeroptera (58%) and consisting mostly of *Ephemerella* sp. and *Baetis* sp., Diptera (26%) and Trichoptera. Gorgopis was the only site where Plecoptera were collected. At Anthofito the percentage occurrence of Diptera (Simuliidae pupae) was very high (92%). In July, the benthic community was more diverse than in May and mainly consisted of Ephemeroptera (*Baetis* sp. and *Caenis* sp.), Chironomidae, Trichoptera (mainly *Hydropsyche* sp. and *Ceraclea* sp.), Odonata and Amphipoda. The dipteran family Chironomidae and the order Mysidacea constituted

TABLE 2

Biotic scores and indices at 10 sites on the river Axios in May and July 1997 sites

Sites	Abund	High-score	Low-score	BMWP	ASPT	Lincoln Quality Index			IBMWP		IASPT
						O.Q.I.		Inter-pretation		Class	
1 May	231	0	2	18	3,6	3	E	Moderate	16	IV-V	3,2
2 May	102	0	2	14	3,5	2,5	F	Poor	12	IV-V	3
3 May	242	0	2	8	2,66	1,5	H	Very poor	10	IV-V	2,5
4 May	3290	6	3	109	5,7	5,5	A+	Excellent	125	I	5
5 May	116	0	2	19	3,8	3	E	Moderate	21	IV-V	3,5
6 May	105	0	2	10	3,33	2,5	F	Poor	10	IV-V	3,33
7 May	33	0	2	8	2,66	1,5	H	Very poor	8	V	2,66
8 May	763	0	2	3	1,5	1	I	Very poor	11	IV-V	2,75
9 May	395	0	2	23	3,83	3	E	Moderate	21	IV-V	3
1 July	1176	3	2	46	5,11	4	C	Good	41	III	4,55
2 July	1184	4	5	91	5	4,5	B	Good	90	II	4,74
3 July	182	2	3	44	4,4	4	C	Good	47	III	3,92
5 July	45	2	1	26	5,2	3,5	D	Moderate	27	IV	4,5
7 July	275	5	5	92	5,41	5	A	Excellent	82	II	4,82
8 July	2502	0	5	31	3,44	3	E	Moderate	33	III-IV	3
9 July	429	2	2	43	4,77	4,5	B	Good	42	III	3,82
10 July	441	1	2	38	4,75	4,5	B	Good	36	III-IV	4,5

a significant part of the benthic community at the Delta site and Anatoliko. In general, more pollution-sensitive taxa were present at the upstream sites, whereas at the downstream sites (6-10) tolerant taxa were dominant, similarly to the study in the summer of 1998 (Drouin et al., 1999). Vardarovasi contained a quite different macroinvertebrate benthic community, consisting mainly of Oligochaeta, Chironomidae, *Cloeon dipterum* (Ephemeroptera), that prefers eutrophic waters (Elliot et al., 1988), plus a few Gastropoda, Coleoptera and Diptera. At site Delta in May, no benthic macroinvertebrates were sampled.

Statistical analyses

Fuzzy analysis produced three clusters on the basis of the benthic community (Fig. 2). These clusters separate July sites and Gorgopis of 'good'/'excellent' water quality from May sites of 'poor'/'very poor' water quality. Site Anthofito in May constituted a cluster on its own. Twinspan classification of the sites was, in general, in agreement to Fuzzy clustering. According to CANOCO, July sites and high-scoring taxa were placed on the left side of the diagram correlated to flow and pH, which characterized the first axis (Fig. 3). May sites and low-scoring

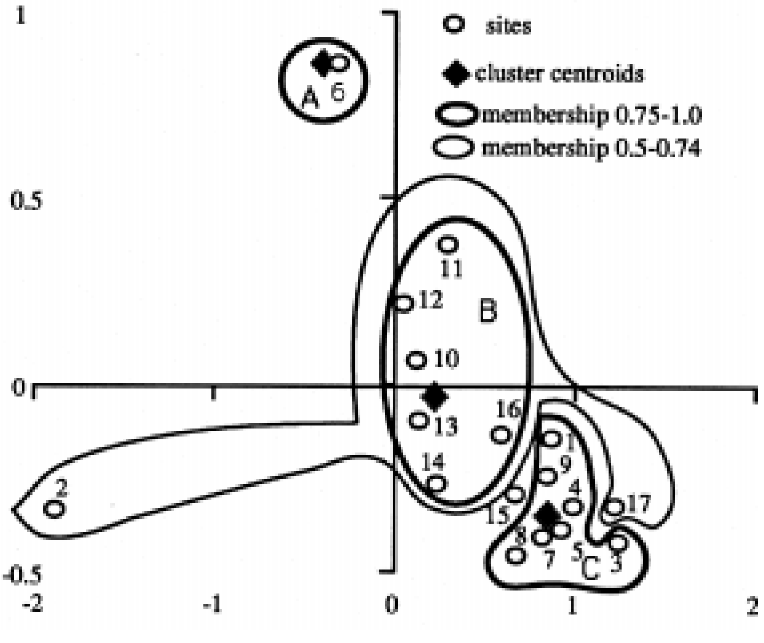

Fig 2. – Clustering of sites along the Axios in May and July 1997 according to Fuzzy analysis.

The characteristics of the analysis are:

partition coefficient	.6999
membership sum of squares:	
between	.00196/within 1.17198
eigenvalues	.451/.456/.391/.289
no.of sites	17
no.of taxa	67
no.of clusters	3
tranformation	ln(x+1)
axes	c-means(5)
norm	diagonal
convergence criterium	.00010000

Where: 1. Evzoni May, 2. Gorgopis May, 3. Axioupoli May, 4. Polykastro May, 5. Akropotamos May, 6. Anthofito May, 7. Fr. Ellis May, 8. Vardarovasi May, 9. Anatoliko May, 10. Evzoni July, 11. Axioupoli July, 12. Polykastro July, 13. Akropotamos July, 14. Fr. Ellis July, 15. Vardarovasi July, 16. Anatoliko July, 17. Delta July.

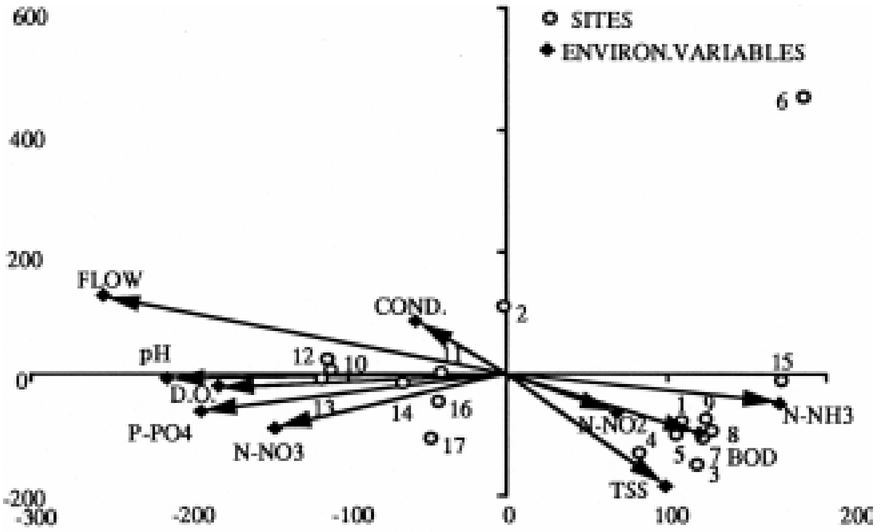

Fig. 3. – Ordination of sampling sites and environmental variables along the river Axios in May and July 1997 according to CANOCO. The characteristics of the analysis are:

axes	1	2
eigenvalues	.357	.330
species-environ.correlation	.988	.965
cumulative percentage:		
variance of species data	19.0	36.6
of species-environ.relation	23.3	44.9

The Interset correlations of the environmental variables with axes are:

axes	1	2
BOD	.3975	-.3227
DO	-.5968	-.0719
pH	-.7053	-.0323
conduct.	-.1896	.2668
tss	.3287	-.5953
flow	-.8354	.4063
N-NO3	-.4817	-.2930
N-NO2	.2275	-.2103
N-NH3	.5644	-.1618
P-PO4	-.6326	-.2016

taxa were placed on the right side of the diagram correlated to TSS and BOD_5, which characterized the second axis. Vardarovasi in both seasons was correlated to ammonia. Discriminant analysis confirmed the Fuzzy clustering, but the unusually high value of the discriminant coefficient (100%) may be due to the small sample of sites processed. According to the pooled within-groups correlations, site Anthofito constituted a separate group because of its high conductivity. Vardarovasi in July was outgrouped mainly because of its low flow and pH. Finally, July and May sites were separated due to differences in TSS and temperature values.

DISCUSSION

The macroinvertebrate benthos in the 10 stations along Axios during the high and low flow season of 1997 was, in general, not very diverse due partly to the typically lowland character of the river stretch, but also resulting from anthopogenic effects. Physicochemical characteristics, biotic indices and statistical analyses indicated poor water quality in spring and moderate to good quality in summer. In the high flow season, lower quality was due to diffuse pollution in the form of suspended solids along the main river course, concentrations of which were well above current E.U. guidelines for waters capable of supporting cyprinids (78/659/EU). The same kind of pollution was detected along the main course of the Axios in April and May 1997 during a study of monthly water quality assessment of only four stations (LANGRICK et al., 1998). Heavy rainfall and the lack of aquatic and bankside vegetation led to excessive flooding of the river and to the transport of suspended solids into the Axios, which were consequently deposited as silt. The main sources of suspended solids were nearby fields that were recently ploughed in preparation for the spring sowing. Organic pollution load was significant in the tributaries Vardarovasi and Anthofito, because of better self-purification capacity of the river during the high flow season. The high BOD_5 values were most probably related to the diffuse organic matter accompanying the suspended solids and not to point source organic pollution. In May, sampling took place at silt-substrata at the edges of the riverbed, and some of the sites were perhaps just recently covered by water. The fauna consisted mainly of Chironomidae and Oligochaeta, which are taxa tolerant to suspended solids and silt-substrate habitats (Hynes, 1970). In sandy and silty areas, which have low resistance to high discharge because of lack of suitable refugees, only burrowing animals such as Oligochaeta and Chironomidae can survive without being swept down (Hynes, 1970). Furthermore, following a flood, the fauna of the recovering stages is dominated by Chironomidae that have short life cycles (Hynes, 1970). Very diverse fauna and excellent water quality, however, characterized the Gorgopis tributary. In the low flow season, nitrates and conductivity were high due to farming and the release of industrial and urban effluents, which is in accordance with previous measurements in the Axios (Vasilikiotis et al., 1991). High BOD_5 values at Anatoliko and the Delta were clearly due to organic pollution. The increased amounts of orthophosphate during summer were due to urban run-off and industrial effluents discharged in the river, as noted in a previous study (Moustaka et al., 1992). Lower discharge and the closing of the dam, both noted during this research and as described in Argiropoulos (1991), contributed to the accentuation of pollution and to the lower self-purification capacity of the river especially downstream of the dam. Additionally, Anatoliko and the Delta were burdened with organic load, nitrates and orthophosphates. The fauna in July was more diverse reflecting mainly the more diverse substrate.

Biotic indices functioned satisfactorily but, overall, the physicochemical and the benthic macroinvertebrate data were better explained by the statistical analyses. At Fragma Ellis in July, water quality was overestimated, as the use of the biotic indices do not take abundance into consideration. Therefore, it is suggested that the development of a Greek index should include both abundance and diversity. The performance of the Iberian system was more in agreement with the physicochemical status of the system. The British system (BMWP and ASPT scores and Lincoln Quality Index) seemed to overestimate water quality on some occasions, probably because it does not take into account several pollution-tolerant families (mainly Dipteran families) that are abundant in Greek systems. The statistical analyses functioned very well as they separated the two seasons because of differences in biotic and physicochemical parameters (mainly temperature and TSS). The clean site Gorgopis was grouped separately. Anthofito was not grouped with any of the other sites because of its high conductivity. Vardarovasi was correlated mainly to its high load of ammonia, low flow and low pH. Site Vardarovasi was still characterized by high levels of ammonia in the summer of 1998 (Drouin et al., 1999). Anatoliko and the Delta of lower water quality in July, were placed at a distance from the centroid of their group of clean sites. Low scoring taxa such as Oligochaeta, Chironomidae, *Physa* spp. and Diptera were correlated to TSS and BOD_5. High scoring taxa (Ephemeroptera, Plecoptera, Trichoptera, Odonata) were correlated to dissolved oxygen and flow.

This study indicates that it is essential that bankside vegetation is developed and not cut, and the irrigation system is improved in order to prevent excessive soil erosion and to reduce flooding. The anthopogenic regulation of the discharge of the Axios seems to be affecting the ecological status of the river and its self-purification capacity. Additionally, during summer, the amount of water taken up for irrigation needs to be controlled. This is necessary as the low discharge due to irrigation coincides with seasonal pollutant inputs from adjacent industries and urban centers resulting in organic pollution of the Axios.

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ZOOPLANKTON ABUNDANCE IN THE ALIAKMON RIVER, GREECE

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ABSTRACT. The zooplankton of the River Aliakmon was studied from February 1995 to January 1996 at two sampling stations. Seventy nine different taxa were recorded, including a possible new species (*Pleurotrocha* n.sp.). The zooplankton community was dominated by rotifers, which made up to 96% of the total abundance. The two stations differed in terms of abundance ($p<0.05$) and species composition. Upstream, abundance was less than 1131 ind m^{-3}, while it varied from 712 to 13870 ind m^{-3} downstream. The highest zooplankton densities were recorded in autumn but no seasonal pattern was observed. Water temperature, dissolved oxygen and the nitrogen and phosphorus nutrients were the main environmental parameters which were found to influence zooplankton abundance in the Aliakmon.

KEY WORDS: Zooplankton, River Aliakmon, Greece.

INTRODUCTION

Relatively little attention has been paid to river zooplankton compared with lake zooplankton, although data on zooplankton composition and seasonal dynamics exist for some rivers in Europe and America (e.g. KLIMOWICZ, 1981; POURRIOT et al., 1982; KRZECZKOWSKA-WOLOSZYN, 1985; SAUNDERS & LEWIS, 1988a; FERRARI et al., 1989; PACE et al., 1991; VAN DIJK & VAN ZANTEN, 1995).

In Greece, although some information is available on the zooplankton community in lakes (ZARFDJIAN et al., 1990; MICHALOUDI et al., 1997), the present work is the first contribution to the ecology of river zooplankton. This paper describes the seasonal and spatial distribution of zooplankton in the River Aliakmon, as well as the possible influence of environmental parameters on the zooplankton community.

STUDY AREA AND METHODS

The Aliakmon river is 310 km long, and its springs are situated in the Grammos and Vernon mountains, in north-western Greece. It discharges into the Thermaikos gulf. It has a catchment area of 8443 km^2 and a mean discharge, at the mouth, of 80 m^3 sec^{-1}. The upper part of the catchment drains a mountainous area before entering a series of artificial lakes resulting from the construction of three hydroelectric dams. The river receives urban, rural and industrial wastes, which are mainly confined to the lower reaches of the river below the influence of the irrigation canal, T-66.

Samples were collected at biweekly intervals from February 1995 to January 1996 at two stations. One station was situated above the dams (upstream) (St. 1) and one (downstream) after the dams and canal T-66 (St. 2). A total of 105 l of water were taken with an electric pump at approximately 30% of the measured depth at each station. The zooplankton were retained with a 35 µm mesh size and preserved in a 4% formalin solution. All zooplankton were identified and counted.

In parallel with the zooplankton sampling, selected water quality parameters were measured *in situ* (temperature, oxygen, pH, water discharge), while water samples were taken for the analysis of nitrogen ($N\text{-}NO_3$, $N\text{-}NO_2$, ammonia-N) and phosphorus ($P\text{-}PO_4$), total-P, chl-a and suspended solids. For the analytical results of these

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Corresponding author: E. Michaloudi, e-mail: tholi@bio.auth.gr parameters see BOBORI & MOURELATOS (1999) and MONTESANTO et al. (in press).

In order to identify the homogeneity of the taxonomic units, cluster analysis using the Bray-Curtis similarity coefficient (BRAY & CURTIS, 1957) was applied to the (species abundance) x (samples) table for both stations and for each station separately. Principal components analysis (PCA) was also employed in order to reduce the environmental parameters in factors that may correlate with the zooplankton abundance.

RESULTS

Species composition

A total of 79 zooplankton taxa were identified in the River Aliakmon (Table 1). The majority were found at the downstream station. Twenty three species (29%) have not previously been recorded in Greece and one may be an entirely new species (*Pleurotrocha* n.sp., R. Pourriot pers. communication). Although the list of species is large, only

TABLE 1

Zooplankton taxa identified in the Aliakmon river, February 1995-January 1996

ROTIFERA

- *Ascomorpha ecaudis* (Perty)
- *Asplanchna priodonta* Gosse
- *Brachionus angularis* Gosse
- *B. calyciflorus* Pallas
- [n] [b] *B. quadridentatus* f. *brevispinus* (Ehrb.)
- [b] *B. urceolaris* Müller
- [b] *Cephalodella gibba* (Ehrb.)
- *C.* cf. *catellina*
- [b] *C.* sp.
- [b] *Colurella adriatica* (Ehrb.)
- *C. colurus* (Ehrb.)
- [b] *C. uncinata* (o.f.m.)
- [n] [b] *Dicranophorus uncinatus* (Milne)
- [b] *D. lütkeni* (Berg.)
- [n] [b] *Encentrum* cf. *putorius*
- [n] *Euchlanis deflexa* (Gosse)
- *E. dilatata* Ehrb.
- [n] *E. lyra* Hudson
- [n] *Epiphanes* sp.
- *Kellicottia longispina* (Kell.)
- *Keratella cochlearis* (Gosse)
- *K. c.* v. *tecta* Gosse
- *K. quandrata* (Müller)
- [b] *Lecane bulla* (Gosse)
- [n] [b] *L. closterocerca* (Schmarda)
- [b] *L. luna* (o.f.m.)
- [b] *L. lunaris* (Ehrb.)
- [n] [b] *L. quadridentata* (Ehrb.)
- [b] *Lepadella ovalis* (o.f.m.)
- [n] [b] *Lindia torulosa* Dujardin
- *Lophocharis salpina* (Ehrb.)
- [b] *Mytilina mucronata* (Müller)
- [n] [b] *M. bisulcata* (Lucks)
- [n] *Notholca acuminata* (Ehrb.)
- [n] *N. foliacea* (Gillard)
- *N. squamula* (Müller)
- [n] [b] *Notomata pseudocerberus* De Beauch.
- [b] *Platyias quadricornis* (Ehrb.)
- [n] [b] *Pleurotrocha petromycon* Ehrb.
- [n] [b] *Pleurotrocha* n.sp.
- *Ploesoma* cf. *hudsoni*
- *Polyarthra dolichoptera* Carl.
- *P. major* (Burck.)

[n] new for the Greek fauna [b] benthic and/or littoral

ROTIFERA

- *P. vulgaris* Carl.
- [n] [b] *Proales theodora* (Gosse)
- *Ptygura* sp.
- *Rotaria* sp.
- [n] [b] *Scaridium longicaudum* (o.f.m.)
- [n] *Sinantherina* sp.
- [n] [b] *Synchaeta litoralis* Rouss.
- *S. pectinata* Ehr.
- *S.* cf. *oblonga*
- [b] *Testudinella patina* (Herm.)
- [n] [b] *Trichocerca elongata* (Gosse)
- *T. longiseta* (Schrank)
- [b] *T. pusilla* (Lauterb.)
- [b] *T. rattus* (o.f.m.)
- [n] [b] *Trichotria tetractis* (Ehrb.)
- [b] Bdelloidea

CLADOCERA

- *Acroperus* sp.
- [n] [b] *Alona guttata* Sars
- [n] [b] *Biapertura affinis* (Leydig.)
- *Bosmina longirostris* (o.f.m.)
- *Ceriodaphnia pulchella* Sars
- *C. quadrangula* (o.f.m.)
- [b] *Chydorus sphaericus* (o.f.m.)
- *Daphnia cucullata* Sars
- *Diaphanosoma mongolianum* Ueno
- [b] *Eurycercus lamellatus* (o.f.m.)
- [b] *Ilyocryptus sordidus* Lievin
- *Pleuroxus* sp.
- *Simocephalus exspinosus* (Koch)
- *S. vetulus* (o.f.m.)

MOLLUSCA

- *Dreissena polymorpha* Pal.

COPEPODA

- *Cyclops* sp.
- *Eucyclops* sp.
- *Eudiaptomus gracilis* Sars
- *Megacyclops* sp.
- *Thermocyclops crassus* (Fischer)
- Harpacticoida

[n] new for the Greek fauna [b] benthic and/or littoral

a few were common and many were recorded only once or twice. More than half of them were benthic and/or littoral (Table 1). The dominant genera are shown in Fig. 2.

Seasonal variations

Abundance was generally very low. Upstream, zooplankton densities ranged from 19 to 1131 ind m^{-3}. No animals were found from May to June, while numbers peak in November 1995 (Fig. 1a). Downstream (Fig. 1b) abundance was much higher and varied from 713 to 13870 ind m^{-3}. From May to June abundance increased, and maximum densities occured in September 1995.

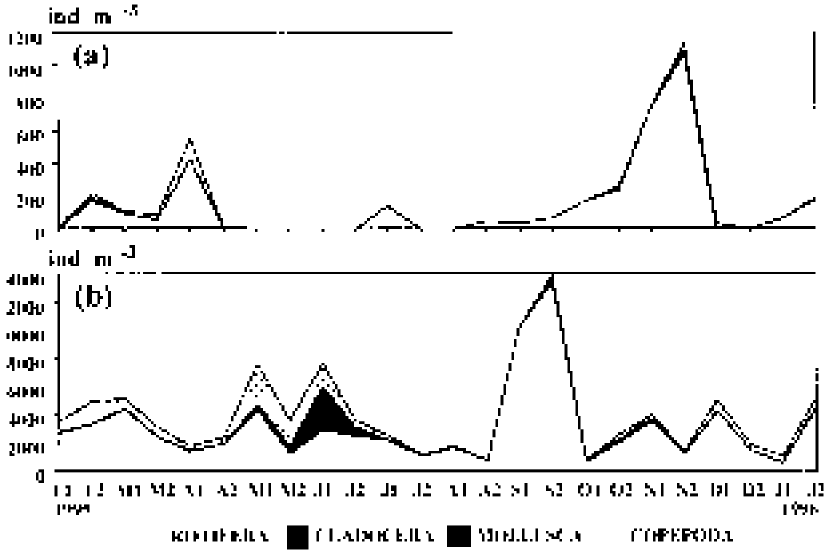

Fig. 1. – Seasonal variations of the total abundance (ind m^{-3}) and percentage contribution of the zooplankton groups in (a) St. 1 and (b) St. 2.

Rotifers dominated throughout the year, except for December 1995 at St. 1 and May 1995 at St. 2 when nauplii increased to 75 and 42%, respectively. Mollusca larvae of *Dreissena polymorpha* were present only at the downstream station (Fig. 1b), in February and from May to November of 1995, with their highest abundance (2670 ind m^{-3}) in June. Cladocera were generally only present in low numbers at both stations (0.2-11.4%), except for St. 1 in February when this group contributed 50% to the total abundance.

Spatial distribution

Abundance of zooplankton, differed significantly between the two stations (t-test=7.01, p=0.0001, DF=46). Cluster analysis also separated the two stations (Fig. 2a). All the samples of St. 2 formed group I. Group II consisted of samples of the upstream station (St. 1). Three samples from the same station formed three further groups (III, IV and V) because of the different species that dominated them compared to other samples (Fig. 2b). Group VI included all samples from the upstream station containing no animals. Cluster analysis was also performed on samples from each station separately (Fig. 2b,c). It is clear that samples were grouped mainly in relation to species dominance, and no seasonal pattern was observed.

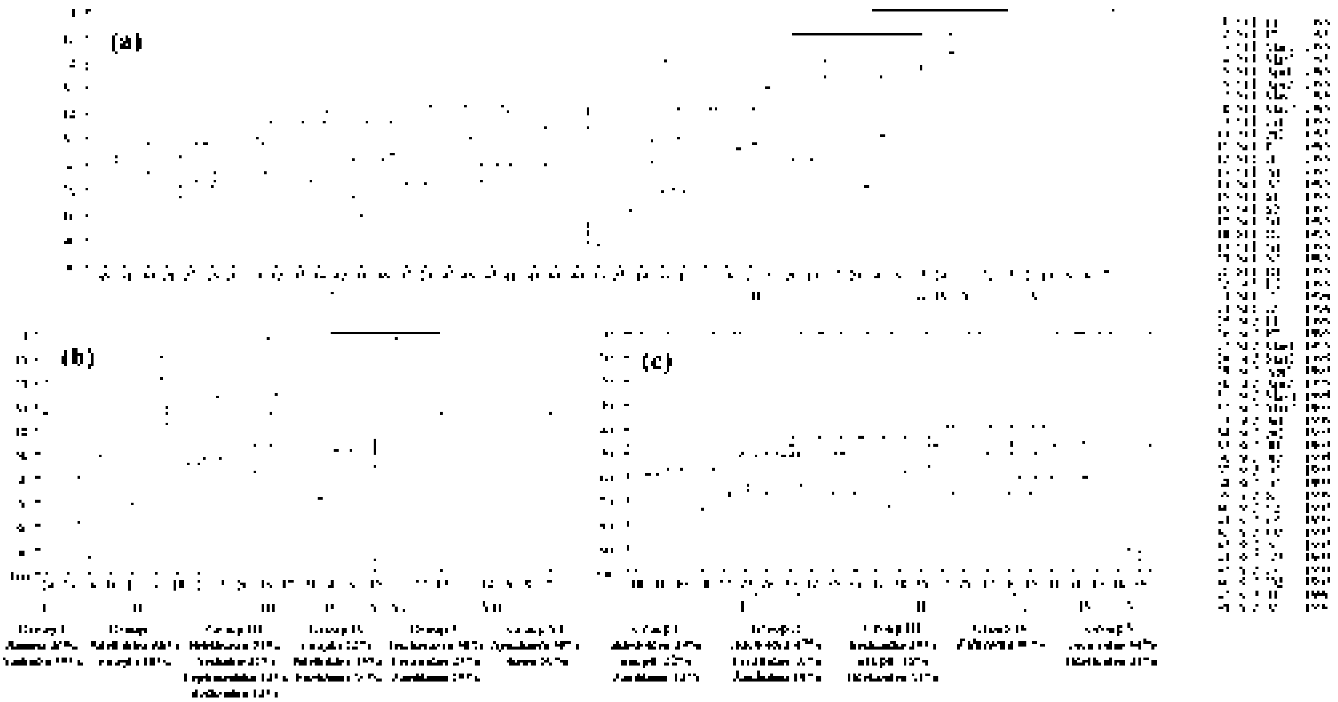

Fig. 2. – Dendrogram for clustering, based on Bray-Curtis similarities for (a) both sampling stations, (b) St. 1 and (c) St. 2, February 1995-January 1996. The species x samples combinations 1 to 48 are shown in the legend.

Influence of environmental parameters on zooplankton abundance

Principal components analysis (PCA) (Fig. 3a) extracted two main factors that explained 51% of the total variance at the upstream station. The first factorial axis (F1) associated mainly with oxygen, temperature and phosphate. Along this axis the zooplankton groups produced positive scores with oxygen and negative scores with temperature and phosphate. Nitrate, total nitrogen and rotifers showed stronger loading on the second factorial axis (F2). For the downstream station (Fig. 3b) the first two factors extracted by PCA explained 52% of the total variation. Nitrate, total nitrogen, N/P ratio and oxygen were the variables that had the stronger weighting on the first axis (F1). The distribution along the second axis (F2) was explained by nitrites and ammonium, and to a smaller degree, by pH and phosphate. The zooplankton groups were distributed on the positive part of the F1 axis. Copepods and Cladocera had positive scores on the F2 axis and were positively related to pH, while they were inversely related to nitrite, ammonia and phosphate, along the same axis. The opposite was found for rotifers and mollusc larvae.

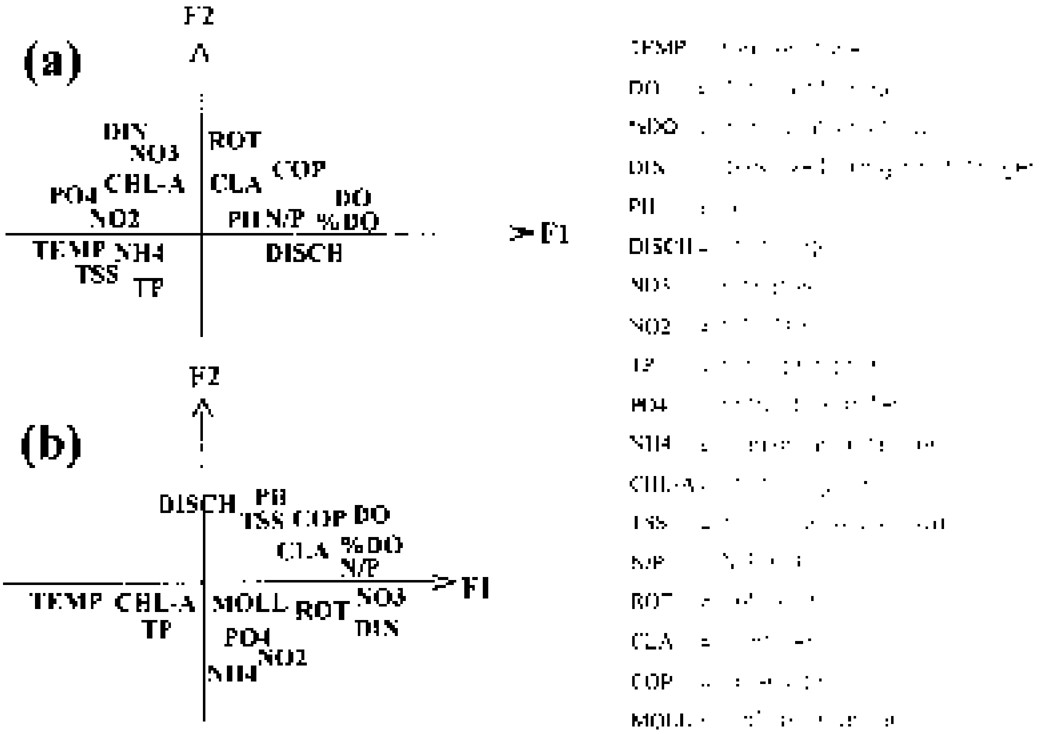

Fig. 3. – Principal components analysis of environmental parameters and abundance of the main zooplankton groups.

DISCUSSION

River zooplankton, commonly, consist of numerous species with the majority being benthic and only a few dominating (Klimowicz, 1981; Krzeczkowska-Woloszyn, 1985; Saunders & Lewis, 1988a; Brown et al., 1989; Vasquez & Rey, 1989; Zurek & Dumnicka, 1989). The same species composition was found in the river Aliakmon. However, seasonal variations in the Aliakmon exhibited no similarities with those of other rivers, where the zooplankton is characterized by low abundance in winter and high numbers in spring and summer (Klimowicz, 1981; Pourriot et al., 1982; Saunders & Lewis, 1988a,b; Van Dijk & Van Zanten, 1995). Moreover, the maximum abundance recorded in the Aliakmon was 13870 ind m^{-3}, while in large rivers densities often exceed 10^6 ind m^{-3} (Klimowicz, 1981; Ferrari et al., 1989; Van Dijk & Van Zanten, 1995). The main difference is that large rivers have high water discharge values [e.g. the river Rhine 1000-8000 m^3 s^{-1} (Van Dijk & Van Zanten, 1995)] so they provide a larger water volume where animals can develop. Small rivers like the Aliakmon and the Illinois (Brown et al., 1989) with low discharge have correspondingly lower zooplankton abundance. Although water discharge is considered to be one of the main parameters affecting zooplankton seasonal variations in rivers (Saunders & Lewis, 1988a,b; Brown et al., 1989; Pace et al., 1991; Van Dijk & Van Zanten, 1995; Vranovsky, 1995), it did not explain the variations in the Aliakmon. Chlorophyll a was also of minor importance, since the majority of the species found during the present study were benthic and thus do not feed on phytoplankton. According to our results, the factors that explained the greatest percentage of the variations were nitrogen and phosphorus, which is also noted for the river Po (Ferrari et al., 1989), as well as water temperature and oxygen which are also known to influence zooplankton abundance (Allan, 1976; Wetzel, 1983).

Seasonal variations in zooplankton abundance (Fig. 1), could be partly explained by invertebrate predation since maximum abundance was recorded when the macroinvertebrates in the Aliakmon decreased in numbers (Lazaridou-Dimitriadou et al., 1999). This kind of impact has been also observed by Zurek & Dumnicka (1989) and Brown et al. (1989). Fish predation is another factor affecting the zooplankton community (Pourriot et al., 1997; Pourriot et al., 1982; Brown et al., 1989). The Aliakmon river has a rich fish fauna (Economidis et al., 1981), that could have contributed to the small numbers of cladocera.

Generally, the zooplankton community of the Aliakmon was dominated by rotifers, which due to their short generation time and their high reproductive rate (Allan, 1976), dominate in rivers (Klimowicz, 1981; Pourriot et al., 1982; Saunders & Lewis, 1988b; Van Dijk & Van Zanten, 1995).

Considering the differences found between the two stations (Fig. 2), it seems that the distribution of zooplankton along the Aliakmon follows the general pattern noted along the course of other rivers, with higher abundance and more diverse species composition downstream (Krzeczkowska-Woloszyn, 1985; Brown et al., 1989). In the case of the Aliakmon the downstream station is also affected by water bodies connected to the river, such as the reservoirs, which may contribute to the species composition and abundance (Krzeczkowska-Woloszyn, 1985; Pourriot et al., 1997; Zurek & Dumnicka, 1989). The presence of mollusc larvae downstream is likely to be due to the presence of adults in the reservoirs since they are found nowhere else (Lazaridou-Dimitriadou et al., 1999). The domination of *Kellicottia* downstream coincided with the highest discharge values, probably indicating the contribution of either the resevoirs or other water bodies as was found by Zurek (1985) in the river Brynica.

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Contribution to the study of the genetic variability and taxonomic relationships among five lizard species of the family Lacertidae from Greece

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ABSTRACT. The present study examines the genetic variability and the taxonomic relationships among five lacertid species, i.e. *Podarcis taurica*, *P. milensis*, *P. peloponnesiaca*, *Lacerta graeca* and *Algyroides moreoticus*, representing the three main genera of this family in Europe. The last four of the above species are endemic to Greece and three of them live sympatrically in Peloponnisos. These relationships were studied by allozyme analysis. Of the loci analyzed, the Mpi-1 locus was found to be a convenient molecular marker for discrimination of the genera *Podarcis* (allele a), *Lacerta* (allele b) and *Algyroides* (allele c). The values of Nei's genetic distances between the examined species ranged from 0.025 to 0.484. According to the UPGMA-dendrogram plotted using the Nei's genetic distances, two species groups are formed indicating that the genera *Lacerta* and *Algyroides* show a stronger relationship to one another than either does to *Podarcis*. These results are in agreement with DNA sequence data but are not in accordance with previous electrophoretic and immunological studies, which suggest that *Lacerta* is more closely related to *Podarcis* than to *Algyroides*. The studied *Podarcis* taxa were found to be close relatives (Nei's diastances <0.18), especially *P. taurica* and *P. milensis*. These values are lower than those usually given in the litterature for the distinction of lacertid species.

INTRODUCTION

Although many studies have been carried out on the systematics of the family Lacertidae dating from the last quarter of the 19th century, well-supported estimations of the overall phylogeny of the family on the basis of morphological and molecular evidence have only appeared quite recently (ARNOLD, 1989; HARRIS et al., 1998; HARRIS & ARNOLD, 1999). According to ARNOLD (1989) Lacertidae falls into two parts: (a) a paraphyletic Palaearctic and Oriental group of primitive forms, also including nearly all Mediterranean species, and (b) a holophyletic assemblage of Ethiopian and advanced Saharo-Eurasian taxa derived from the former part.

Among the members of the Palaearctic-Oriental group, the collective genus *Lacerta* is one of the most diverse lacertid genera, with more than 50 species. The phylogeny and taxonomy of this widespread genus has been the subject of by far the most attempts (for a review see ARNOLD, 1989, 1993). Cladistically, it is evident that the traditional genus *Lacerta* is a paraphyletic group consisting of a number of smaller clades and species groups; so its systematics is still unclear and many conflicts occur. On the other hand *Podarcis* and *Algyroides* are much more homogeneous entities: The well-defined Mediterranean group of *Algyroides* is clearly holophyletic, and there is little or no doubt of the holophyletic character of *Podarcis* (ARNOLD, 1989; MAYER & LUTZ, 1990; HARRIS & ARNOLD, 1999). Taxonomically, *Algyroides* was long ago thought to be a clearly delineated genus while *Podarcis*, although formerly considered as a subgenus of *Lacerta* (more recently this opinion is also expressed by MAYER & TIEDEMANN, 1982 and Guillaume and Lanza, 1982), now is widely accepted as a definite genus composed of closely related species.

Within the clearly paraphyletic taxon *Lacerta* s. lat. as defined by ARNOLD (1989), *Archaeolacerta* is considered

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as a rather informal and not well-defined assemblage of primitive lizards; according to this author it seems better, for the present, to treat it as a subgenus of *Lacerta* s. lat., although Harris et al. (1998) do not give enough evidence for the monophyly of archaeolacertas as a whole.

For the clarification of the phylogenetic relationships of the European lacertids, apart from the morphological studies, biochemical and immunological techniques have been used extensively (for a review see Arnold, 1989 and Harris et al., 1998). Recently, mt-DNA and r-RNA sequence data have also been used (Gonzalez et al., 1996; Douris et al., 1997; Fu et al., 1997; Harris et al., 1998; Harris & Arnold, 1999). In spite of all this accumulated information many ambiguities and controversies concerning the affinities of certain taxa still remain.

The present article constitutes a contribution intended to reveal or confirm the relationships among *Lacerta* (*Archaeolacerta*), *Podarcis* and *Algyroides*. Such a direct, simultaneous comparison of these three genera by means of allozyme electrophoresis has been lacking from the literature. It is well known theoretically that a large number of species (if not the total) of the three genera compared should be studied in order to acquire definitive conclusions on their relationships. However, in practice, acceptable results have been obtained by many authors who used smaller numbers of species. In the present work five species (*L. graeca, P. peloponnesiaca, P. taurica, P. milensis* and *A. moreoticus*) were considered as representatives of the above three genera because they are interesting members of the Balkan lacertids. Apart from *P. taurica* all others are endemic to confined continental and/or insular parts of Greece (Chondropoulos, 1986; Valakos & Mylonas, 1992). These particular species were studied since it is generally known that the relationships of the Mediterranean lacertids have been greatly influenced by the major palaeogeographic events in the area (Bohme & Corti, 1993); so endemism and other biogeographical consequences of those events constitute very useful material to illuminate phylogenetic affinities and test the taxonomic hypotheses proposed. On the other hand the faunistic importance of the endemic Mediterranean lizards is obvious as they largely contribute to the local and the whole European biodiversity.

MATERIAL AND METHODS

A total of 53 lizards of the five studied species were used. The sampling areas and the respective sample size of each species are shown in Table 1. The collection of this material has been carried out on the basis of rules and limitations of the Greek legislation (Presidential Decree 67/1981). For the electrophoretic procedure liver, kidneys and muscles were taken from the properly anesthetized animals. The electrophoretic analysis was carried out on already prepared cellulose acetate plates using the set of devices, both provided by Helena Laboratories house. The preparation of the tissue samples, the electrophoretic conditions and the protein-specific staining were performed according to Searle (1983) modified by Fraguedakis-Tsolis. In the present study 17 putative genetic loci also used in previous studies on lacertid lizards, were examined (Table 2). For technical reasons in *P. milensis* only 8 loci were examined. The electrophoretic results were treated with the biostatistical package BIOSYS-1 (Swofford & Selander, 1981).

TABLE 1

The sampling areas and the sample size of the species examined

Taxa	Sample size	sampling area
Podarcis peloponnesiaca (Bibron & Bory, 1833)	15	Stymfalia, Korinthia Pref.
Podarcis milensis (Bedriaga, 1882)	10	Milos archipelago, Kyklades Pref.
Podarcis taurica (Pallas, 1814)	7	Stymfalia, Korinthia Pref.
Lacerta graeca (Bedriaga, 1886)	6	Stymfalia, Korinthia Pref.
	4	Kardamyli, Messinia Pref.
Algyroides moreoticus (Bibron & Bory, 1833)	3	Zarouchla, Korinthia Pref.
	8	Vouraikos Gorge, Achaia Pref.

TABLE 2

The loci examined, the respective enzymes and the tissues from which they were extracted

Locus	Enzyme	Tissue
Aco-1,2	Aconitase	Liver
Ak-1	Adenylate kinase	Liver
Ck-1	Creatine kinase	Liver
Got-1,2	Glutamate oxaloacetate transaminase	Liver
Gpi-1s	Glucose phosphate isomerase	Muscle
Idh-1,2	Isocitrate dehydrogenase	Kidneys
Ldh-1,2	Lactate dehydrogenase	Muscle
Mod-1,2	Malic enzyme	Kidneys
Mor-1	Malate dehydrogenase	Kidneys
Mpi-1	Mannose phosphate isomerase	Liver
Np-1	Nucleoside phosphorylase	Liver
Pgm-1	Phosphoglygomutase	Muscle

RESULTS

As a consequence of factors mentioned above for the material of *P. milensis*, there are two sets of results stemming from the statistical treatment of the electrophoretic raw data: the first set includes the results obtained from the comparison of 4 species (*P. milensis* is excluded), on the basis of all the 17 loci, while the second one includes the results derived from the comparison of all the 5 species, taking into account 8 loci.

In the case of the first data set 13 of the 17 loci examined were polymorphic (Table 3). The polymorphic locus Mpi-1 was fixed for three different alleles, one for each of the three lacertid genera examined: namely allele a in *Podarcis*, allele b in *Lacerta* (*Archaeolacerta*) and allele c in *Algyroides* (Table 3). The mean value of the fixation index $F_{(ST)}=0.618$ implies that 61.8% of the total genetic variability observed was due to differences between the species studied, and the remaining 38.2% was due to intraspecific differences. The values of the Nei's genetic distances between the species studied ranged from 0.123 to 1.426, while those of Roger's genetic identity ranged from 0.597 to 0.796 (Table 4). According to the UPGMA-dendrogram representing the taxonomic relationships between the four taxa considered in this case (Fig.1), the lizards *P. peloponnesiaca* and *P. taurica* are the most closely related, while *L. (A.) graeca* and *A. moreoticus* form another group of more remote relatives.

TABLE 3

Allele frequencies of the 17 genetic loci examined in 5 of the lacertid species studied (dashes indicate the loci not examined in *P. milensis* – see Materials and Methods).

Locus	*P. pel/ siaca*	*P. taurica*	*P. milensis*	*L. graeca*	*A. moreo- ticus*
Aco-1		.214a			.091a
	b	.786b	b	b	.909b
Aco-2		.071a	.150a	.050a	
	b	.857b	.600b	.200b	.545b
		.071c	.250c	.750c	.455c
Ak-1	a	a	a		.818a
					.182a
Ck-1	a	.429a	.800a		
		.571b	.200b	b	.364b
					.636c
Got-1	.643a			a	
	.357b				b
		c	c		
Got-2	.214a				
	.786b	b	b	b	b
Gpi-1s	a	.786a	-	a	.875a
		.214b			.125b
Idh-1	.233a	.857a	-	a	a
	.767b	.143b			
Idh-2	a	a	-	.950a	a
				.050b	
Ldh-1	a	a	-	a	a
Ldh-2	a	a	-	a	a
Mod-1	.466a	.357a	-	.400a	.125a
	.267b				
	.267c	.643c		.600c	.875c
Mod-2		.286a	-	a	a
	b	.714b			
Mor-1	a	a	-	a	a
Mpi-1	a	a	a	b	c
Np-1	a	a	a	a	a
Pgm-1			-	a	.938a
	b	b			.063c

TABLE 4

Values of Nei's genetic distances (above diagonal) and values of Roger's genetic identity (below diagonal) between the 5 lacertid species studied. In parentheses are indicated the respective Nei's and Roger's values between 4 lacertid species studied (except *P. milensis*).

Species	*P. pel/ siaca*	*P. taurica*	*P. milensis*	*L. graeca*	*A. mor/ ticus*
P. pel/ siaca	***	0.179 (0.123)	0.142 (-)	0.466 (0.426)	0.437 (0.426)
P. tau- rica	0.750 (0.796)	***	0.025 (-)	0.451 (0.318)	0.449 (0.308)
P. milen- sis	0.795 (-)	0.898 (-)	***	0.484 (-)	0.471 (-)
L. graeca	0.582 (0.619)	0.586 (0.678)	0.593 (-)	***	0.437 (0.181)
A. mor/ ticus	0.567 (0.597)	0.597 (0.684)	0.601 (-)	0.596 (0.780)	***

Fig. 1. – The UPGMA-dendrogram representing the relationships of 4 of the species studied, based on the Nei's genetic distances (*P.p*: *Podarcis peloponnesiaca, P.t*: *Podarcis taurica, L.g*: *Lacerta graeca, A.m.*: *Algyroides moreoticus).*

In the second case seven of the eight presumptive loci analyzed were polymorphic (Table 3*)*. The Mpi-1 locus in *P. milensis* was found monomorphic for the same 'a' allele as in the other *Podarcis* taxa examined. The mean value of the fixation index $F_{(ST)}=0.646$ implies that 64.6% of the total genetic variability observed was due to interspecific differences, while the remaining 35.4% was due to intraspecific ones. The values of the Nei's genetic distances between the taxa examined ranged from 0.025 to 0.484, while those of Roger's genetic identity ranged from 0.582 to 0.898 (Table 4). The UPGMA-dendrogram expressing the relationships between the 5 species studied is shown in Fig.2. This dendrogram clearly indicates that *P. milensis* and *P. taurica* are the most closely related species and *P. peloponnesiaca* is the next most related one

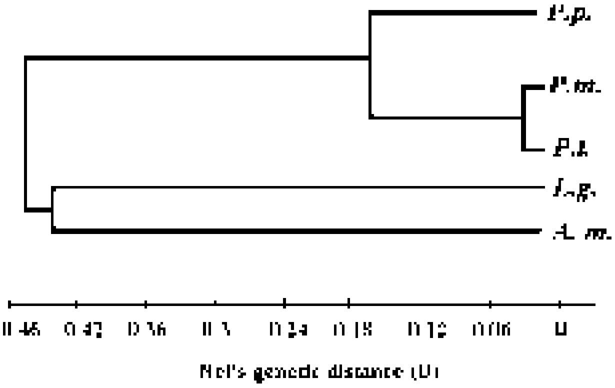

Fig. 2. – The UPGMA-dendrogram representing the relationships of all 5 species studied, based on the Nei's genetic distances (*P.p*: *Podarcis peloponnesiaca, P.m.*: *Podarcis milensis, P.t*: *Podarcis taurica, L.g*: *Lacerta graeca, A.m.*: *Algyroides moreoticus).*

to them. The two remaining taxa, *L. (A.)graeca* and *A. moreoticus*, show much more remote affinities to the previous species, being more closely related to each other than to any of the *Podarcis* species.

DISCUSSION

Morphological analysis indicates that *Lacerta* (*Archaeolacerta*), *Podarcis* and *Algyroides* are closely interrelated taxa as compared to other, more distant members of the primitive Palaearctic and Oriental lacertids (Arnold, 1989). Such a close phylogenetic affinity of the above three taxa is also corroborated by karyological and DNA sequence data (Odierna et al., 1993; Olmo et al., 1993; Harris et al., 1998). Bohme & Corti (1993) suggest that *Archaeolacerta, Podarcis* and *Algyroides* belong to the radiation that also produced the taxa *Teira* and *Timon*, about 18-20 million years ago, but these authors also stress that the interrelations of the above taxa are unresolved.

According to the dendrogram of Fig.1 our data seems to properly separate the three genera examined. This separation is emphasized by the existence of a genus-specific molecular marker, i.e. the protein coded by the Mpi-1 locus where a different allele (a) was found to be fixed in each genus. On the basis of at least the representative species studied it was found that the relationships among *Lacerta* (*Archaeolacerta*) and *Algyroides* appear to be stronger than the relationships of each of them to *Podarcis*. Morphology does not give evidence for an unequivocal pattern of relations among most of the Palaearctic lizard taxa on either generic or specific level (Arnold, 1989). Molecular data (electrophoretic, immunological and DNA sequencing) offer additional information but they are often in conflict each other and frequently also with the morphological data.

Our results are in accordance with DNA sequence data of Harris et al. (1998) who found that archaeolacertas (*L. (A.) graeca* included) are closer to *Algyroides* (represented in their material by *A. marchi* only) than to *Podarcis* (represented by *P. muralis* and *P. taurica*). On the contrary the data of the present study are not supported by chromosomal and immunological data, which suggest that archaeolacertas are closer to *Podarcis* than to *Algyroides* (Mayer & Lutz, 1990; Odierna et al., 1993; Olmo et al., 1993).

The addition of another species, *Podarcis milensis*, to interspecific comparison procedure of our electrophoretic data let us confirm and strengthen our results of the first data set concerning the interrelationships among archaeolacertas*, Algyroides* and *Podarcis* (Fig.1). Furthermore, it makes clear that within *Podarcis* there are stronger relationships of *P. milensis* to *P. taurica* than to *P. peloponnesiaca*; this finding corroborates previous data for the close relations of the former two species and agrees with the opinion that the third one is taxonomically closer to *P. erhardii* than to *P. taurica* (Arnold, 1973; Mayer & Tiedemann, 1982; Lutz & Mayer, 1985; Mayer, 1986).

Concluding this paper we have to underline the frequently conflicting results of studies on the lacertid interrelationships, even within relatively well-studied lineages of this family. This fact is indicative of the complicated taxonomic relations of Lacertidae and makes necessary more detailed and exhaustive studies in order to elucidate the pattern of the lacertid affinities, especially those of the Mediterranean basin.

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Seasonal changes in distribution and abundance of the fish fauna in the two estuarine systems of Strymonikos gulf (Macedonia, Greece)

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ABSTRACT. Fifty-five fish species representing 20 families were recorded in the two estuarine systems of Strymonikos Gulf. Twelve of them were freshwater, while 43 were estuarine and near-shore marine species. Forty-three species were caught in the Strymon estuarine system, nine of which were freshwater (20.9%), and 29 in the Rihios estuarine system, five of which were freshwater species (17.5%). The euryhaline *Pomatoschistus marmoratus* was the dominant species throughout the whole sampling period in the Strymon system but the highest relative abundance was recorded for *Liza ramada* in May 1999 (4,738 ind./1,000 m^2). The highest species richness in the Strymon system was observed in September 1997 with 16 species and the lowest in January 1998 with only two species. In the Rihios system, the freshwater species *Rhodeus sericeus* was the most abundant one during the months of its presence and exhibited the highest CPUE value (38,833 ind./1,000 m^2) recorded in this study. Species richness in the Rihios system peaked in July 1998 (18 species). Species richness and total abundance in the two sites were both found to increase during the warm seasons of the year (summer and autumn) following the water temperature fluctuations almost immediately. The seasonality of temperature changes was also found to determine the temporal changes of species composition of the overall fish community.

KEY WORDS: estuaries, euryhaline fish, CPUE, Strymonikos Gulf, Greece.

INTRODUCTION

Estuarine fish are mainly euryhaline forms that are able to exist in unstable surroundings surviving variable salinities, currents, and food supplies. Apart from these 'true' estuarine species, estuaries are also occasionally used by passage migrants or by marine and freshwater species for feeding and breeding or as nursery grounds (DANDO, 1984). Thus, estuarine fish communities are highly variable, and in some cases they experience large temporal changes in species composition and abundance (TREMAIN & ADAMS, 1995).

Although the estuarine fish communities have been extensively studied in other parts of the world (MARAIS & BAIRD, 1980; MARAIS, 1981; WHITFIELD, 1980; POTTER et al., 1983; WHITFIELD, 1983; LONERAGAN et al., 1986; TREMAIN & ADAMS, 1995 and others) literature concerning the distribution and abundance of fish fauna in European and Mediterranean estuarine systems is rather limited. Relevant information is available for River Po (VITALI & BRAGHIERI, 1981), Severn Estuary (CLARIDGE et al., 1986), Porto-Lagos lagoon (KOUTRAKIS et al., in press) and Gialova lagoon (DOUNAS & KOUTSOUBAS, 1996). Moreover, there are data referring to the distribution and abundance of commercially important fish species, mainly of Mugilidae (BOGRAD, 1961; ALBERTINI-BERHAUT, 1978; KATAVIC, 1980; TORRICELLI et al., 1982; VIDY & FRANC, 1992; KOUTRAKIS et al., 1994).

The two estuarine systems where the study was carried out are those of Strymon and Rihios, which are the two main rivers flowing into the Strymonikos Gulf. The Strymonikos Gulf is located at the northwestern part of the Aegean Sea occupying an area of 540 km^2. Strymon River, one of the three most important rivers in Northern Greece, originates from Bulgaria and flows to the northern part of the gulf. The river is embanked throughout its course, and

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towards its mouth it forms a small lagoon and a number of channels. Rihios is a small river with a steady flow throughout the year that drains the lake Volvi. It flows to the western part of the gulf, creating a small estuarine system. According to the Greek habitat project Natura 2000 both rivers are ecologically important sites and moreover, Rihios is part of the Lake Koronia-Volvi Ramsar site.

The aim of this study was to describe the seasonal patterns of distribution and abundance of the fish fauna in these estuarine systems. More specifically, the objectives were to provide a quantitative checklist of the fish species caught in the two areas and to detect the temporal changes in species richness and abundance and their relationship to the abiotic parameters of the water (temperature, salinity, oxygen and pH). The ecological importance of the two areas makes such data valuable for sustainable management.

MATERIAL AND METHODS

Sampling was carried out on a monthly basis from September 1997 to May 1999. Four sampling stations were selected, two in the Strymon estuarine system and two in the Rihios one (Fig. 1). A bag seine net of 20 m length and 2 mm mesh size was used for the collection of freshwater, euryhaline and near-shore marine species. Moreover, a fyke-net of 2 mm mesh size was placed every month for a period of 24 hours in the Rihios river, in order to collect species difficult to catch with a bag seine, either due to the time of the regular sampling or to behavioural characteristics of the fish. After collection, samples were preserved in 6% formalin until total length and weight were measured in the laboratory. During sampling, water temperature, salinity, pH and dissolved oxygen were also measured *in situ.*

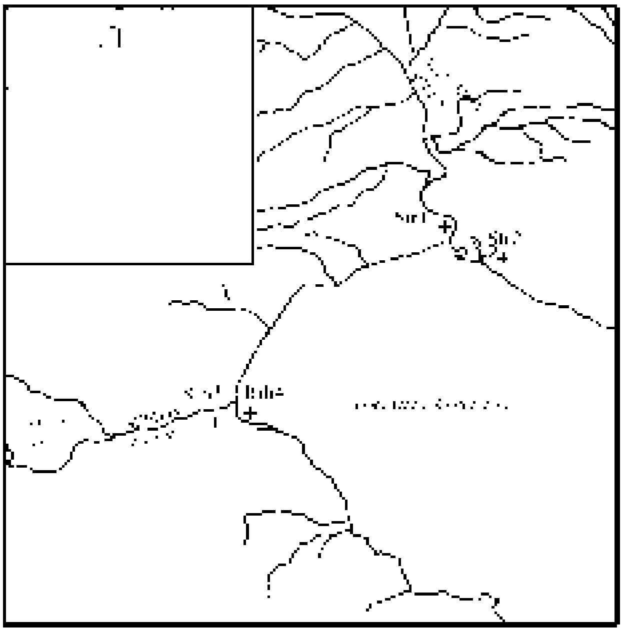

Fig. 1. – The Strymonikos Gulf (Macedonia, Greece) with the two rivers and their estuarine systems. The four sampling stations are indicated.

In order to estimate relative abundance, the catch per unit effort method (CPUE) was used (Gulland, 1964; Bannerot & Austin, 1983). The unit of effort was defined as the area covered by hauling of the seine net (Marais & Baird, 1980; Koutrakis, 1994; Koutrakis et al., 1995) and was converted to 1,000 m^2. Abundance was not estimated for the species caught in the fyke-net.

Regarding data analysis, Pearson's correlation coefficient was used in order to detect relations of fish relative abundance and species richness with the water physicochemical parameters, while in order to reveal any synchronisation in the monthly changes of the above biotic and abiotic parameters, cross-correlation analysis were used (Chatfield, 1975). The pattern of temporal changes in the structure of the overall fish community was explored by Correspondence Analysis, a multivariate method primarily introduced by Benzecri (1969) and developed independently later by several other authors (e.g. 'Reciprocal averaging' by Hill, 1973). The computations used here are presented in Greenacre (1984). For this analysis monthly samples from all experimental stations were treated together, while in order to avoid bias of the results, species that occurred only once or twice in the sampling period were excluded as outliers.

RESULTS

Fifty-five fish species, representing 20 families, were recorded in the two estuarine systems (Table 1 and 2). Twelve of them (21.8%) were freshwater, while 43 (78.1%) were estuarine and near-shore marine species. Forty-three species were caught in the Strymon estuarine system, nine of which (20.9%) were freshwater, and 29 were caught in the Rihios system, five of which (17.2%) were freshwater species. The freshwater species *Anguilla anguilla* and *Knipowitschia caucasica* were only caught in the fyke net.

With respect to the Strymon system (Table 1), members of the Mugilidae family were among the most abundant species, accounting for 43.4% of the total catch. The common goby *Pomatoschistus marmoratus* was the dominant species throughout the whole sampling period (31.2%), being present for 19 months. The second most frequently caught species was *Liza aurata* (14 months of presence), followed by *L. ramada* (13 months of presence). The highest relative abundance was recorded for *L. ramada* in May (4,738 ind./1,000m^2) and March 1999 (3,112 ind./1,000 m^2), followed by *P. marmoratus* in September 1998 (3,388 ind./1,000 m^2).

Regarding the Rihios system (Table 2), the most abundant species (56.5% of the total catch) were members of the Cyprinidae family. The thick lip mullet *Chelon labrosus* was present throughout the study period, but the freshwater species *Rhodeus sericeus* was the most abundant one during the months of its presence (42.8% of the total catch), reaching its maximum relative abundance in September

TABLE 2

The species caught in the Strymon estuarine system and their relative abundance

Family	Species	S-97	O-97	N-97	D-97	J-98	F-98	M-98	A-98	M-98	J-98	J-98	A-98	S-98	O-98	N-98	D-98	J-99	F-99	M-99	A-99	M-99
Clupeidae	*Alosa fallax*				2																	
	Sardina pilchardus	3								493	112				13							51
Cyprinidae	*Alburnus alburnus*				147											43	24		7	37	24	53
	Barbus plebejus										10											
	Carassius auratus				2		8															
	Chodrostoma nasus																4					
	Leuciscus cephalus			2			49			7	2130	9										35
	Rhodeus sericeus										7											25
	Vimba melanops									3												
Belonidae	*Belone belone*	23		42							4			176	12							
Syngnathidae	*Hippocampus ramulosus*	3																				
	Syngnathus abaster						5		12				4	20	10							
	Syngnathus variegatis									4												
	Syngnathus typhle			5																		
Atherinidae	*Atherina boyeri*	613	46		48		6				8	206	371	336	23	626						2
	Atherina hepsetus	29								3												
Mugilidae	*Chelon labrosus*	13							5	99	245	31		24		17					120	134
	Liza aurata			275	96		6		1718	1190		3			212	1238	252	8	4	48	1388	127
	Liza ramada		6				3		210	265	579	3		4			392	112	135	3112	2260	4738
	Liza saliens	48										112	30	4	4	108	16	20			12	
	Mugil cephalus			150	15										16	439	548					
Moronidae	*Dicentrarchus labrax*								305	113	229	15								4		245
Percidae	*Perca fluviatilis*						3															
Sparidae	*Diplodus annularis*	5																			1708	
	Diplodus vulgaris	5																				
	Lithognathus mormyrus	8							170	152				8	3						1080	332
	Sparus aurata									7											124	
Mullidae	*Mullus barbatus*	19										29	3									
Callionymidae	*Callionymus maculatus*									4												
	Callionymus risso	30	13										117	12	7						4	
Centrarchidae	*Lepomis gibbosus*				2																	
Blennidae	*Parablennius sanguinolentus*	29	2									124	73	44								
Clinidae	*Clinitrarchus argentatus*		2																			
Gobiidae	*Gobius cobitis*	134	45	5	2							52	53	32								
	Gobius niger												3									
	Gobius paganellus															4						
	Pomatoschistus marmoratus	456	1225	361	309	3	130		667	82	475	2148	1805	3388	136	315	500	81		196	1680	840
Triglidae	*Lepidotrigla cavillone*								10													
Scophthalmidae	*Psetta maxima*					3														4		
	Scophtalmus rhombus	6																			24	
Soleidae	*Solea kleinii*		2													4				28	640	36
	Solea nasuta													4								
	Solea vulgaris								15													

TABLE 1

The species caught in the Rihios estuarine system and their relative abundance (*: species caught in the fyke-net)

Family	Species	S-97	O-97	N-97	D-97	J-98	F-98	M-98	A-98	M-98	J-98	J-98	A-98	S-98	O-98	N-98	D-98	J-99	F-99	M-99	A-99	M-99
Cyprinidae	*Alburnus alburnus**																67					
	*Barbus plebejus**	133	50								17	33	767	433	183	33						
	*Leuciscus cephalus**	1267	100							167	67	1033	9567	2800	1350							
	*Rhodeus sericeus**	6717	1017			17	100	17			17		4033	38833	7700	33						
	Vimba melanops														17							
Anguillidae	*Anguilla anguilla**																					
Poecilidae	*Gambusia affinis**	4383	517							117	167	17	567	8000	2017	500						
Atherinidae	*Atherina boyeri**			33	17					25	25	1375	525		50	25				1700	700	
	Atherina hepsetus															775						
Mugilidae	*Chelon labrosus**	67			50	50	17	167	17	92	100	675	200	100	50	500			50			
	Liza aurata	67		250		133	517	225	5142	2133	825	17			25	50		183	275		325	
	*Liza ramada**								2850	117	383	300					300	625		33	233	
	*Liza saliens**	58	25				17					333	10467	4375	1133	1200						
	*Mugil cephalus**			100	33		17	25					333	1867	633	2292	92	33			33	
Moronidae	*Dicentrarchus labrax**										900	200	25	25		25			25	50		
Sparidae	*Diplodus sargus*									25		25										
	Lithognathus mormyrus									25												
	Sarpa salpa										175	100										
	Sparus aurata									50												
Mullidae	*Mullus barbatus*											200										
Blennidae	*Aidablennius spynx*																				25	
	*Blennius fluviatilis**											100		67								
	Lipophrys pavo		100							17												
	Parablennius gattorugine											275	33					25				
	*Parablennius incognitus**	183	17	50																		
	*Parablennius sanguinolentus**	17	1175					33		25	67	500	275	500	133	25						
	Parablennius tentacularis									33												
Gobiidae	*Gobius cobitis*	50	225	33						25	75	225	75	150	275							
	Gobius cruentatus											25										
	*Knipowitschia caucasica**																					
	*Pomatoschistus marmoratus**										50			50	25				25	25	25	
Scophthalmidae	*Psetta maxima*											25										

1998 (38,833 ind./1,000 m^2), which was the highest CPUE value recorded in this study. *Liza saliens* and *Leuciscus cephalus* also exhibited high CPUE values in August 1998 (10,467 and 9,567 ind./1,000 m^2, respectively).

As far as the physicochemical parameters of the water are concerned (Figs 2-3), temperature displayed a strong seasonality in both areas, ranging from 7.9 to 29.4°C. Seasonal oscillations were also obvious in the case of dissolved oxygen, which ranged from 3.03 to 14.2 mg/l in Strymon and from 1.95 to 13.56 mg/l in Rihios. Salinity varied according to station location. Thus, at stations Str1 and Rih3, which were located in the mainstream, salinity ranged from 0 to 13 psu, while at stations Str2 and Rih4, which were located in the estuaries, it ranged from 4 to 35 psu. Finally, spatial and temporal variations of pH did not display any trend, ranging from 7.67 to 8.35.

The temporal changes in total relative abundance and species richness in the two areas are shown in Fig. 4. The highest species richness in the Strymon system was observed in September 1997 and May 1998, with 16 and 14 species respectively, and the lowest in January 1998 and February 1999, with 2 and 3 species respectively. Species richness in the Rihios system peaked in July 1998 (18 species). This was also the highest species richness observed in this study. The lowest values were observed in winter of both years (3 species).

In general, species richness at both sites was higher during the warm period of the year, i.e. summer and autumn. The same seasonal pattern was also displayed by the total relative abundance, being almost parallel to the one shown by the water temperature and dissolved oxygen fluctuations. Indeed, temperature was significantly correlated with species richness ($r=0.83$, $p<0.001$) and total abundance ($r=0.54$, $p<0.05$). In contrast dissolved oxygen and species richness were negatively correlated ($r=-0.52$, $p<0.05$). Further analysis of cross-correlations showed that species richness and abundance followed the changes of temperature and dissolved oxygen with a time lag of one month at most. No correlation of species richness with salinity ($r=0.25$, $p>0.05$) and pH ($r=-0.46$, $p>0.05$) was found, nor of total abundance with pH ($r=0.24$, $p>0.05$) and dissolved oxygen ($r=-0.28$, $p>0.05$).

The results of the correspondence analysis (Fig. 5) where the structure of the overall fish community is shown, indicated that species distribution in the monthly samples is generally determined by seasonality, since summer and autumn months together with most of the species were distributed in the right half of the two-dimensional plot, while the winter and spring months were placed towards the left end of the first axis together with *L. ramada, L. aurata, Lithognathus mormyrus* and *Sardina pilchardus*. The two most frequently caught species of the two estuarine systems, *P. marmoratus* and *C. labrosus*, were ordinated in the middle of the plot. Moreover, there was a partitioning of summer from autumn months along the second axis. The species com-

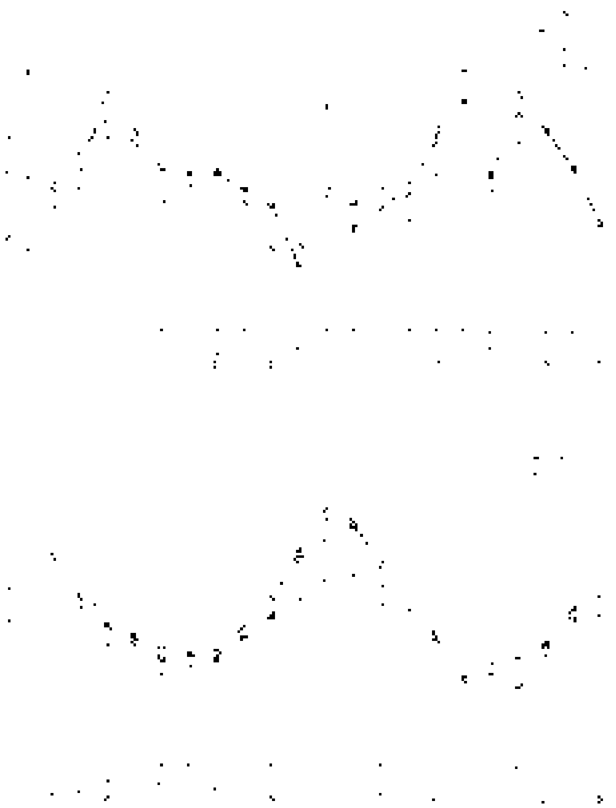

Fig. 2. – Monthly changes of dissolved oxygen (a) and temperature (b) in the sampling stations of Strymon (STR 1,2) and Rihios (RIH 3,4).

Fig. 3. – Monthly changes of salinity (a) and pH (b) in the sampling stations of Strymon (STR 1,2) and Rihios (RIH 3,4).

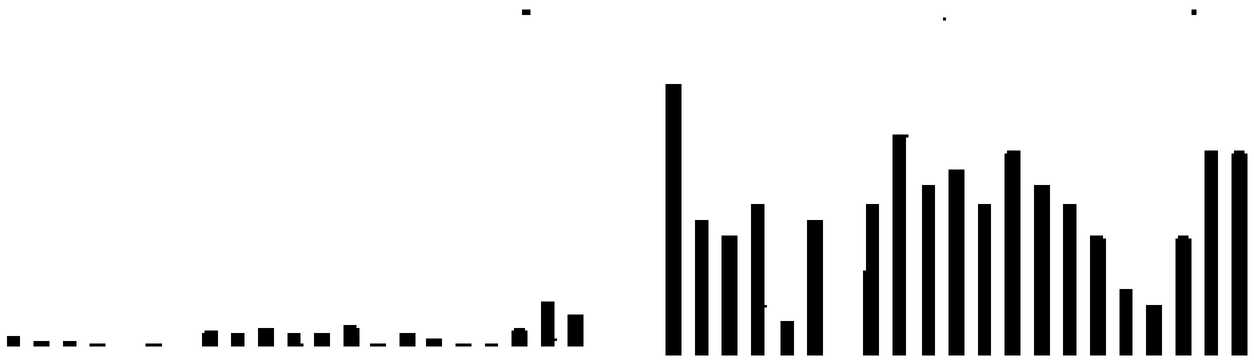

Fig. 4. – Monthly changes of total relative abundance (a) and species richness (b) in the estuarine systems of Strymon and Rihios.

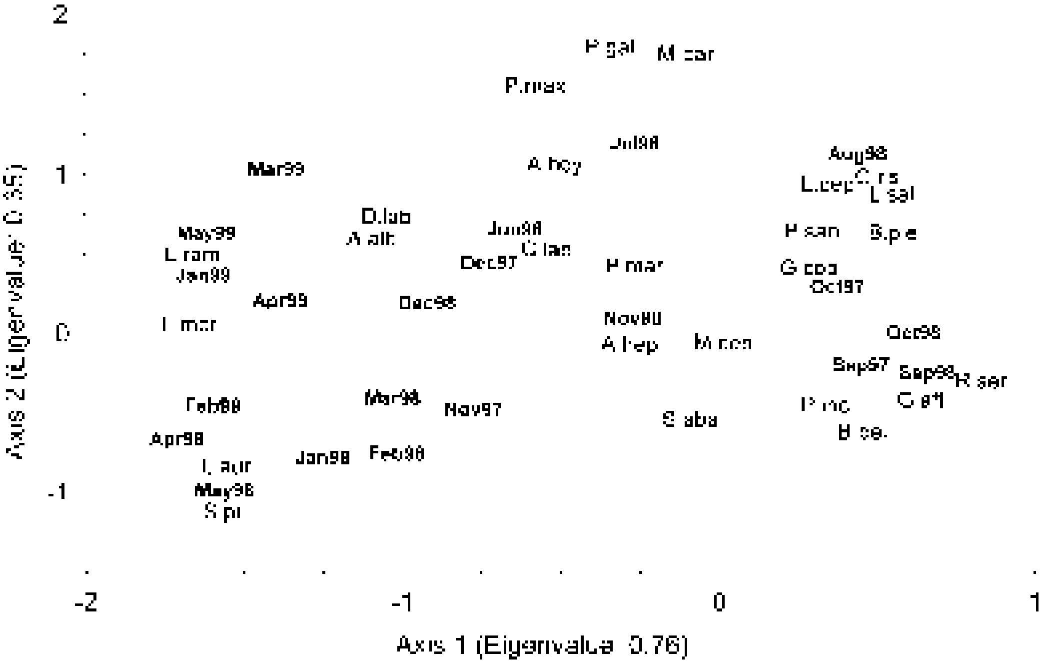

Fig. 5. – Ordination of monthly samples and fish species on the plane of the two first axes of Correspondence Analysis (A.alb: *Alburnus alburnus*, A.boy: *Atherina boyeri*, A.hep: *Atherina hepsetus*, B.ple: *Barbus plebejus*, B.bel: *Belone belone*, C.ris: *Callionymous risso*, C.lab: *Chelon labrosus*, D.lab: *Dicentrarchus labrax*, G.aff: *Gambusia affinis*, G.cob: *Gobius cobitis*, L.cep: *Leuciscus cephalus,* L.mor: *Lithognathus mormyrus,* L.aur: *Liza aurata,* L.ram: *Liza ramada,* L.sal: *Liza saliens,* M.cep: *Mugil cephalus,* M.bar: *Mullus barbatus,* P.gat: *Parablennius gattorugine,* P.san: *Parablennius sanguinolentus,* P.inc: *Parablennius incognitus,* P.mar: *Pomatoschistus marmoratus,* R.ser: *Rhodeus sericeus,* S.pil: *Sardina pilhardus,* S.aba: *Syngnathus abaster,* P.max: *Psetta maxima*).

position changed evenly from autumn to winter and spring while there is a more abrupt transition from spring to summer. Indeed, the ordination of November and December of both years in the middle of the plot indicates that the community displays intermediate faunistic characteristics during these months, i.e. species composition is similar to that of both autumn and winter.

DISCUSSION

Our results showed that the fish fauna of the Rihios system displayed higher values of relative abundance, compared with those of the Strymon system. This is partly due to the smaller sampling area in the Rihios River, a fact that may render the catch of certain species easier. Moreover, it is known that in estuarine systems, water temperature often reaches extreme values because of the shallow waters. This is true for the Strymon estuarine system, but it does not hold for the Rihios estuary, where the high and continuous freshwater flow is responsible for the absence of temperature extremes. However, species richness was generally higher in the Strymon especially during spring. Indeed, shallow waters, as is the case with the Strymon estuary and lagoon, warm up sooner in the season, thus attracting more species (Breber, 1996). The species richness observed in Strymon estuarine system (43 species) was, as far as we know, the highest recorded in the Mediterranean. Thirty-five species were recorded in the Porto-Lagos lagoon (KOUTRAKIS et al., in press), 29 in Rihios estuarine system (present study), 28 in Po estuary (VITALI & BRAGHIERI, 1981) and 16 species in Gialova lagoon (DOUNAS & KOUTSOUBAS, 1996). Only Severn Estuary in UK showed a remarkable species richness of 97 species (CLARIDGE et al., 1986).

Species richness and total relative abundance at the two study sites depended strongly on water temperature fluctuations, increasing during the warm period of the year. Similar results were also reported by WHITFIELD (1980) and LONERAGAN et al. (1987). The negative correlation between species richness and dissolved oxygen found in this study is probably of no biological meaning and it can be attributed to the strong dependence of oxygen concentration on the water temperature. Thus, any positive effect of dissolved oxygen on species richness and abundance is masked by the stronger effect of water temperature.

According to the results of Correspondence analysis, seasonality seemed also to determine the temporal distribution of the fish species. The abrupt change of species composition from spring to summer is probably because *L. ramada* and *L. aurata*, which are the dominant species in winter and spring, even though they are 'estuary-depended' species (DANDO, 1984), disappear from early summer samples. As these species grow larger than 70-80 mm they become faster and hence their collection with a bag seine becomes very difficult. Moreover, during the same period, juveniles of *L. mormyrus* and *S. pilchardus* leave the estuary and migrate towards the sea. On the other hand, *P. marmoratus*, which dominates the community of the Strymon system, spends its entire life cycle in the estuary under euryhaline conditions and is characterised as a 'true' estuarine fish according to DANDO (1984). *P. marmoratus* was also the second most abundant species in Porto-Lagos lagoon (12.7 % of the total catch) present in almost the whole study period (KOUTRAKIS et al., in press) and among the 15 most abundant fish species in the Severn Estuary (CLARIDGE et al., 1986). Likewise *C. labrosus* individuals cannot live in wholly freshwater habitats for long periods but are better adapted in poly and mesohaline waters (LASSERRE & GALLIS, 1975), as is the case with stations Str2 and Rih4 (Fig. 1). KOUTRAKIS et al. (in press) observed similar behaviour in the Porto-Lagos lagoon where *C. labrosus* was present during the whole study period (20 months of presence in a 21 month survey).

The importance of the studied estuarine systems as nursery grounds is supported by the presence of young individuals of several species such *as L. mormyrus*, *S. pilchardus* etc. Apart from the commercially important fish species that inhabit estuaries, the estuarine fish fauna has also a significant ecological value for the fish-eating birds that live and feed in estuarine systems. More specifically it has been reported (GOUTNER et al., 1997) that species of the families Gobiidae and Mugilidae were the dominant prey of the great cormorant (*Phalacrocorax carbo*). Moreover, some of the species found are either protected by the 92/43 EEC Directive and the Bern Convention (*R. sericeus*) or referred to the Red Data Book of Threatened Vertebrates of Greece (*Alburnus alburnus*, *L. cephalus*, *Vimba melanops*, *K. caucasica*). Therefore, it is necessary to obtain more information on their distribution and abundance in order to be able to provide more efficient means of protection.

ACKNOWLEDGEMENTS

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Biochemical systematics and patterns of genetic divergence between the *Troglophilus* species of Crete and Rhodos (Orthoptera, Rhaphidophoridae)

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ABSTRACT. Four populations of *Troglophilus spinulosus* from Crete, one population of *T. lagoi* from Rhodos and one population of *Dolichopoda paraskevi* from Crete have been studied for genetic variation at 19 enzymatic loci by electrophoresis. Three different levels of genetic divergence emerged: mean Nei's genetic distance was 0.025 among *T. spinulosus* populations, 0.347 between *T. spinulosus* and *T. lagoi* and 0.837-0.918 between *D. paraskevi*-*T. spinulosus* and *D. paraskevi*-*T. lagoi* respectively. Relationships among *T. spinulosus* populations were studied by Principal Component Analysis in order to clarify their debated taxonomic position; relationships among all study populations were assessed by two different methods of tree reconstruction, Maximum Likelihood and Neighbor-Joining. The low degree of genetic differentiation among *T. spinulosus* populations and the different data analyses all indicate that this is the only *Troglophilus* species of Crete.

KEY WORDS: *Troglophilus*, electrophoresis, genetic distance, biochemical systematics.

INTRODUCTION

Rhaphidophoridae is an Orthopteran family including, in the Mediterranean area, only two genera, *Troglophilus* and *Dolichopoda*, both colonising cave environments. In this paper we report data on the levels of genetic differentiation and genetic variability in five populations of the cave crickets belonging to the genus *Troglophilus*. In particular, we have studied two species, *T. spinulosus* Chopard, 1921 and *T. lagoi* Menozzi, 1934 endemic of Crete and Rhodos islands respectively. The cavernicolous Orthopteran fauna of Greece, in spite of its biogeographic interest, is relatively unknown, mainly because species identification has been often based on the study of few individuals and in some cases subadults (WILLEMSE, 1985; KOLLAROS et al., 1991).

Particularly debated have been the systematics of the *Troglophilus* of Crete: *T. spinulosus* was described by Chopard (1921). Throughout the years two other species have been recognised *T. roeweri,* Werner, 1927 and *T. petrochilosi,* Boudou-Saltet, 1978. The validity of these species has been often questioned (KOLLAROS et al., 1987); finally KOLLAROS et al. (1991) studying 30 morphological characters were not able to obtain a pattern of discrimination that could account for the presence of three species in Crete, leading them to conclude that *T. spinulosus* is the only species present on this island.

Allozyme markers have proved to be a powerful tool in analysing levels of genetic affinities; we used this approach to inquire into the taxonomic status of the *Troglophilus* of Crete. Moreover we studied a population of *T. lagoi* for evaluating the degree of genetic divergence between morphologically distinct species within the genus and a population of *Dolichopoda paraskevi* Boudou-Saltet, 1973 from Crete to assess the degree of genetic divergence within the family.

MATERIAL AND METHODS

Sampling

For each collecting site we report the cave name and location, the collecting period, the number (N) of study samples and the population symbol. The *Troglophilus* populations of Crete were sampled in the following four caves: Katholiko cave, Khania peninsula, Western Crete, VII-1995, N=14 (SPR); Doxa cave, Iraklion, Central Crete, VII-1995, N=5 (SPS); Milatos cave, Aghios Nikolaos,

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Eastern Crete, IV-1994, N=3 (SPP1); Mikro Katafygi cave, Sitia, Eastern Crete, VII-1995, N=12 (SPP2). The population of *T. lagoi* of Rhodos was from the Tolomeo cave, Rodino, VII-1996, N=19 (LAG); the population of *D. paraskevi* of Crete was from the Atzigano cave, Aghios Nikolaos, Eastern Crete, VII-95, N=3 (PAR). The samples were transported alive to the laboratory and frozen at -80°C. Sampling localities are shown in Fig. 1.

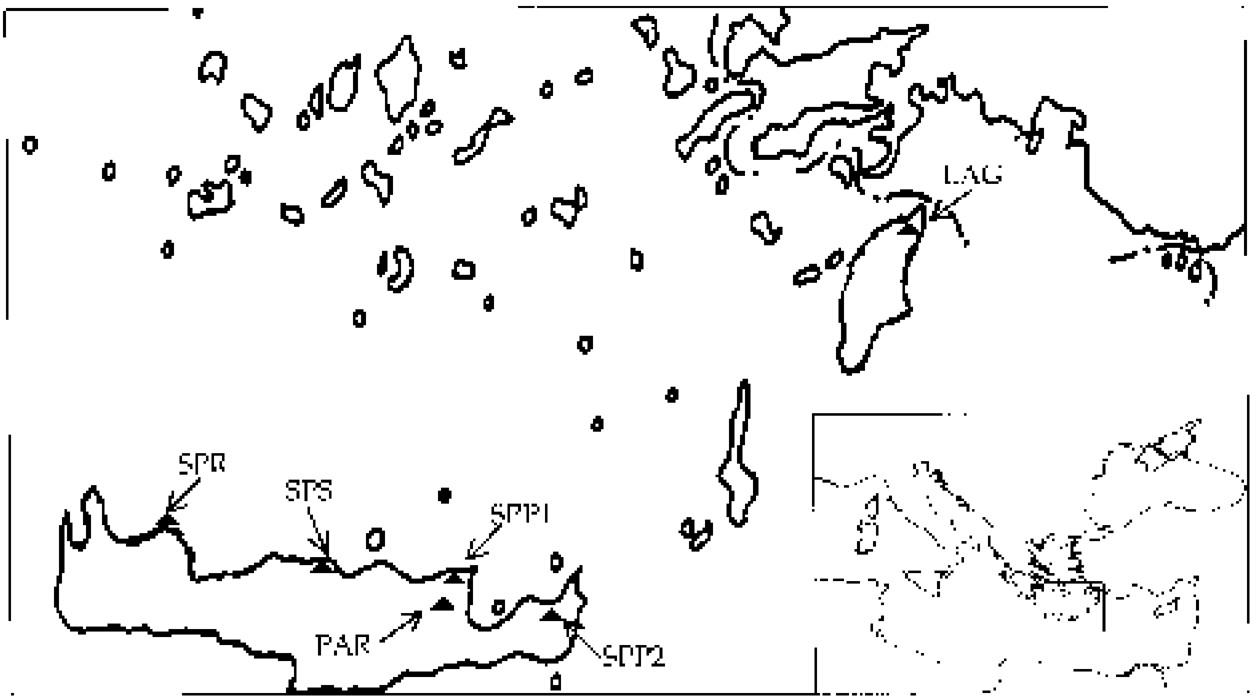

Fig.1. – Sampling locations of study population, for symbols see Materials and Methods.

Electrophoresis

Horizontal electrophoresis was performed on 12% starch gel by using crude homogenates of caudal femor muscle. Fourteen enzymatic proteins were investigated, encoded by 19 loci, namely: Acid phosphatase (EC 3.1.3.2; *Acph*); a- Amylase (EC 3.2.1.1; *a- Amy*); Aldeyde oxidase (EC 1.2.3.1; *Ao-1, Ao-2*); Carbonic anhydrase (EC 4.2.1.1; *Ca-1, Ca-2, Ca-3*); Creatine kinase (EC 2.7.3.2; *Ck*); Esterase (EC 3.1.1.1; *Est-1, Est-2*); Exochinase (EC 2.7.1.1; *Hk*); Isocitrate dehydrogenase (EC 1.1.1.42; *Idh*); Lactate dehydrogenase (EC 1.1.1.27; *Ldh*); Mannose phosphate isomerase (EC 5.3.1.8; *Mpi*); Peptidase (EC 3.4.11; *Pep-1, Pep-2*); Phosphoglucomutase (EC 2.7.5.1; *Pgm*); Phosphohexose isomerase (EC 5.3.1.9; *Phi*); Tetrazolium oxidase (EC 1.15.1.1; *To*). Details for buffer systems and staining procedures are reported in CHRISTENSEN (1977), DE MATTHAEIS et al. (1998) and KETMAIER et al. (1998).

Statistical analysis

From electrophoretic data, allele frequencies, variability parameters (H_e= expected mean heterozygosity under Hardy-Weinberg equilibrium; H_o= observed mean heterozygosity; P= proportion of polymorphic loci according to the 5% criterion; A= mean number of alleles per locus) and genetic distances (D; Nei, 1978) were calculated.

Principal Component Analysis (PCA) was performed on multilocus genotype profiles to investigate relationships among *Troglophilus* populations from Crete. Genetic heterogeneity among these populations was investigated by means of F-statistics: we calculated θ values (θ corresponds to F_{ST} in Wright's notation) for pairs of populations, according to WEIR & COCKERHAM (1984). From θ values indirect estimates of gene flow were obtained, by using the relation Nm = 1/4 $[(1/F_{ST})-1]$ (WRIGHT, 1965). Relationships among all study populations were assessed by two different methods of tree reconstructions: Maximum Likelihood (FELSENSTEIN, 1993; ML) and Neighbor-Joining (SAITOU & NEI, 1987; NJ); trees were rooted by using PAR as outgroup. Robustness of the obtained trees was tested by the bootstrap method (FELSENSTEIN, 1985) with 1000 replications.

The programs BIOSYS-1 (SWOFFORD & SELANDER, 1981), PHYLIP 3.5 (FELSENSTEIN, 1993), FSTAT (GOUDET, 1995) and STATISTICA for Windows were employed for data analyses.

RESULTS

Six loci were polymorphic in one population at least (*Ao-1*; *Ao-2*; *Idh*; *Mpi*; *Pep-2*; *Pgm*), six loci were monomorphic and fixed for the same allele in all study populations (*a- Amy*; *Est-1*; *Est-2*; *Ldh*; *Phi*; *To*), while the remaining seven loci showed fixed alternative alleles between groups of populations (*Acph*; *Ca-1*; *Ca-2*; *Ca-3*; *Ck*; *Hk*; *Pep-1*); no fixed alternative alleles were detected within the Crete populations. Allele frequencies are reported in Table 1, which also shows variability estimates for all study populations. The most polymorphic population was LAG (H_o = 0.055); on the contrary SPS and PAR showed no genetic variation. D (Nei, 1978) values are reported in Table 2. Three levels of genetic differentiation were highlighted. D ranged from 0.805 to 0.918 comparing different genera (*Troglophilus* vs *Dolichopoda*), from 0.346 to 0.399 comparing two *Troglophilus* species (*lagoi* vs *spinulosus*), finally D varied from 0.006 to 0.041 among Crete populations. Within these populations SPP1 was the most differentiated in terms of D values (D_{mean}= 0.038). PCA produced a somewhat similar result, emphasising also the separation of SPP2 (Fig. 2); the cumulative proportion of total variance for Factor 1 and Factor 2 was 0.704. Crete populations proved to be genetically structured, Nm not exceeding 0.825 (Table 2), indicating a general reduction of gene flow. ML and NJ analyses produced trees with identical topologies and very robust in terms of bootstrap values (Fig. 3).

TABLE 1

Allele frequencies and variability estimates in 5 *Troglophilus* and 1 *Dolichopoda* populations at 19 loci.

Loci/Pop.	**SPS**	**SPP1**	**SPP2**	**SPR**	**LAG**	**PAR**
Acph						
A	0.000	0.000	0.000	0.000	1.000	1.000
B	1.000	1.000	1.000	1.000	0.000	0.000
a-Amy						
A	1.000	1.000	1.000	1.000	1.000	1.000
Ao-1						
A	0.000	0.000	0.000	0.167	0.447	1.000
B	1.000	1.000	1.000	0.833	0.553	0.000
Ao-2						
A	0.000	0.000	0.000	0.000	0.000	1.000
B	0.000	0.000	0.000	0.417	0.000	0.000
C	1.000	1.000	1.000	0.583	1.000	0.000
Ca-1						
A	0.000	0.000	0.000	0.000	0.000	1.000
B	1.000	1.000	1.000	1.000	1.000	0.000
Ca-2						
A	1.000	1.000	1.000	1.000	0.000	1.000
B	0.000	0.000	0.000	0.000	1.000	0.000
Ca-3						
A	1.000	1.000	1.000	1.000	1.000	0.000
B	0.000	0.000	0.000	0.000	0.000	1.000
Ck						
A	0.000	0.000	0.000	0.000	0.000	1.000
B	0.000	0.000	0.000	0.000	1.000	0.000
C	1.000	1.000	1.000	1.000	0.000	0.000
Est-1						
A	1.000	1.000	1.000	1.000	1.000	1.000
Est-2						
A	1.000	1.000	1.000	1.000	1.000	1.000
Hk						
A	0.000	0.000	0.000	0.000	1.000	0.000
B	1.000	1.000	1.000	1.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	1.000
Idh						
A	0.000	0.667	0.000	0.250	0.000	0.000
B	1.000	0.333	1.000	0.750	1.000	0.000
C	0.000	0.000	0.000	0.000	0.000	1.000
Ldh						
A	1.000	1.000	1.000	1.000	1.000	1.000
Mpi						
A	0.000	0.667	0.278	0.000	0.214	0.000
B	1.000	0.333	0.722	1.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.786	0.000
D	0.000	0.000	0.000	0.000	0.000	1.000
Loci/Pop.	SPS	SPP1	SPP2	SPR	LAG	PAR
Pep-1						
A	0.000	0.000	0.000	0.000	0.000	1.000
B	1.000	1.000	1.000	1.000	1.000	0.000
Pep-2						
A	1.000	1.000	1.000	1.000	0.947	1.000
B	0.000	0.000	0.000	0.000	0.053	0.000
Pgm						
A	0.000	0.000	0.250	0.125	0.000	0.000
B	1.000	1.000	0.750	0.875	1.000	0.000
C	0.000	0.000	0.000	0.000	0.000	1.000
Phi						
A	1.000	1.000	1.000	1.000	1.000	1.000
To						
A	1.000	1.000	1.000	1.000	1.000	1.000
Variability estimates						
H_e^*	0.000	0.056	0.043	0.075	0.050	0.000
H_o	0.000	0.035	0.010	0.022	0.055	0.000
P^{**}	0.000	10.5	10.5	21.1	15.8	0.000
A	1.000	1.1	1.1	1.2	1.2	1.000

* *unbiased estimate (see Nei, 1978)*

** *a locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95*

TABLE 2

Above diagonal, genetic distance D (Nei, 1978) for all study populations; below diagonal θ (upper) and Nm (lower) values for *T. spinulosus* populations.

Pop.	**SPS**	**SPP1**	**SPP2**	**SPR**	**LAG**	**PAR**
SPS	*****	0.044	0.006	0.013	0.399	0.865
SPP1	0.586 0.176	*****	0.031	0.041	0.397	0.836
SPP2	0.543 0.210	0.237 0.804	*****	0.017	0.356	0.805
SPR	0.232 0.825	0.264 0.697	0.326 0.516	*****	0.346	0.843
LAG	- -	- -	- -	- -	*****	0.918
PAR	- -	- -	- -	- -	- -	*****

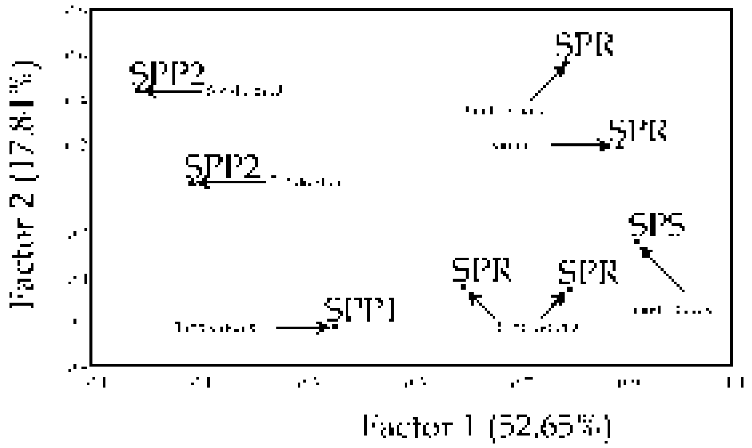

Fig. 2. – Two dimensional plot of multilocus genotype profiles based on Principal Component Analysis. Percentages refer to portion of the overall variance explained by each Factors.

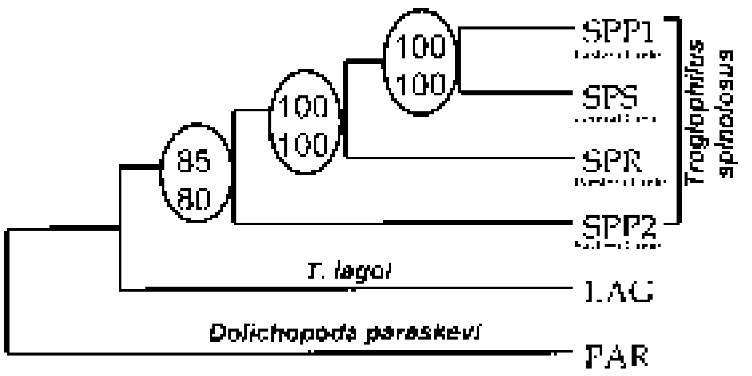

Fig. 3. – Majority rule consensus tree obtained by ML and NJ bootstrap analyses. Circled nodes include bootstrap percentages of 1000 replications for ML and NJ (first and second value, respectively). Bootstrap values are shown only for nodes for which the two phylogenetic methods had a bootstrap support of 70% or greater.

DISCUSSION

The allozyme data on *Troglophilus* populations from Crete indicate a low degree of genetic differentiation among them, the highest D value being 0.044 between SPS and SPP1. D values of this order of magnitude are frequently reported for comparisons among local populations belonging to the same species, either considering surface or cave organisms (Ayala, 1983; Sbordoni, 1982). Moreover, in the only other genetic study on *Troglophilus*, comparable D values are reported between conspecific populations of *T. cavicola* and *T. andreinii* respectively (Sbordoni et al., 1981). The patterns of relationships among the four Crete populations here studied, as assessed by PCA, ML and NJ analyses, always indicate a close affinity among SPS/ SPP1/ SPR, while SPP2 was relatively more isolated. It is interesting to note that SPP1 and SPP2 do not group together, SPS is linked to SPP1 while SPR and SPP2 are subsequently nested to them. Thus, it means that phyletic relationships among these populations do not match a geographic trend; this evidence is in disagreement with the pattern of species distribution proposed by Boudou-Saltet (1978). She stressed that, supporting the existence of three *Troglophilus* species in Crete, *T. roeweri*, *T. spinulosus* and *T. petrochilosi* should be distributed in Western, Central and Eastern Crete respectively. On the contrary, genetic data here presented strongly support the hypothesis of Kollaros et al. (1991) that *T. spinulosus* is the only species of *Troglophilus* in Crete. F- statistics analyses revealed a considerable degree of genetic structuring among *T. spinulosus* populations, θ values ranging from 0.232 (SPS vs SPR) to 0.586 (SPS vs SPP1), to which correspond Nm values of 0.825 and 0.176. Also analysing the levels of gene flow, it is not possible to recognise a clear geographic pattern, since Nm is higher between populations from Eastern and Western Crete than between populations from Central and Eastern Crete (Table 2). Anyhow, these data are just a rough indication of the degree of genetic structuring of *T. spinulosus*, due to the scattered distribution of study populations. Further research on a higher number of *T. spinulosus* populations is needed to understand the importance of the availability of routes for dispersal (in particular bioclimatic conditions outside the caves), hopefully considering both very close populations and more distant ones in order to calibrate rates of dispersal and to discriminate between present and historical gene flow.

The mean genetic distance between *T. spinulosus* and *T. lagoi* is 0.374, and the same value was found by Sbordoni et al. (1981) between *T. cavicola* and *T. andreinii*. If we use the equation proposed by Nei (1975) to date the beginning of independent evolution of *T. lagoi* and *T. spinulosus* we obtain an estimate of T= 1.870 Myr. This dating does not agree with the geological time estimates regarding the separation of Crete and Rhodos; according to Meulenkamp et al. (1972) Crete became isolated at the end of Messinian (about 5 Mya) and has remained isolated ever since. We propose the following explanations to account for these results: a) *T. lagoi* and *T. spinulosus* may not be sister species: only studying levels of genetic affinity among several other *Troglophilus* species such as the Anatolian ones could we resolve this point; b) if *T. lagoi* and *T. spinulosus* are actually sister species and their mutual separation is related to the breakdown of the connection between Crete and Rhodos, this probably means that the Nei's formula underestimates evolutionary times in this group of cave crickets and a calibration *ad hoc* is needed.

We are presently studying the degree of genetic differentiation among several *Troglophilus* species from

Anatolia, Greece and Italy in order to provide a more accurate evolutionary scenario for this genus.

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Vertical distribution of Collembola in deciduous forests under mediterranean climatic conditions

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ABSTRACT. This study was conducted in the deciduous forests in the delta of the river Nestos. Soil samples were taken in various seasons and were divided into three layers: O layer, 0-2.5 cm soil depth and 2.5-5 cm soil depth. The Collembola caught were counted and identified and the biomass was determined. Soil moisture and temperature were also recorded.

In most seasons the majority of the animals was found in the O and the upper soil layer. Under adverse conditions in summer (low soil humidity) a vertical migration to deeper layers was observed. No similar response was observed in winter. These patterns persist at the level of the whole Collembola population and at the species level as well. Only minute differences, if any, were observed in the vertical distribution pattern of the most abundant species, irrespectively of the life form they belong to. Generally a preference of smaller animals for the deeper layers was observed.

KEY WORDS: Collembola, vertical distribution, life forms, body size.

INTRODUCTION

A number of studies state that the majority of Collembola live in the few upper centimeters of the soil habitat, notably in the litter and humus layer (TAKEDA, 1978; HÅGVAR, 1983; WOLTERS, 1983; BERG et al., 1998). The migration of Collembola to deeper layers is a well known avoidance behaviour, which is attributed to different factors according to the climatic characteristics of the areas where the different studies were conducted (HOPKIN, 1997).

The classification of Collembola to ecomorphological life forms was introduced by GISIN (1943) and has been widely used ever since. Based on morphological characteristics, such as presence and number of eyes, pigmentation, length of the legs and the furca, species are characterised as euedaphic (species that are permanent soil dwellers), hemiedaphic (species that that live in the superficial soil layers and leaf litter) and atmobios (species that live in the surface and on vegetation). It was originally assumed that the morphological characteristics of a species reflect the soil layer preferred by it, but certain species have a life strategy that does not correspond to their life form (HÅGVAR, 1983, TAKEDA, 1995). On the other hand, a differentiation in the vertical preferences within a species has been reported (WOLTERS, 1983) indicating that smaller individuals tend to inhabit deeper layers.

In this study the following questions were addressed: are there vertical preferences for Collembola and under what circumstances does an avoidance behaviour occur? Are there species specific preferences and are they related to life forms? Does an intraspecific differentiation in vertical preferences occur that can be related to body size?

MATERIAL AND METHODS

Site description

The investigation site is located in north-eastern Greece, in the western part of the delta of the river Nestos (24° 43′ N, 40° 53′ E). The climate in the area is of the subcool-subhumid Mediterranean type, as defined by NAHAL (1981). Four types of deciduous forests can be distinguished in the area: a. Hardwood riparian forest, dom-

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inated *by Quercus pedunculiflora*, *Populus alba*, *Fraxinus angustifolia* and *Fraxinus oxycarpa*. b. Softwood-softwood riparian forest, dominated by *Populus alba*, *Salix alba*, *Alnus glutinosa* and *Ulmus minor*. c. Mixed stands without contact to the river, dominated by *Populus alba*, *Quercus pedunculiflora*, *Alnus glutinosa* and *Ulmus minor*. and d. Plantations of man-introduced hybrid poplars (*Populus canadensis*) plantations and d. mixed stands without contact to the river. All the stands grow on alluvial soil.

Sampling

The sites were sampled in July 1991, April 1992, January, April, August, September and November 1993, except for the softwood forest, which was sampled only in April and November 1993. In each sampling occasion 7 samples were taken from each forest type. The sampling design was stratified random. The samples consisted of the O-layer and a soil core 5 cm in diameter and 5 cm deep. Each sample was divided into three layers: the O layer, a layer 0-2.5 cm soil depth and 2.5-5 cm soil depth. The animals from each layer were extracted by means of a Berlese-Tullgren funnel.

Collembola animals were identified and counted and their body lengths measured. The biomass was calculated using the equations given by Tanaka (1970); Petersen (1975) and Teuben & Smidt (1992). When no equation was given for a certain species the one given for the taxonomically closest one was used (Wolters, 1983).

Soil temperature was measured immediately after each sample was taken at 5 cm depth with a digital point thermometer. Soil samples adjacent to those used for the extraction of the animals the ones mentioned above were taken to the laboratory and dried overnight at 103°C to determine water content. Biomass, soil temperature and soil water content were not determined in July 1991.

Statistics

Since the samples of the three layers resulted from the splitting of single soil cores, the differences in population densities in the different depths were tested with the Wilcoxon signed-rank test for tied samples (Sachs, 1972). Data from all samples were pooled for the purpose of this analysis.

The same procedure was undertaken with the data from the most abundant species to check whether the patterns observed reflect a tendency of the whole Collembola population or if there are species specific strategies. The results for four species that exhibit distinct seasonal strategies and belong to different families and life forms are presented here to illustrate the findings. Three of the species are classified as hemiedaphic (*Isotoma notabilis* Schäfer, 1896, *Cryptopygus thermophilus* Axelson, 1900 and *Ceratophysella engadinensis* Gisin, 1949) and one as euedaphic (*Protaphorura sp.*). The differences between mean individual biomass at different depths were tested for significance with the U-test.

Results

Soil water content and temperature data for the different sampling occasions are presented in Table 1. When the whole Collembola population is considered, most of the

TABLE 1

Mean soil water content and temperature ± Standard Deviation and Mean abundance per sample ± Standard Deviation of *Collembola* in the different layers. Significance levels (Wilcoxon-Test) *: p < 0.05, **: p < 0.01, ***: p < 0.001. Asterisks in the 2.5–5 cm and 0-2.5 cm columns concern differences between these layers and the 0-2.5 cm and O layers respectively. Asterisks in the O layer columns concern differences between this layer and the 2.5–5 cm one.

	Soil water content (%)	Soil temperature (°C)	Individuals			Biomass (µg)		
			2.5-5 cm soil	0-2.5 cm soil	O layer	2.5-5 cm soil	0-2.5 cm soil	O layer
July 91	-	-	20±31 **	27±37	42±85	-	-	-
April 92	32,3±12.5	15,5±3,6	19±32 ***	56±82	32±41 *	23,4±48,2 **	50,7±64,9	54,2±66,7 *
Jan. 93	33,3±16,6	2,4±1,6	14±23 **	27±29	32±44 **	27,9±43,8 *	55,1±66,1	77,5±84,6 ***
April 93	32,1±12,0	10,6±3,4	15±27 ***	56±76	46±57 ***	16,6±23,7 **	68,0±90,0	86,8±110,0 ***
Aug. 93	7,3±6,6	24,8±5,7	40±68	10±10 **	2±3 ***	41,2±70,0	21,1±22,7	6,3±10,7 ***
Sept. 93	21,1±20,0	19,0±1,2	17±22	29±27	49±53 *	16,0±17,8	43,1±50,5	91,3±137,8 **
Nov. 93	28,1±15,8	5,8±2,9	8±13 ***	27±26	29±27 ***	16,7±35,0 **	50,8±46,1	112,1±170,4 ***

TABLE 2

Mean abundance per sample ± Standard Deviation of four Collembola species in the different layers. Significance levels (Wilcoxon-Test) *: $p < 0.05$, **: $p < 0.01$. Asterisks in the 2.5–5 cm and 0-2.5 cm columns concern differences between these layers and the 0-2.5 cm and O layers respectively. Asterisks in the O layer columns concern differences between this layer and the 2.5–5 cm one.

	Isotoma notabilis			***Cryptopygus thermophilus***			***Ceratophysella engadinensis***			***Protaphorura sp.***		
	2.5-5 cm soil	**0-2.5 cm soil**	**O layer**	**2.5-5 cm soil**	**0-2.5l cm soil**	**O layer**	**2.5-5 cm soil**	**0-2.5l cm soil**	**O layer**	**2.5-5 cm soil**	**0-2.5 cm soil**	**O layer**
July 91	0±1 *	3±3	11±22 **	0±1	6±9	49±87	8±10	5±6	2±2	1±1	4±1	10±7
April 92	3±5 **	13±12	11±16 *	0	7±0	0	1±2	2±1	4±4	9±12	5±5	2±2
Jan. 93	4±8 **	9±13	8±12	3±1	18±8	8±7	1±2	3±3	6±8	4±6	8±8	9±12 *
April 93	5±10 **	16±22	12±11 **	7±9	21±13	44±48	8±14	34±56	17±51	2±5	8±9	12±24
Aug. 93	9±17 *	2±7	0±1 *	83±133	1±2	0	1±1	1±1	0	2±2	1±2	1±1
Sept. 93	6±8	16±24 *	32±32	28±28	8±7	36±44	2±2	1±3	2±3	1±2	12±20	29±51
Nov. 93	4±11 **	8±10	9±18 *	2±3	27±35	7±10	0±1 *	2±1 *	9±9 *	2±4	7±7	7±12

individuals as well as the greatest biomass were found in the upper soil layer and in the O layer (Table 1). The differences between the deeper soil layer and the upper ones were in most cases statistically significant. Differences were found between the upper soil and the O layer too and in most cases the population in the O layer was larger. These differences were statistically not significant and much smaller than the ones observed in the deeper soil layer. A single exception was August 1993. In this case most of the animals were found in the deeper layer. Generally the proportion of the individuals found in the deeper layer is greater than the proportion of the biomass found in the same layer, which indicates that the deeper layer is preferred by the smaller animals.

The pattern of vertical distribution observed at the species level coincides with the one observed for the whole Collembola population (Table 2). In August 1993 all species, regardless of their life-form classification retreated to the deeper layer. During all other sampling occasions, all species were generally more abundant in the surface layers, once again regardless of their life-forms. The large standard deviation observed is largely due to differences in the population between samples, and not to deviations from the observed vertical patterns.

Differences in mean biomass of the individuals inhabiting different layers were in most cases statistically significant and show that, for all species studied here, body size decreases with increasing depth, regardless of life forms (Table 3). There are two important features of this pattern. Firstly, results are consistent for all species and that invariably mean biomass decreases with increasing depth. Secondly, large standard deviation also exists that can be partly attributed to the pooling of data from different seasons and partly to an existing overlap in depth preferences.

TABLE 3

Mean biomass of individuals (µg of dry weight) ± Standard Deviation. Significance levels (U-Test) *: $p < 0.05$, **: $p<0.01$, ***: $p < 0.001$. Asterisks in the 2.5–5 cm and 0-2.5 cm columns concern differences between these layers and the 0-2.5 cm and O layers respectively. Asterisks in the O layer columns concern differences between this layer and the 2.5-5 cm one.

Species	**2.5-5 cm soil**	**0-2.5 cm soil**	**O layer**
Isotoma notabilis	1.17±0.96	1.29±1.34 ***	1.51±1.42 ***
Cryptopygus thermophilus	0.94±0.51 ***	1.52±1.32	1.59±1.16 ***
Ceratophysella engadinensis	1.05±1.10	1.15±1.11 ***	1.39±1.30 ***
Protaphorura sp.	3.56±4.06	3.58±3.15 *	4.03±3.96 *

DISCUSSION

The majority of Collembola was found in the upper soil layer and in the O layer in all cases except in August 1993. It has been suggested that the maximum population density is observed there, where the maximum decomposition activity takes place (WALLWORK, 1970). HASSALL et al. (1986) suggested that the hyphal mass is more easily accessible to Collembola in the litter and fermentation layers. A preference might also exist for early colonising microorganisms. If changes in their habitat occur, Collembola can react quickly and change their vertical distribution within a few hours (Whitford et al., 1981; Hassall et al., 1986). Drought seems to be the factor that triggered the downward migration in August 1993 (Takeda, 1978; Hassall et al., 1986; Berg et al., 1998 but see Faber & Joosse, 1993).

In a comparative study Sgardelis et al. (1993) found that in Mediterranean ecosystems Collembola survived the summer drought period in an inactive state, which results in a population density close to zero. On the contrary in temperate ecosystems no significant population reduction was observed but rather a retreat of Collembola to deeper soil layers. The results of this study indicate that the Collembola population of the Nestos forests lies between these two extremes: a significant reduction in the population density is observed (although without coming close to zero) as well as a retreat to deeper layers.

In the two winter sampling occasions in January and November 1993 most animals were found in the surface layers. The air temperatures during sampling in November and January 1993 were much lower than the mean air temperature for these months and the long term average; in the latter case the soil was even partly covered by snow. Usher (1970) observed a downward migration in winter that was attributed to low temperatures. The lowest temperatures that occurred in the area during this study should present no substantial problem for Collembola.

All four species studied show the same pattern as the whole population. No vertical specialisation was observed. At the most, there was a slight tendency of *Ceratophysella engadinensis* and *Protaphorura sp.* to live somewhat deeper than the rest. In other studies too, only minor differences on the scale of millimetres were found in the "mean depth" for a number of species. They were so minor that a large overlap always existed (Usher, 1970; Faber & Joosse, 1993). Hågvar (1983) also observed a great overlap and a periodical appearance of almost all species in the higher layers.

Although according to its morphological characteristics *Protaphorura sp.* is classified as euedaphic, there were only minor differences in its vertical distribution compared to the other species. It was already observed in other studies (Hågvar, 1983) that some Collembola species, which are classified as euedaphic, do prefer the surface soil layers. Takeda (1978; 1995) observed that species with different life forms reach their maximum abundance level in the same depth. *Protaphorura sp.* is a large species, which is a common feature of species that prefer the upper part of the soil profile (Takeda, 1978; Hågvar, 1983). All the above do not suggest that species with euedaphic life forms generally prefer the surface soil layers, but that some of them do, and that life form is not necessarily restrictive in this context.

A general tendency of the smaller individuals of the same species to live deeper was observed (Wolters, 1983). Therefore, there are two possible explanations. The first is directly related to body size: the larger animals cannot find enough space in the deeper layers and stay in the surface layers where crevices are bigger (Hågvar 1983), whereas smaller animals, being more sensitive to desiccation have to stay deeper. The second explanation is that the eggs are laid in deeper layers and so the proportion of juveniles there is larger (Dunger, 1983). It seems that, apart from the drought avoidance behavior, body size is the decisive factor controlling vertical distribution. This applies to the whole Collembola population and to the populations of the different species as well.

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Benthic communities of the inner Argolikos Bay

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ABSTRACT. Benthic assemblages of the inner Argolikos Bay were investigated as an indication of the quality of the marine environment of the area. Samples were taken with a Ponar grab at 5 and 10 m. The assemblages found corresponded to two communities of the infralittoral (SVMC and SFBC) and one of the circalittoral (VTC). Communities of the two zones were clearly separated. The variety of substrata offered by the presence of plants is the most probable cause for the higher diversity found in the shallower stations. Although diversity is relatively high, the abundance of *Capitella capitata* and *Levinsenia gracilis* indicates that the shallow stations are moderately disturbed, probably due to organic loading.

KEY WORDS: Greece, benthic assemblages, sewage effluent, organic loading.

INTRODUCTION

Argolikos is an open and relatively shallow bay in the southeastern Peloponnese (Fig. 1). It receives the discharges of Argolikos basin, an area of intensive industrial and agricultural activities, as well as sewage from various towns along its shores. The discharge of mainly untreated effluent in the inner bay, in combination with its shallow depth, may have a significant effect on the quality of the marine environment, with severe ecological and financial consequences. The aim of the present study is to investigate the benthic assemblages of the inner Argolikos Bay, as an indication of the quality of the marine environment.

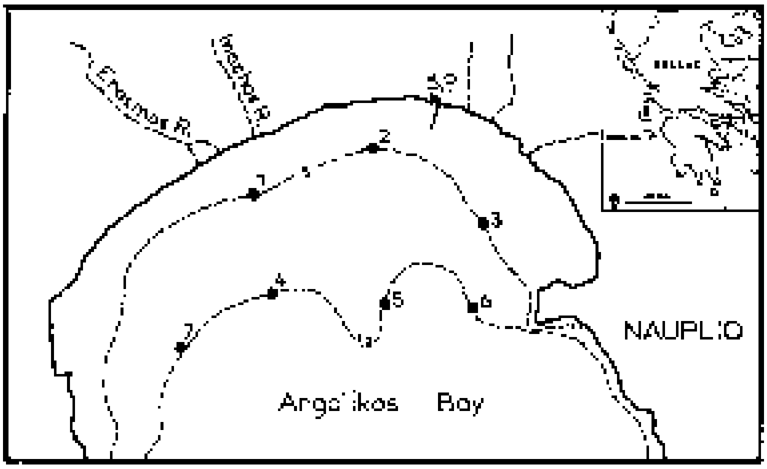

Fig. 1. – Argolikos Bay and the sampling stations

MATERIAL AND METHODS

To study the benthic community of Argolikos Bay, sampling took place in September 1998, a year after the operation of the sewage treatment plant of four main cities of the Argolikos basin, Argos, Nauplio, Nea Kios and Medeia. Three stations were chosen at 5 m (station 1 near the mouth of rivers Inachos and Erasinos, station 2 near the sewage pipe and station 3 on the course of the effluent according to the prevailing currents) and four at 10 m (stations 4, 5, 6 and 7). Three replicate samples were taken at each station using a Ponar grab, which collects 0.05 m^2 of substrate. Each sample was sieved through a 1 mm sieve, fixed in 4% formalin and dyed with Rose Bengal. To assess plant coverage, plants were removed from the samples, washed and then dried to a constant weight at 60° C.

For the mathematical analysis of the samples the Shannon-Wiener Diversity Index (SHANNON & WEAVER, 1968) and the Evenness Index (PIELOU, 1969) were calcu-

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lated. Clustering was calculated with the Bray-Curtis Similarity Index, after a log(x+1) transformation of the data. Classification was performed with the "group average" method and ordination with non-metric Multi-Dimensional Scaling (MDS). To assess differences in ecological parameters (N, S, H' and J) between stations at 5 m and 10 m, a one-way Analysis of Variance (ANOVA) was run between the values of the parameters of stations 1, 2 and 3 at 5 m and 4, 6, and 7 at 10 m. Values of N and S were log transformed to normality [log(x+1)].

RESULTS

The sediment in all sampling stations was mainly muddy. At 10 m (stations 4, 5, 6, 7) shell debris was found deeper within the sediment, while at 5 m (stations 1, 2, 3) there was a distinctive presence of the green algae *Caulerpa prolifera*. Plant biomass was higher at station 2, while at station 3 apart from whole plants there was also a high concentration of plant debris – mainly from *Caulerpa* and possibly from *Posidonia oceanica* – that reached more than 26 gr/0.05 m^2 in sample 3a.

A total of 3062 animals were counted, belonging to 151 species. Polychaetes were the dominant phylum (57 % of all taxa), while 16 % were mollusks, 14 % crustaceans, 3% echinoderms and 10% of all taxa belonged to minor phyla. Total abundance (N) and number of species (S) for each station are shown in Fig. 2, while Diversity (H') and Evenness (J) indices for each station are shown in Fig. 3. Station 3 had the highest number of species and individuals and the highest Diversity Index. High diversity was also found at station 5, which should be attributed not so much to its high species number as to its high Evenness. The lowest number of species was observed at station 6, which also had the lowest Diversity (Figs 2-3).

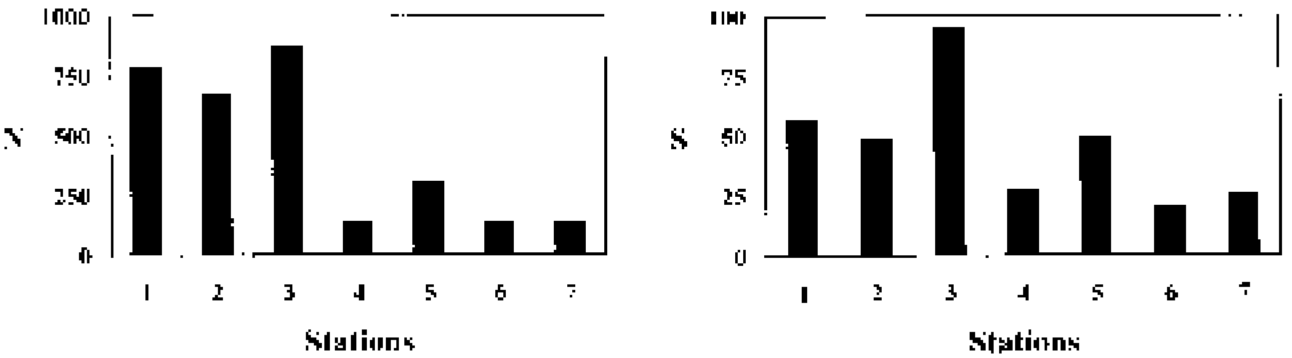

Fig. 2. – Total abundance (N) and total number of species (S) for each sampling station

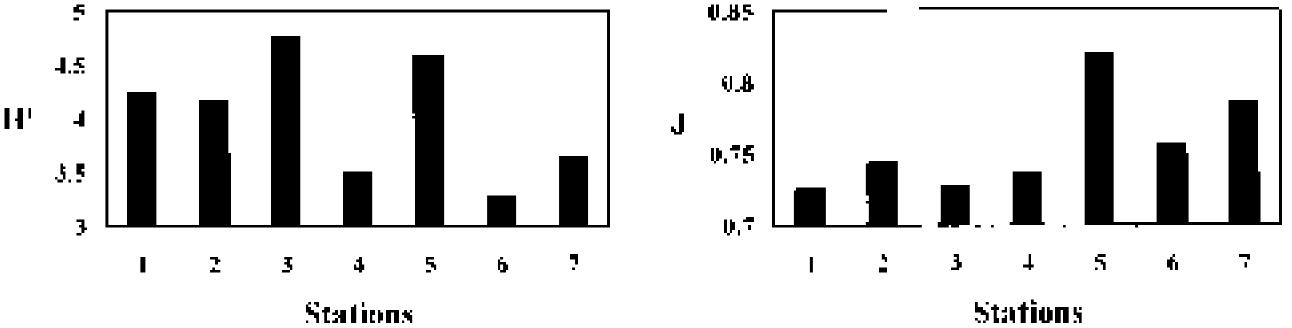

Fig. 3. – Diversity (H') and Evenness (J) Indices for each sampling station

Many of the species identified characterise Mediterranean benthic communities, as these were described by Peres (1967). In the Infralittoral, the bivalve *Loripes lacteus* and the polychaete *Paradoneis lyra*, abundant in stations 1, 2 and 3, are characteristic of the Biocoenosis of Muddy Sand in Sheltered Areas (SVMC). The bivalves *Tellina pulchella* and *T. fabula* and the polychaete *Prionospio malmgreni*, found in relative abundance in stations 1, 2, 3 and 5, are characteristic of the Biocoenosis of Fine, Well Sorted Sand (SFBC). In stations 4, 6 and 7 we observed the polychaetes *Nephthys hystricis* and *Sternaspis scutata*, the bivalve *Abra nitida* and the crab *Goneplax rhomboides*, which are characteristic of the Biocoenosis of Terrigenous Mud (VTC) of the Circalittoral Zone.

The polychaete *Capitella capitata*, which indicates organic enrichment and/or environmental disturbance (Peres, 1967; Pearson & Rosenberg, 1978), was abundant in stations 1, 2, 3 and 5. Its highest number (108 per 0.15 m^2) was observed in station 2 and its lowest (30 per 0.15 m^2) in station 5. The ascidian *Clavellina lepadiformis*, a species of hard substrata, which also appears in eutrophic areas (Tursi, 1980), was also found in stations 1 to 3. Further, the polychaete *Levinsenia gracilis*, which is also

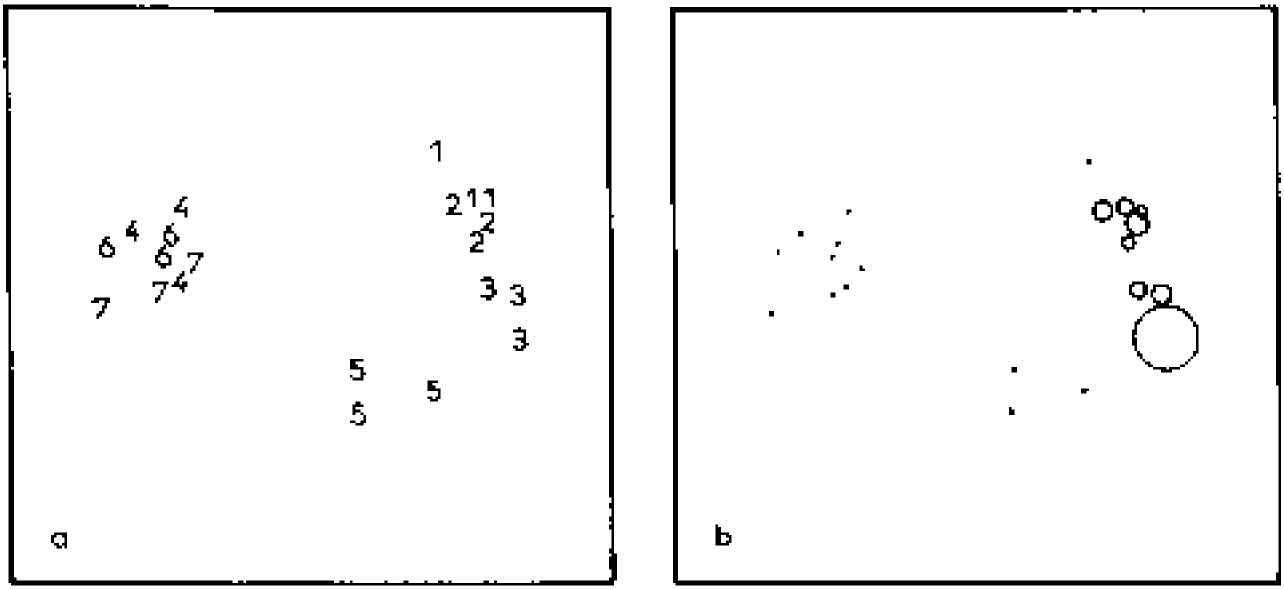

Fig. 4. – a.Ordination of samples with Multi-Dimensional Scaling (MDS). b. Schematic representation of dried plant biomass superimposed on the MDS results.

considered resistant to most pollutants (Chang *et al*., 1992), was found in relative abundance in stations 2 and 3.

Ordination of the samples (Fig. 4a) showed two groups according to depth: one with stations 4, 6 and 7 (10 m depth) and one with stations 1 to 3 (5 m depth). The three samples of station 5 were placed in between but closer to the 5 m stations although taken at 10 m. The dried plant biomass superimposed on the MDS results (Fig. 4b), shows that the presence of plants is most probably the factor separating stations 1 to 3 from the rest.

Comparing the ecological parameters between stations 1, 2, 3 (5 m) and 4, 6, 7 (10 m) showed significant differences in all but Evenness (Table 1). Thus, the number of species (S), abundance (N) and Diversity Index (H’) were significantly higher for stations taken at 5 m (1-3), while Evenness Index was marginally lower for stations taken at 10 m (4, 6, 7).

TABLE 1

One-way ANOVA results between stations of 5 and 10 m for the parameters S, N, H’ and J

	R_a^2	F	df	p	5 m mean ± s.d.	10 m mean ± s.d.
S	0.971	34.10	1, 16	0.000	35.444 ± 13.380	13.667 ± 2.828
N	0.978	44.61	1, 16	0.000	211.33 ± 101.59	46.00 ± 14.12
H’	0.943	16.45	1, 16	0.001	3.877 ± 0.516	3.097 ± 0.259
J	0.834	5.03	1, 16	0.039	0.768 ± 0.062	0.829 ± 0.053

DISCUSSION

Results of the present study show that the benthic fauna of inner Argolikos Bay does not correspond to only one of the communities described by Peres (1967) for the Mediterranean. In stations 1 to 3 there are species that characterise two communities of the Infralittoral Zone, that of Muddy Sand in Sheltered Areas (SVMC) and that of Fine, Well Sorted Sand (SFBC). In this area we also find species characteristic of disturbance and/or eutrophication. In stations 4, 6 and 7 we find characteristic species of the Biocoenosis of Terrigenous Mud (VTC) of the Circalittoral Zone. Both classification and ordination, as well as the ecological parameters N, S, H’ and J show that there are two distinctly separate benthic assemblages, one at 5 m and one at 10 m.

It is interesting to note that station 5, although it was taken at 10 m, is placed –in both clustering and the MDS-closer to the group of stations taken at 5 m, i.e. stations 1, 2 and 3. Further, the presence of species *Tellina fabula*, *T. pulchella* and *Prionospio malmgreni*, which are characteristic of the Biocoenosis of Fine, Well Sorted Sand (Peres, 1967) in relative abundance in station 5, also places it at the Infralittoral Zone. It is thus possible that station 5 represents an intermediate situation between the more sheltered inner stations 1, 2 and 3 at 5 m, and the more exposed outer stations 4, 6 and 7 at 10 m.

An examination of the values of N, S and H’ per sample shows that they are significantly higher for samples taken at 5 m, while Evenness J is slightly higher for samples at 10 m. The presence of *Caulerpa prolifera* and of a significant amount of dried plant biomass at 5 m appears to be responsible for the increased number of species and animals and the high Diversity. It is possible that the rhizomes of *Caulerpa* act as hard substrate for many organisms, as is proved by the presence of species like the ascidian *Clavellina lepadiformis* and various polychaetes of the family Syllidae that are characteristic of hard substrata (Tursi, 1980 and Simboura et al., 1995, respectively). This variety of available substrata results in an increase of microniches, and thus of Diversity at the shallower stations, while the homogeneity of the substrate at 10 m could be responsible for the lower Diversity and higher Evenness found at the deeper stations (Gray, 1974). Therefore, station 3, which had the highest amount of dried plant biomass (app. 40 gr.), showed the highest number of species and individuals, the highest Diversity and the lowest Evenness.

Although diversity is high, the abundance of polychaete species *Capitella capitata* and *Levinsenia gracilis* indicate that the shallow stations 1, 2 and, in a lesser degree, 3 show some signs of environmental disturbance. On the contrary, no such signs are obvious in any of the deeper stations (4-7). This disturbance is most probably due to organic loading from the two small rivers Inachos and Erasinos (station 1) and a sewage pipe outlet (station 2), in addition to the presence of an important amount of plants and plant debris (station 3). The Diversity values of these stations (H' = 3.4-4.4) vary within the limits of other Greek areas with moderate disturbance. For example, in Atalanti Bay (Central Greece) diversity ranged from 2.8 to 4.5 (Bei et al., 1990), while in Geras Bay (Mytilini, E. Aegean) the values were 4.4 to 5.3 (Zenetos & Papathanassiou, 1989). On the contrary, diversity of the highly polluted area near the mouth of the sewage pipe of Athens at Saronikos Bay (30 m), was 0.93 (Nicolaidou et al., 1993).

	Stations						
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Porifera							
Craniella cranium (Muller)	0	0	1	0	0	0	0
Polychaeta							
Amphiglena mediterranea Leidig, 1851	0	0	2	0	0	0	0
Amphinomidae sp.	0	1	0	0	0	0	0
Aonides oxycephala (Sars, 1862)	15	30	41	0	0	0	0
Aphroditidae sp.	0	0	0	0	1	0	0
*Aphylochaeta marioni (*Saint-Joseph, 1894)	4	15	13	1	1	0	1
Arabella iricolor (Montagu, 1804)	0	0	1	0	0	0	0
Aricidea capensis Day, 1965	1	0	2	0	0	0	0
Aricidea fauveli Hartman, 1957	13	7	9	4	9	4	7
Axiothella constricta (Claparede 1868)	0	0	0	0	10	0	0
Capitella capitata (Fabricius, 1780)	78	55	94	0	30	0	2
Capitomastus minimus (Langerhans, 1880)	181	119	0	6	0	0	2
Cauleriella bioculatus (Keferstein, 1862)	0	1	0	0	0	0	0
Chaetopterus variopedatus (Renier, 1804)	0	0	0	1	0	0	0
Chaetozone cf setosa (Malmgren, 1867)	3	1	0	0	2	0	0
Chone collaris Langerhans, 1880	0	2	0	0	0	0	0
Cirratulus filiformis Keferstein, 1862	1	0	8	0	5	0	0
Clymenura clypeata (Saint-Joseph 1894)	5	8	0	2	4	0	0
Cossura coasta (Kitamori, 1960)	0	0	0	3	2	8	8
Ctenodrilus serratus (O.Schmidt)	1	17	21	0	0	0	0
Drilonereis filum (Claparede 1868)	0	0	1	0	0	0	1
Euchone rosea Langerhans, 1884	0	0	6	0	0	0	0
Euclymene oerstedii (Claparede, 1863)	44	0	4	0	2	0	0
Eunice harassii Audouin & M.-Edwards, 1834	4	0	0	0	0	0	0
Eunice oerstedii Stimpson, 1854	0	0	5	0	9	0	0
Eunice pennata (O.F.Muller, 1776)	0	0	4	0	2	0	0
Exogone verrugera (Claparede, 1868)	0	0	1	0	0	0	0
Glycera alba (Muller, 1788)	1	0	1	0	0	1	0
Glycera rouxii Audouin & M.-Edwards, 1834	4	9	1	0	1	0	0
Goniada emerita Audouin & M.-Edwards, 1834	0	2	0	0	0	0	0
Grubeosyllis limbata (Claparede, 1868)	**0**	**0**	**1**	**0**	**0**	**0**	**0**
Harmothoe lunulata (Delle Chiaje, 1841)	0	1	4	1	8	0	3
Harmothoe sp.1	0	0	2	0	0	0	0
Harmothoe sp.2	0	0	1	0	0	0	0
Inermonephthys inermis (Ehlers, 1887)	0	1	0	0	0	0	0
Kefersteinia cirrata (Keferstein, 1863)	1	0	1	0	0	0	0
Lanice conchilega (Pallas, 1778)	0	0	2	0	0	0	0
Levincenia gracilis (Hartman, 1965)	10	82	53	0	3	2	0

	Stations						
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Levincenia oculata Hartman, 1957	0	0	13	0	3	0	0
Lumbrineris coccinea (Renieri, 1804)	1	1	1	0	0	0	0
Lumbrineris gracilis (Ehlers, 1868)	5	8	23	1	29	5	0
Lumbrineris impatiens (Claparede, 1868)	1	1	4	1	5	0	0
Lumbrineris latreilli Audouin & M.-Edwards, 1834	86	29	20	46	42	47	37
Magelona cincta Ehlers1908	0	0	0	0	2	0	0
Maldane sarsi Malmgren, 1865	0	0	0	0	3	0	0
Marphysa bellii Audouin & M.-Edwards, 1834	4	5	6	0	0	0	0
Melinna palmata Grube, 1870	6	12	2	0	8	1	2
Mysta siphonodonta (Delle Chiaje, 1822)	0	0	2	0	0	0	0
Nematonereis unicornis Schmarda, 1861	11	18	16	0	1	0	0
Nephthys hombergii (Savigny, 1820)	3	2	1	0	3	0	0
Nephthys hystricis (Mc Intosh, 1908)	0	0	1	20	0	21	25
Nephthys incisa (Malmgren, 1865)	0	0	2	0	0	0	0
Nereis sp.	0	0	6	0	3	0	0
Nicomache lumbricalis (Fabricius, 1780)	0	0	0	0	3	0	0
Notomastus latericeus Sars, 1851	37	33	50	5	10	4	2
Owenia fusiformis Delle Chiaje, 1844	0	4	3	0	2	0	0
Paradoneis armata Glemarec, 1967	4	2	0	0	0	0	0
Paradoneis lyra Southern, 1914	59	76	96	0	2	0	0
Perinereis cultrifera (Grube, 1840)	0	0	0	1	0	0	1
Pilargis verucosa Saint-Joseph, 1899	0	0	1	0	0	0	0
Pisione remota (Southern, 1914)	0	0	0	1	0	0	0
Pista cristata (Muller, 1776)	0	0	1	0	0	0	0
Polycirrus medusa Grube 1855	0	0	0	5	0	7	0
Polydora ciliata (Johnston, 1838*)*	0	0	0	0	0	0	6
Polydora hoplura Claparede, 1870	0	0	2	0	0	0	0
Polynoe sp.	0	0	1	0	0	0	0
Polyophthalmus pictus (Dujardin, 1893)	0	2	0	0	0	0	0
Prionospio cirrifera Wiren, 1883	8	1	1	0	0	0	0
Prionospio malmgreni Claparede, 1870	15	0	3	0	3	0	0
Protodorvillea kefersteini (McIntosh, 1869)	0	0	11	0	0	0	0
Psammolyce articulata Day, 1960	0	0	1	0	0	0	0
Pseudocapitella incerta Fauvel, 1913	0	1	0	0	0	0	0
Pseudopolydora antennata Claparede, 1870	4	0	1	0	0	0	0
Sabella sp.	0	1	0	0	0	0	0
Schistomeringos rudolphi (Delle Chiaje, 1828)	3	8	6	0	0	0	0
Schistomeringos sp.	0	0	1	0	0	0	0
Scoloplos armiger (O.F.Muller, 1776)	0	0	5	0	0	0	0
Serpula vermicularis L.,1767	0	1	0	0	0	0	0
Sigambra parva Day, 1963	2	1	8	4	3	1	1
Sphaerosyllis pirifera Claparede, 1868	0	0	7	0	0	0	0
Spio filicornis Southern, 1914	20	1	0	0	0	0	0
Spiochaetopterus typicus Sars, 1856	0	0	0	0	1	0	0
Spiophanes bombyx (Claparede, 1870)	0	0	0	1	0	0	0
Sternaspis scutata Renier, 1807	0	1	0	5	1	16	8
Syllidae sp.	0	0	1	0	0	0	0
Syllis cirropunctata Michel, 1909	0	0	1	0	0	0	0
Syllis sp.	0	0	3	0	0	0	0
Trichobranchus glacialis Malmgren, 1866	0	0	0	2	0	7	5
Mollusca							
Abra alba (W.Wood, 1802)	29	7	1	0	3	0	0
Abra prismatica (Montagu, 1808)	0	0	1	18	0	2	12
Cerastoderma glaucum (Poiret, 1789)	0	0	0	0	1	0	0
Corbulla gibba (Olivi, 1792)	0	0	1	3	1	0	1
Cylichna cylindracea (Pennant, 1777)	1	0	0	0	0	0	0
Fasciolaria lignaria (L., 1758)	0	0	1	0	0	0	0
Gastrochaena dubia (Pennant, 1777)	0	0	1	0	0	0	0

	Stations						
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Glans trapezia (L., 1767)	4	2	2	0	0	0	0
Gouldia minima (Montagu, 1803)	0	0	1	0	0	0	0
Hemilepton nitidum (Turton, 1822)	1	0	9	0	0	0	0
Limatula subauriculata (Montagu, 1808)	0	0	1	0	0	0	0
Loripes lacteus (L., 1758)	26	15	18	0	0	0	0
Lucinoma boreale (L., 1767)	0	0	1	0	0	0	0
Mangillella multilineolata (Deshayes, 1835)	0	1	0	0	0	0	0
Modiolus barbatus (L.1758)	0	0	1	0	0	0	0
Musculus costulatus (Risso, 1826)	1	0	0	0	0	0	0
Mysella bidentata (Montagu, 1803)	2	0	11	0	0	0	0
Natica hebraea (Martin, 1784)	0	0	0	0	0	1	0
Neverita josephinia Risso, 1876	4	0	0	0	0	0	0
Rissoa monodonta Philippi, 1836	0	10	0	0	0	0	0
Tellina fabula Gmelin, 1791	0	0	1	0	0	0	0
Tellina pulchella Lamarck, 1818	9	0	1	1	10	1	0
Tricolia pullus (L., 1758)	5	0	0	0	0	0	0
Venus verrucosa (L., 1758)	0	0	1	0	0	0	0
Decapoda							
Alpheus glaber (Olivi, 1792)	1	0	0	0	0	0	0
Anapagurus hyndmanii (Bell, 1845)	0	0	1	0	0	0	0
*Athanas nitescens (*Leach, 1814)	0	2	2	0	0	0	0
Goneplax rhomboides (L., 1758)	0	0	0	1	0	1	1
Inachus dorsettensis (Pennant, 1777)	0	0	1	0	0	0	0
Leiocarcinus arquatus (Leach, 1814)	0	1	0	0	1	0	0
Upogebia tipica (Nardo, 1869)	1	0	0	0	1	0	1
Amphipoda							
Ampelisca tenuicornis Liljeborg, 1855	2	1	1	1	0	0	1
Bathyporeia sp.	0	0	1	0	0	0	0
Caprella linearis (L.)	0	0	2	0	0	0	0
Erichthonius brasiliensis (Dana, 1855*)*	3	0	8	0	0	0	0
Leucothoe incisa (Robertson, 1892)	2	0	0	0	0	0	0
Oedicerotidae sp.	0	0	1	0	0	0	0
Isopoda							
Arctruropsis rudis	0	0	1	0	0	0	0
Tanaidacea							
Apseudes latreilli (M. Edwards, 1828)	1	0	0	0	0	0	0
Cumacea							
Iphinoe sp.	0	0	0	0	2	0	0
Pycnogonida							
Nymphon gracile (Leach)	2	0	0	0	1	0	0
Echinodermata							
Amphiura chiajei Forbes, 1843	0	0	6	1	24	0	4
Holothuria tubulosa Gmelin,1788	1	0	0	0	0	0	0
*Leptopenctata elongata (*Duben & Koren, 1844)	0	0	0	0	1	0	0
Phoronida							
Phoronis sp.	0	0	0	2	0	6	4
Sipuncula							
Aspidosiphon mulleri Diesing, 1851	0	0	0	0	2	0	0
Phascolion strombi (Montagu, 1804)	0	0	2	0	2	0	0
Tunicata							
Clavellina lepadiformis (Muller)	50	70	160	0	32	0	2
Tunicata sp. (juveniles)	0	0	3	0	0	0	0
Nemertea							
Carcinonemertes sp.	0	0	1	0	0	0	0

	Stations						
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Cerebratulus fuscus (McIntosh, 1873-74)	1	0	8	0	6	0	1
Micrura fasciolata (Ehrenb)	0	0	3	0	0	0	0
Micrura purpurea (Dal)	5	5	1	0	0	0	0
Micrura sp.	0	5	1	0	0	2	0
Poliopsis lacazei	0	0	0	0	0	1	0
Turbelaria							
Leptoplana alcinoi	1	0	0	0	0	0	0
Leptoplana tremellaris	0	0	1	0	0	0	0
Miscellanea							
Calanoidea sp.	0	0	0	0	1	0	0
Mysidacea	1	0	0	0	0	0	0
Nematoda sp.	1	1	2	0	0	0	0
Oligochaeta sp.	0	0	41	0	0	0	0
Ostracoda	0	0	0	0	1	0	0
Peachia cylindrica (Reid)	0	0	0	1	0	0	0

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Notes on *Brachioteuthis riisei* (Steenstrup, 1882) and *Onychoteuthis banksi* (Leach, 1817) (Cephalopoda:Teuthoidea) found in the Aegean Sea

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ABSTRACT. The occurrence of *Brachioteuthis riisei* and *Onychoteuthis banksi* in the region of Argosaronikos (Aegean Sea) is reported in this note. Both species were collected by the experimental bottom trawl net used in the framework of the International Bottom Trawl Survey in the Mediterranean (MEDITS).

A single individual of *Brachioteuthis riisei* was caught in June 1997, between 380-418 m of depth. It was a fully mature female with a mantle length of 66 mm, representing the largest individual ever caught in the Mediterranean and the first one recorded in the Aegean Sea. Five specimens of *Onychoteuthis banksi* were found in June 1997 and June 1998 at depths ranging between 685 and 760 m. All specimens were immature their size ranging from 55 to 107 mm DML. This is the first finding of the species in the Aegean Sea and the Eastern Mediterranean (east of 23° E).

KEY WORDS: cephalopoda, teuthoidea, Mediterranean.

INTRODUCTION

Brachioteuthis riisei (Steenstrup, 1882) and *Onychoteuthis banksi* (Leach, 1817) are pelagic oegopsid squids distributed in warm and temperate oceanic waters (ROPER et al., 1984; D'ONGHIA et al., 1995). Records of both species in the Mediterranean Sea have been reported mainly for the western and central part (JATTA, 1896; NAEF, 1923; DEGNER, 1926; CIPRIA, 1935; GAMULIN-BRIDA & ILIJANIC, 1965; TORCHIO, 1965; 1966; CLARKE, 1966; LUMARE, 1970; MANGOLD, 1973; ROPER, 1974; BERDAR & CAVALLARO, 1975; MORALES & GUERRA, 1977; SANCHEZ & MOLI, 1985; BELLO, 1985, 1990; SANCHEZ, 1986; VILLANUEVA,1992; JEREB & RAGONESE, 1994; TURSI et al.,1994; ORSI-RELINI et al.,1994; D'ONGHIA et al., 1995). The only record of *Brachioteuthis riisei* in the Eastern Mediterranean (East of 23° E) goes back to that reported by Degner (1926), whereas its presence in the Aegean Sea has not yet been documented. *Onychoteuthis banksi* has never been reported in the Eastern Mediterranean. It should be noted however, that Degner (1926) in the report of the Danish expedition, referring to the captures of *Onychoteuthis banksi* paralarvae, found also at west of Rhodes island, mentioned that some of those specimens might belong to the genus *Ancistroteuthis*.

New findings concerning these species are reported and discussed in this note, and data on their biometry, sex and maturity are given.

MATERIAL AND METHODS

The present material was collected during two bottom trawl surveys carried out in June 1997 and in June 1998 in the region of Argosaronikos, in the framework of the EU funded project International Bottom Trawl Survey in the Mediterranean (MEDITS). A professional trawler equipped with an experimental net, with a cod end of 10 mm mesh was used. The horizontal and vertical net opening, measured by means of a SCANMAR sonar system, ranged between 13-18,8 m and 1,9-2,8 m respectively. The hauling speed was 2.9-3.1 knots. The sampling design was random stratified (BERTRAND et al., 1997). Hauls lasting from 30 to 60 minutes, were performed in both years, at a total of 22 sampling stations distributed in five depth strata: 10-50, 50-100, 100-200, 200-500 and 500-800 m.

The species were identified following the keys in MANGOLD & BOLETZKY (1987). The weight and the morphometric measurements of the individuals of *Brachio-*

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teuthis riisei and *Onychoteuthis banksi* were taken after fixation in formalin solution 5%. The maturity stage was determined based on the Lipinski's (1979) maturity scale. The beaks of some of the specimens were removed and measured according to Clarke (1962).

RESULTS

One specimen of *Brachioteuthis riisei* and five specimens of *Onychoteuthis banksi* were caught in the study area (Fig. 1). The weight, the sex, the maturity stage and the measurements of the morphometric characters of the specimens caught are presented in Table I.

B. riisei was fished in Epidauros Gulf at a depth between 380 and 418 m, in June 1998 (Fig. 1). The specimen was a fully mature female of 66 mm DML. The oocyte greatest axis varied from 0.95 to 1.15 mm.

A. lichtensteini was found in both years in Argolikos Gulf at two different stations (Fig. 1). Four specimens with a dorsal mantle length (DML) ranging between 55 and 92 mm were caught during a haul at depths ranging between 685 and 713 m in June 1997 and one larger specimen with 106 mm DML at a depth of 760 m in June 1998. All five specimens were immature.

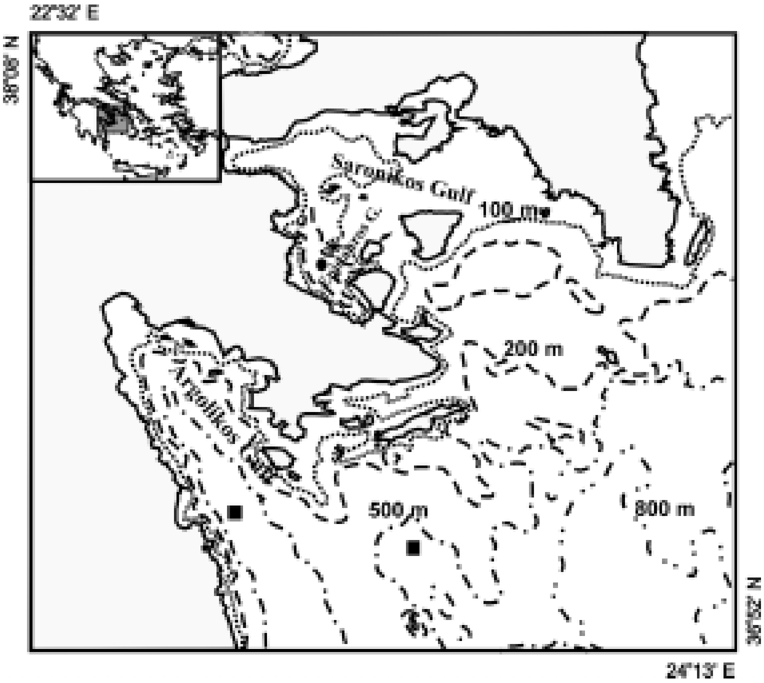

Fig. 1. – Map of the region of Argosaronikos showing place names mentioned in the text and locations of the stations where *Brachioteuthis riisei* (•) and *Onychoteuthis banksi* (■) were caught during the MEDITS surveys of June 1997 and June 1998.

DISCUSSION

The region of Argosaronikos (Fig. 1) consists of two main gulfs, Argolikos and Saronikos, quite heterogeneous in respect to their bottom topography and hydrology.

TABLE I

Measurements of morphometric characters in *Brachioteuthis riisei* (1) and *Onychoteuthis banksi* (2-6) caught in the region of Argosaronikos, after fixation.

No	Sex and maturity stage	DML (mm)	VML (mm)	BW (gr)	MW (mm)	HL (mm)	HW (mm)	FL (mm)	FW (mm)	AL-RI (mm)	AL-RII (mm)	AL-RIII (mm)
1	female V	66	63,5	9	36	11	13	31,5	34	35	54	56
2	male II	106	102	35	55	17	24	63	71	52	63	58
3	female I	90	87	22	50	15	21	55	56	38	50	47
4	male II	65	62	9	39	14	13,5	37	39,5	25	34	34
5	female I	64										
6	unsexed	55										

No	AL-RIV (mm)	AL-LI (mm)	AL-LII (mm)	AL-LIII (mm)	AL-LIV (mm)	TCL (mm)	URL (mm)	UHL (mm)	UCL (mm)	LoRL (mm)	LoHL (mm)	LoCL (mm)
1	43	34	55	58	42	22				2,67	2,45	3,90
2	61	51	61	57	61	36	4,50	9,77	13,67	3,22	2,74	5,08
3	49	39	48	49	50	30	3,36	8,73	10,20	2,66	2,16	4,03
4	32	26	34	32	32	24	1,92	5,53	8,50	2,24	1,78	3,27
6							2,12	6,01	8,79	2,24	1,72	3,31

DML = Dorsal mantle length; VML = Ventral mantle length; BW = Body weight; MW = mantle width; HL = Head length; HW = Head width; FL = Fin length; FW = Fin width; AL = Arm length; R = Right; L = Left; TCL = Tentacular club length; U = Upper beak; Lo = Lower beak; RL = Rostral length; HL = Hood length; CL = Crest length.

Argolikos Gulf is a narrow deep-water basin open to the Aegean Sea. Saronikos Gulf is a semi-enclosed sea which has depths lower than 200m in its greatest part, forming at south-west the deep depression (>400 m) of Epidauros Gulf. The replacement time for the upper waters (<200m) of the western Saronikos Gulf has been estimated 1-2 years, whereas the minimum turnover time for the whole water mass about 8 years. Temperature and salinity values remain remarkably constant below the sill depth (80-100 m) at about 14^o C and 38.6‰ respectively, whereas reduced oxygen levels down to 1,5 ml/L are found to occur in the deeper layers (FRILIGOS, 1985).

The presence of the oceanic *B. riisei* in the isolated basin of Epidauros Gulf, is rare and most probably related to the circulation of the upper water originating from the Aegean Sea (FRILIGOS, 1985). Although a wide bathymetrical distribution of *B. riisei* is indicated by the literature (DEGNER, 1926; MANGOLD, 1973; ROPER et al., 1984; ROPER & SWEENEY, 1992), the reduced oxygen levels found to occur in the deeper layers of Epidauros Gulf (Friligos, 1985),lead us to suggest that the single individual presently caught was rather collected in the water column while the trawl net was lifted.

B. riisei is a rather small sized species. Individuals with a DML ranging between 2,7 and 65 mm have been recorded from the western Mediterranean (JATTA, 1896; NAEF, 1923; DEGNER, 1926; MANGOLD, 1973; ROPER, 1974), the Ionian Sea (DEGNER, 1926, BERDAR & CAVALLARO, 1975; D'ONGHIA et al., 1995) and the Levantine basin (DEGNER, 1926). The specimen presently found is the largest one reported in the Mediterranean so far and represents the first record of *B. riisei* in the Aegean Sea and the second one in the Eastern Mediterranean (East of 23^o E) since 1910 (DEGNER, 1926).

Numerous were the records of *A . lichtensteini* in the Mediterranean sea since the mid of the nineteenth century (CLARKE, 1966) and it was considered an endemic species till its finding by Voss (1956) in the Gulf of Mexico (TORCHIO, 1968). Although *A. lichtensteini* specimens are found stranded in large numbers (Torchio, 1965, 1968; Berdar and Cavallaro, 1975) and are fairly common in the stomach content of swordfish and dolphins (ORSI-RELINI et al., 1994; BELLO, 1996), the catches of the species by sampling devices and fishing gears are rather scarce. Sanchez and Moli (1985) cited one paralarva (DML: 19 mm) caught with Bongo device off the Mediterranean coast of Spain, and Orsi-Relini et al. (1994) listed one juvenile of *A. lichtensteini* between the species caught by an open Isaac Kid midwater trawl in the Ligurian Sea. A small number of immature individuals (DML: 100-150 mm) has been reported in bottom trawl catches from the western Mediterranean (MORALES, 1962; LUMARE, 1970; MORALES & GUERRA, 1977; SANCHEZ, 1986; VILLANUEVA, 1992;) the strait of Sicily (JEREB & RAGONESE, 1994) and the Ionian Sea (BELLO, 1985; Tursi et al., 1994). The relatively smaller size of the individuals presented here could be attributed to the small mesh size of the MEDITS trawl. The size range of pelagic cephalopods has been shown to differ a lot depending on the sampling unit used (ROPER, 1977; D'ONGHIA et al., 1995).

The vertical distribution of *A. lichtensteini* is quite broad ranging between surface and 1270 m (VILLANUEVA, 1992). In the strait of Messina *A. lichtensteini* has been taken by squid-jigs, harpoons and as bycatch in trammel nets (CAVALLARO & BERDAR,1969), which indicates that the species is probably more abundant in the upper layers. However daytime distribution of the species can not be determined on the basis of the scant information available. The capture of *A. lichtensteini* in Argolikos gulf in both surveys reinforces the view that it is not rare in this area.

The non-finding of both species presently recorded, in the Aegean Sea till now, is most probably be due to insufficient sampling in deep and middle waters.

Over the last 30 years, information on the distribution of mesopelagic cephalopods in the Eastern Mediterranean and Greek seas was based mainly on the results of bottom trawl surveys aiming to study the demersal resources (SALMAN et al., 1997; STERGIOU et al., 1977; LEFKADITOU et al., 1999). Thus information concerning the mesopelagic populations is extremely limited because of inappropriate sampling gear employed and entirely different orientation of the research, taking into account that a) sampling depth rarely exceeded 500 m; b) the mesh size used was 18 mm resulting in a significant loss of relatively smaller individuals and/or species, and c) mesopelagic organisms were caught occasionally, while the net is on its way up, and as a consequence it was not possible to know their vertical distribution.

In Table II the number of mesopelagic cephalopod species by family in the western Mediterranean

TABLE II

Number of mesopelagic species of the cephalopod families in the Western Mediterranean, the Levantine basin and the Aegean Sea.

	Western Mediterranean	**Levantine basin**	**Aegean Sea**
Brachioteuthidae	1	1	1
Chiroteuthidae	1	1	1
Cranchiidae	2	1	-
Ctenopterygidae	1	1	1
Enoploteuthidae	5	3	4
Histioteuthidae	3	-	2
Octopoteuthidae	1	1	1
Ommastrephidae	5	3	4
Onychoteuthidae	3	1	1
Thysanoteuthidae	1	-	1
Sepiolidae	3	1	1
Argonautidae	1	1	1
Ocythoidae	1	1	1
Tremoctopidae	1	1	1

(Mangold & Boletzky, 1988; Orsi-Relini & Massi, 1991), the Levantine basin (Salman et al., 1997) and the Aegean Sea (Lefkaditou et al., 1999; unpublished data from MEDITS surveys) is shown. The mesopelagic species found in the Aegean Sea are still considerably fewer than found in the western Mediterranean, but more than the ones in the Levantine basin where research on cephalopods is less systematic. The capture of mesopelagic cephalopod species in the Aegean Sea seems to be favored by trawling in deeper waters and the use of a trawl net with a wide horizontal and vertical opening during the MEDITS surveys, as suggested by Maiorano et al. (1999).

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Distributional patterns in the Greek species of the terrestrial isopod genus *Armadillidium* Brandt, 1833

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ABSTRACT. The Mediterranean genus *Armadillidium* has radiation centres in Italy and Greece, with around 55 species in each of the two countries. Three of the Greek species are found in most parts of the Mediterranean region, and about 40 species are endemic to Greece. Island endemism is, however, low; five species are known from only a single island. Six species occur on several Ionian islands but not on the adjacent mainland. On the Aegean islands, with the exception of Crete, endemic species are lacking. The bulk of the Greek species have restricted distribution ranges on the mainland and the Peloponnese. A gradient of species density from west to east is correlated to climatic factors and a substitution by other conglobating genera in the east.

INTRODUCTION

The genus *Armadillidium* Brandt, 1833 comprises around 180 nominal species, and the question of its monophyly is still open to debate. Its autochthonous distribution covers Europe, the coasts of the Mediterranean and the Black Sea, and the Caucasus area (Fig. 1). The highest species numbers occur in Italy and Greece, with more than 50 species in each country. In the Aegean region there is an abrupt and rather enormous drop in the number of species numbers, with only five species known from the Turkish mainland, contrasting with around 55 species recorded from the Greek territory (compare ARCANGELI, 1914; STROUHAL, 1927; 1928 a; 1928 b; 1929 a; 1929 b; 1936; 1937 a; 1937 b; 1937 c; 1937 d; 1938; 1939; 1956; 1966; VANDEL, 1958, SCHMALFUSS, 1981, 1982, 1985 a, 1985 b; SFENTHOURAKIS, 1992; 1993; 1994; 1995; SFENTHOURAKIS & GIOKAS, 1998; around 8 undescribed species from Greece are in the Stuttgart collection).

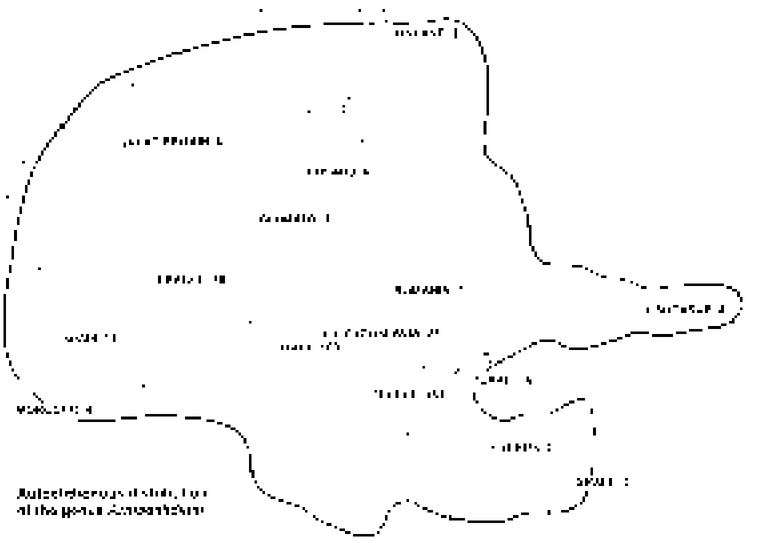

Fig. 1. – Autochthonous distribution of the genus *Armadillidium*, with species numbers of selected regions.

DISTRIBUTION TYPES

The 55 Greek species exhibit a number of distribution types that can be ascribed to the following categories (listed in Table 1):

Holomediterranean

Armadillidium vulgare probably originated in the eastern mediterranean region and has today a rather cosmopolitan distribution, having been introduced by human activities to most parts of the world. But even these widely distributed species with obviously broad

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ecological tolerances have ecological limits beyond which they cannot exist. In the Aegean *A. vulgare* is missing on a number of larger islands, e. g. on Kárpathos and Ródhos (Fig. 2), where it should not have been overlooked. In a former paper I have interpreted this situation as competitive exclusion between *A. vulgare* and *Schizidium oertzeni* (Budde-Lund, 1896) (SCHMALFUSS, 1983: 15, 17). Since then, both species have been found sympatricly on several islands, e. g. Náxos, and I have dismissed the idea of direct competition that excludes *A. vulgare* from these islands. Probably climatic factors, e. g. very hot and dry summers, in combination with the lack of suitable biotopes, keep these islands free of *A. vulgare*.

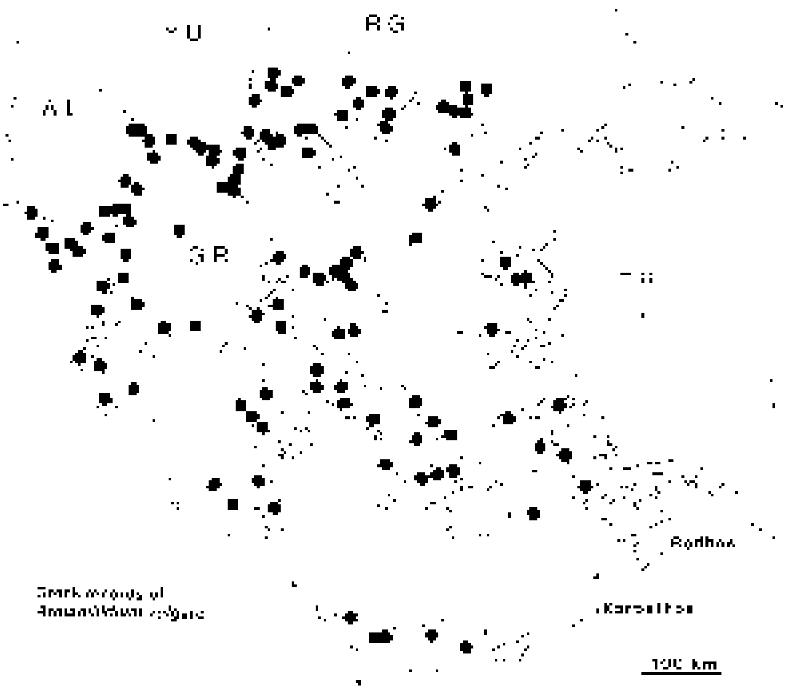

Fig. 2. – Greek records of *Armadillidium vulgare* (literature records and samples in the Stuttgart collection). Note the absence of the species on the islands Kárpathos and Ródhos.

Two more Greek species, both connected to litoral biotopes, exhibit an overall distribution throughout most of the Mediterranean and even along the Atlantic coast of Europe. *Armadillidium granulatum* occurs from the Atlantic coast of Portugal and France through the Mediterranean, also along the North African coast, to the Aegean and the southwestern Black Sea (Fig. 3). It is missing in the easternmost part of the Mediterranean, and it has not been found in the northwestern Aegean (Fig. 3). In the eastern Mediterranean it is substituted by a vicariant species, *Armadillidium fallax* (compare Schmalfuss 1989). Searching for an explanation for its absence in the northwestern Aegean I found a correlation with the 5 mm precipitation line for July (see PHILIPPSON, 1948). Since this correlation does not exist in the more western distribution area of the species it should not be a direct causal correlation, but a secondary regional phenomenon.

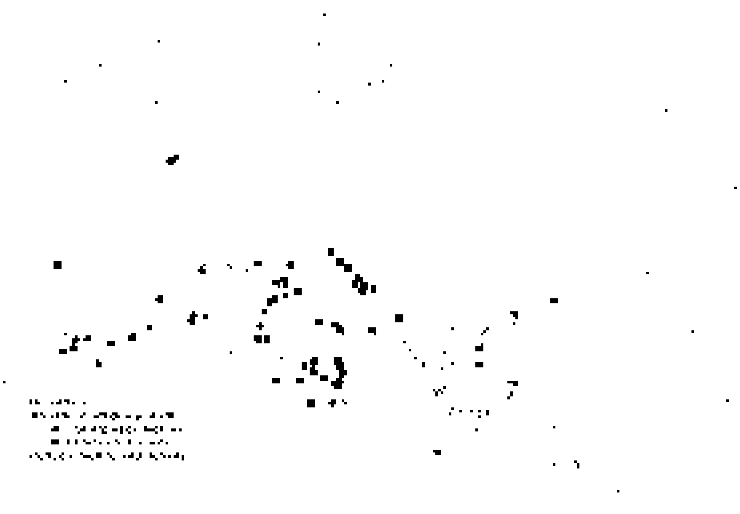

Fig. 3. – Recorded overall distribution od *Armadillidium granulatum*, including literature records and samples from the Stuttgart collection.

The second species of this distribution type is *Armadillidium album*, of which we have two new unpublished records from Greece. The species reaches up the Atlantic coast to the British Isles and the Netherlands. It is small and buries itself deep into the sand, so it is easily overlooked and may turn up in many other places of the Mediterranean.

Eastern Mediterranean

Armadillidium marmoratum is known from the coasts of the Aegean, reaching the Black Sea at the Bosporus, from Cyprus, Israel and Egypt (Fig. 4). It is still an open question whether it is conspecific with taxa described from Italy. In any case it is the only species distributed throughout the eastern Mediterranean.

Northeastern Mediterranean

Armadillidium pallasii lives along the shores of the Adriatic, the northeastern Aegen and the Black Sea (Fig. 5). If we suppose the southern distribution boundary to be formed by temperature and/or precipitation factors we can imagine a continuous distribution along the coasts

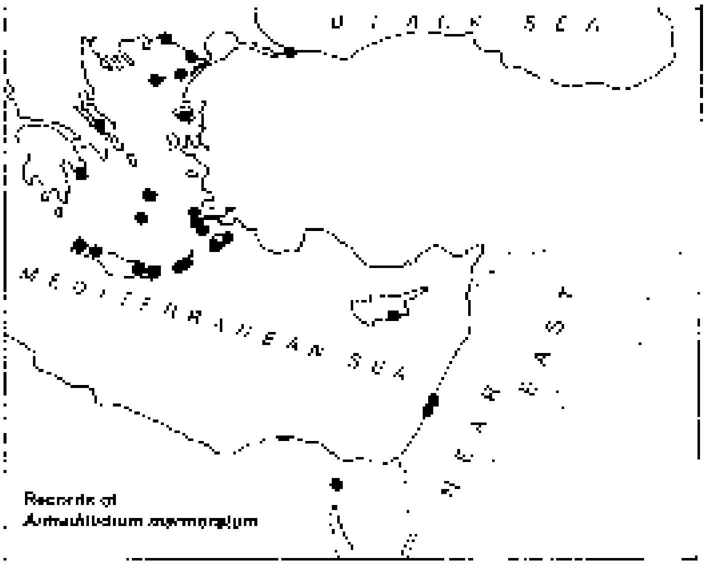

Fig. 4. – Recorded overall distribution of *Armadillidium marmoratum* (literature records and samples in the Stuttgart collection). Some species described from Italy may be synonyms of *A. marmoratum*.

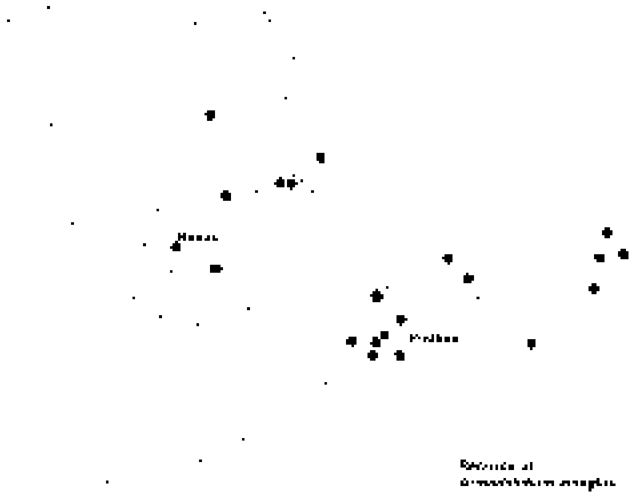

Fig. 5. – Safe records of *Armadillidium pallasii* (literature records and samples in the Stuttgart collection; the type locality Crimea is mentioned with a question mark). The subspecific classification of this species group has yet to be clarified.

of the Greek mainland during colder and wetter periods of the Ice Age.

Southwestern Balkans

Five species of *Armadillidium* are known from Albania and western Greece. One of these is *Armadillidium bicurvatum*, whose eastern distribution limit seems to coincide with the 100 mm precipitation line for December (after Philippson 1948) (Fig. 6).

Aegean

Armadillidium insulanum populates the northern Aegaean islands (compare Schmalfuss, 1985 b: 298, fig. 13) and the adjacent mainland coasts to the north and the east. *Armadillidium ameglioi* is recorded from the southeastern Aegean islands and the adjacent coasts of Asia Minor (Fig. 7).

Greek endemics

The bulk of the Greek species of *Armadillidium*, around 40, have been found only in Greece. Some of them have a rather wide distribution inside Greece, as e.g. *Armadillidium peloponnesiacum* (Fig. 8), most of them are however restricted to a limited region as e.g. *Armadillidium janinense* (see Schmalfuss, 1985 b: 298, fig. 13) or are even known only from the type localities.

Ionian island endemics

Six species are known from several Ionian islands but not from the adjacent mainland (*A. beieri, frontemarginatum, humile, jonicum, justi, werneri*), each species inhabiting several islands. This phenomenon should have been caused by an increased predation or/and competition pressure that extinguished these species on the mainland. A speciation *in situ* can be excluded because the islands were isolated earlier from each other than from the mainland.

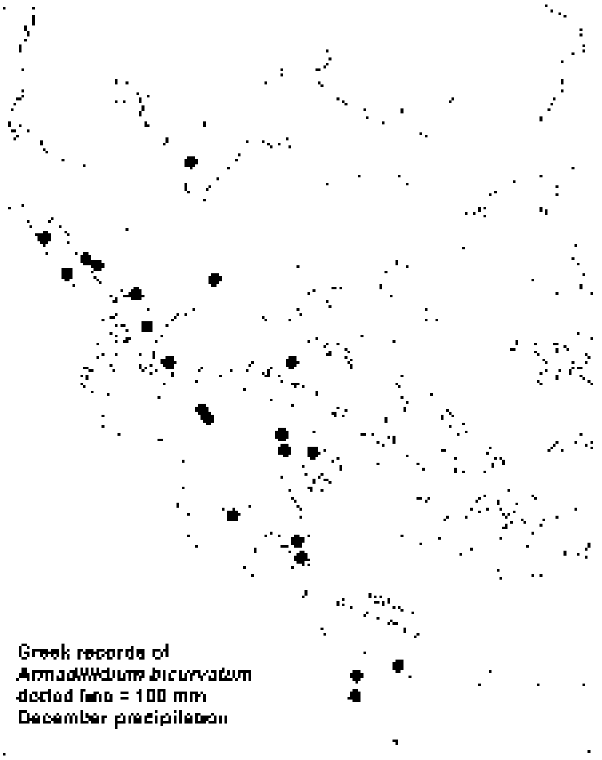

Fig. 6. – Greek records of *Armadillidium becurvatum*. the eastern distributional border seems to be formed by the 100 mm December precipitation line. From Schmalfuss 1985 b.

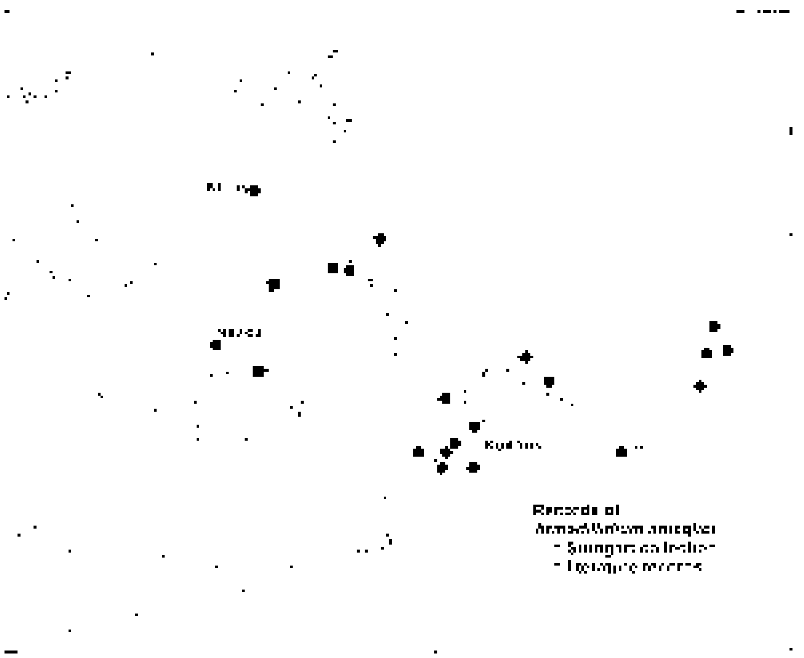

Fig. 7. – Distributional records of *Armadillidium ameglioi* (literature records including the synonyms *A. ephesiacum* Strouhal, 1927 and *A. samium* Strouhal, 1929, and samples from the Stuttgart collection).

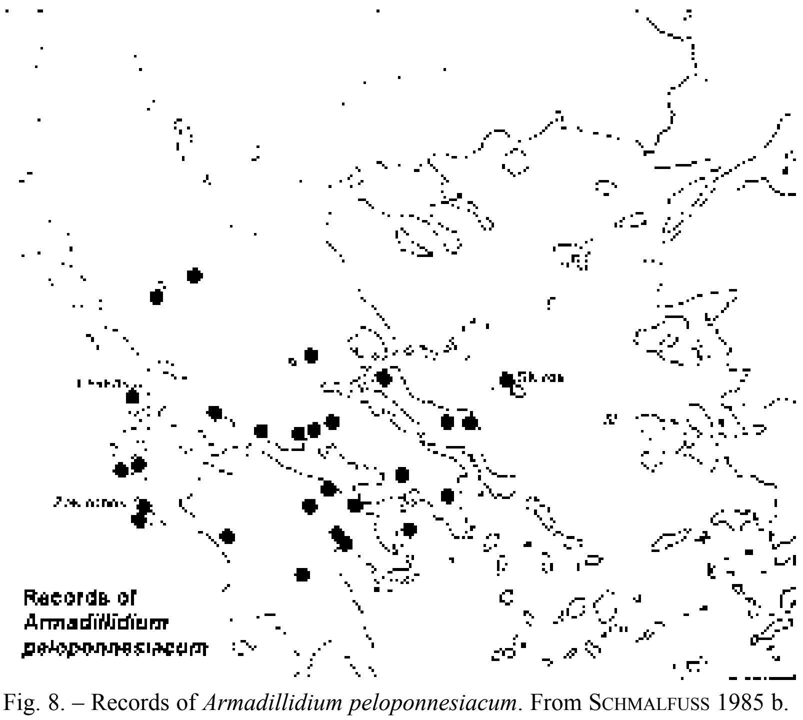

Fig. 8. – Records of *Armadillidium peloponnesiacum*. From Schmalfuss 1985 b.

Single island endemics

Five species have been, up to now, found only on one island. Two of them live on Crete (*A. cavernarum* and an undescribed species), three are known from single Ionian islands (*A. simile* from Kérkira, *A. hauseni* from Paxí, *A. kalamium* from Kálamos). So single island endemism is low, and in the Aegean it is completely lacking, if we exclude Crete which by its size is not comparable to the other Aegean islands. An explanation would be that the islands are very similar and comparably young so speciation processes have not yet been working.

SPECIES NUMBERS

The species numbers of single islands reflect the general gradient in the region, a decline of numbers from west to east. The Ionian islands house a much higher number than Aegean islands of corresponding size (Fig. 9). On a greater scale the drop from west of the Aegean to east of the Aegean is rather drastic. Looking for an explanation of this phenomenon the following observations can be helpful. The considered region is characterized by rather severe changes from mesic to xeric conditions. The summers become very dry, with practically no precipitation. Under these conditions the plant cover of the ground is greatly reduced, which leads to a reduced number of micro-habitats and thus generally to reduced species numbers. Additionally, the predator spectrum changes, with an increased number of forms with pincer-like gripping appendages, and the attack possibilities increase on open ground. This leads to a substitution of *Armadillidium* by genera with more optimized conglobation facilities, such as *Schizidium* Verhoeff, 1901 and *Armadillo* Duméril, 1816 (compare Schmalfuss, 1988 and 1996). These genera have developed structures that protect the rolled-up animal against shearing forces, such as e.g. grooves on the margin of the first tergite in which the edges of subsequent tergites are locked. The outside of the rolled up animal is completely closed, so it leaves no possibility for predators to open it and to reach the vulnerable ventral parts. The distribution of the genera *Schizidium* and *Armadillo* complies with these contentions. They populate xeric environments in western Asia, reaching their western limit in the southern Aegean, as demonstrated in the map for the genus *Schizidium* (Fig. 10).

CONCLUSIONS

The genus *Armadillidium* is a *sensu latu* mediterranean taxon with radiation centres in Italy and Greece. The fact of more than 40 out of 55 species being Greek endemics reflects the restricted mobility of these animals. Only 12

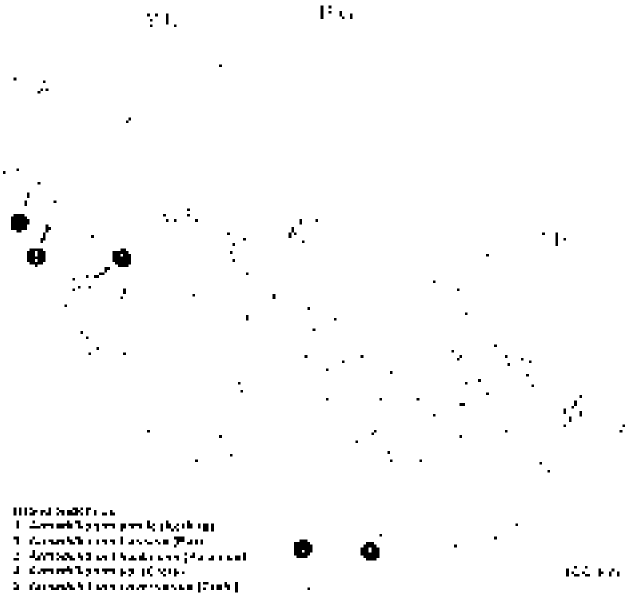

Fig. 9. – Numbers of *Armadillidium* species on selected Greek islands.

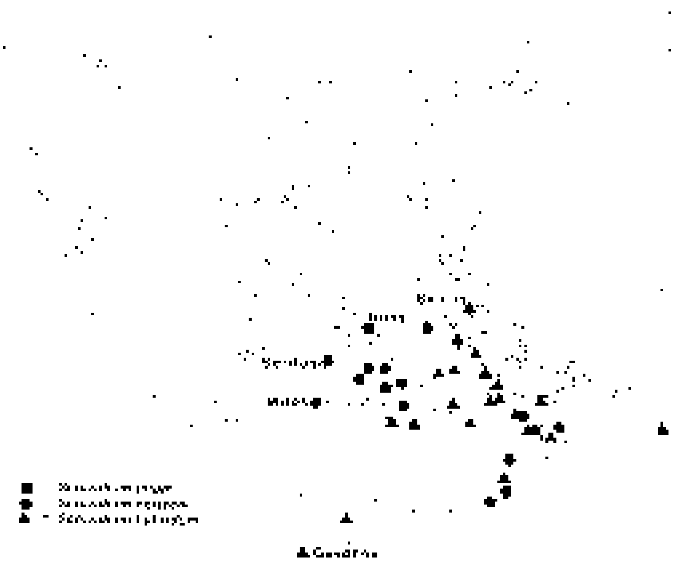

Fig. 10. – Greek records of epigean species of the genus *Schizidium* which is supposed to partly substitute *Armadillidium* east of the Greek mainland.

TABLE 1

Distributional categories to which the Greek species of *Armadillidium* can be ascribed.

HOLOMEDITERRANEAN
A. album Dollfus, 1887 *A. granulatum* Brandt, 1833 *A. vulgare* (Latreille, 1804)
EASTERN MEDITERRANEAN
A. marmoratum Strouhal, 1929
NORTHEASTERN MEDITERRANEAN
A. pallasii Brandt, 1833
SOUTHWESTERN BALKANS
A. albanicum Verhoeff, 1901 *A. bicurvatum* Verhoeff, 1901 *A. frontetriangulum* Verhoeff, 1901 *A. humectum* Strouhal, 1937 *A. laminigerum* Verhoeff, 1907
AEGEAN
A. ameglioi Arcangeli, 1913 *A. insulanum* Verhoeff, 1907
GREEK ENDEMICS
A. aegaeum Strouhal, 1929 *A. arcadicum* Verhoeff, 1902 *A. argolicum* Verhoeff, 1907 *A. artense* Strouhal, 1956 *A. atticum* Strouhal, 1929 *A. cephalonicum* Strouhal, 1929 *A. corcyraeum* Verhoeff, 1907 *A. cythereium* Strouhal, 1937 *A. epiroticum* Strouhal, 1956 *A. fossuligerum* Verhoeff, 1902 *A. inflatum* Verhoeff, 1907 *A. irmengardae* Strouhal, 1956 *A. janinense* Verhoeff, 1902 *A. kalamatense* Verhoeff, 1907 *A. lobocurvum* Verhoeff, 1902 *A. messenicum* Verhoeff, 1902 *A. parvum* Strouhal, 1938 *A. pelionense* Strouhal, 1928 *A. peloponnesiacum* Verhoeff, 1901 *A. pseudovulgare* Verhoeff, 1902 *A. stolikanum* Verhoeff, 1907 *A. tripolitzense* Verhoeff, 1902 *A. versluysi* Strouhal, 1937 *A. xerovunense* Strouhal, 1956 *A. zuellichi* Strouhal, 1937 plus the two following categories plus ± 8 undescribed species
IONIAN ISLAND ENDEMICS
A. beieri Strouhal, 1937 *A. frontemarginatum* Strouhal, 1927 *A. humile* Strouhal, 1936 *A. jonicum* Strouhal, 1927 *A. justi* Strouhal, 1937 *A. werneri* Strouhal, 1927
SINGLE ISLAND ENDEMICS
A. sp. (Crete) *A. cavernarum* Vandel, 1958 (Crete) *A. hauseni* Schmalfuss, 1985 (Paxí) *A. kalamium* Strouhal, 1956 (Kálamos) *A. simile* Strouhal, 1937 (Kérkira)

species surpass the boundaries of Greece, half of which are ecologically connected to litoral biotopes. The distributional categories to which the Greek species can be ascribed are somewhat arbitrary, since every species has a distribution range different from all others. The most obvious result of this analysis is the gradient of species density from west to east of the Greek territory, with a drastic decline on the Turkish mainland. This gradient is correlated to a decrease of precipitation and an increase of seasonality from west to east. This leads to a reduction of niches and of biodiversity, i. e. species density. The gradient is enhanced by the fact that the genus is replaced in the east by morphologically related genera with more opitmized conglobation abilities.

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Abundance and distribution of four-spotted megrim (*Lepidorhombus boscii*) in the Aegean Sea

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ABSTRACT. A two-year study of the abundance and distribution of the four-spotted megrim in the North Aegean revealed a pronounced preference of the species for fine sediment (sandy-muddy) bottoms, in a bathymetric range from 200 to 400 m. The depth of maximum predicted megrim density was found to be 339.8 m. The mean size of specimens in areas displaying higher levels of abundance was significantly smaller than that calculated for specimens in shallower and/or deeper waters. A density-dependent bathymetric pattern of distribution appeared to exist in the study area, with larger fish spreading out from habitats of increased fish stock abundance, where stronger competitive interactions might exist. Such interactions may be of great importance, considering that the Aegean Sea is one of the most oligotrophic areas of the world.

INTRODUCTION

The study of the distribution and abundance of a species provides information on the preferred habitats and on possible spatial and temporal shifts of the fish stock. Among the major factors determining the distribution of fish are prey availability and temperature (ROSE & LEGGETT, 1989), which are often correlated with depth (SWAIN, 1993).

In many aquatic ecosystems, older (larger) demersal fish inhabit deeper and colder water, where they may benefit from lower metabolic costs and greater longevity, than their younger (smaller) conspecifics occupying shallower and warmer waters where food supply and growth rates may be greater (MACPHERSON & DUARTE, 1991). If habitat selection is based on density-dependent resources such as prey abundance, then distribution is also density-dependent (ROSENZWEIG, 1991). SWAIN (1993) reported a density-dependent depth distribution for the Atlantic cod in the southern Gulf of St. Lawrence, suggesting a trade-off between the density-dependent benefits of greater prey resources in warmer shallower waters and the density-independent benefits of lower metabolic costs in colder deeper waters.

The four spotted megrim, *Lepidorhombus boscii* (Risso), is a sinistrally oriented flatfish (Scophthalmidae), which constitutes a significant portion of the Aegean groundfish resource (PAPACONSTANTINOU et al., 1994). It is of commercial interest, especially when longer than 200 mm. Little information exists on the biology and ecology of the four-spotted megrim. DWIVEDI (1964) presented data on morphometric characters, the bathymetrical length-frequency distribution and the length-weight relationship of the species off the Mediterranean coast of France and in the Atlantic. The age and growth of the four-spotted megrim in the Atlantic were studied by FUERTES (1978) and SANTOS (1994), while in the Mediterranean the limited data that are published come from the Adriatic (BELLO & RIZZI, 1987; UNGARO & MARANO, 1995). The feeding habits of the species off the Mediterranean Spanish coast were discussed by MACPHERSON (1979). SANTOS (1994) analyzed the fecundity of the four-spotted megrim off the Portuguese coast. Its larval development was described by SABATES (1991). Finally, landing statistics of the U.K. megrim fishery, referring to *L. boscii* and *L. whiffiagonis* (Walbaum), were reported by BOON (1984) and trawl selectivity for megrims off the Spanish coast was studied by ASTUDILLO & SANCHEZ (1989).

This paper analyzes the abundance and distribution pattern of four-spotted megrim collected in the North Aegean Sea, in order to delineate habitat preferences of the species and examine the role that population density plays in the dispersal of specimens.

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MATERIAL AND METHODS

During eight experimental seasonal fishing cruises, from June 1990 till March 1992, 15323 four-spotted megrims were collected in the north Aegean Sea, from a grid of 33 stations designed in order to be representative of all biotopes and depths of the trawlable area (Fig. 1). Bottom substrate at each station was characterized by the material (organisms and sediment type) that was contained in the cod-end of the trawl net, taking into account the classification scheme suggested by Peres & Picard (1964), as well as that of the benthic communities followed by Augier (1982). The vessel used was a commercial trawler equipped with a net having a cod-end mesh size of 14 mm (bar length).

Fig. 1. – Location of sampling stations in the Aegean Sea.

On board, the total length (TL) was measured to the nearest mm and the sex and the state of maturity of the gonads, according to NIKOLSKY's scale (1976), were recorded in most cases. A representative sample from each station was preserved in a deep freeze for further analysis in the laboratory.

Abundance values of the four-spotted megrim at each station, per season were expressed as number of specimens per hour of trawling (N: nh^{-1}) and were subjected to analysis of variance (ANOVA) for investigating the existence of possible differences. The Tukey multiple comparison procedure (ZAR, 1984) was used in order to locate differences among means.

Similarity of the species' spatial distribution was examined by performing complementary clustering (group average) and non-metric multidimensional scaling (MDS) to the seasonal abundance replicates from each site. Abundance values were subjected to square-root transformation before the analysis. The Bray-Curtis similarity coefficient (BRAY & CURTIS, 1957) was computed and then haul data were clustered with a group average fusion strategy (CLIFFORD & STEPHENSON, 1975) using Primer algorithms (CLARKE & WARWICK, 1989). Discontinuities in data from different sites may be accepted as real when the results of cluster analysis and multidimensional scaling agree (CLARKE & GREEN, 1988).

In order to determine which were the characteristics that could distinguish the groups defined by cluster analysis, discriminant analysis was applied to the data using as variables the depth and the substrate type of each station. The distinctiveness of station-groups was measured using a) the Wilk's λ criterion and its correspondent F-statistic to test the significance of the overall difference between group centroids (TASUAOKA, 1971; RAO, 1973) and b) the squared canonical correlation for each discriminant function, which was interpreted as the part of the total variance in the corresponding discriminant function that accounted for the groups (LEBART et al., 1984). Moreover, the percentage of stations correctly assigned to groups by the analysis was computed and considered as an indirect measure of the adequacy of the classification feature.

The regression relating abundance of megrims with depth was of the form: $Ln(N_i+1)=b_0+b_1D_i+b_2D_i^2$, where N is the abundance of megrim (nh^{-1}) in tow i and D is the depth in tow i (SWAIN & MORIN, 1997). The depth of maximum predicted fish density was calculated from the first derivative of the fitted model with respect to depth (i.e. $D_{max}=-b_1/2b_2$) (SWAIN & MORIN, 1997) for males, females and sexes combined.

RESULTS

The abundance of the four-spotted megrim ranged between 0 and 572 nh^{-1} (mean =60.3 nh^{-1}, ±S.D.=110.6). In relation to season of sampling, abundance values did not appear to differ significantly (ANOVA: F=1.359, P>0.05), whereas the opposite was true of spatial variations (F=10.897, P<0.001).

For further investigation of site-dependent differences in abundance values, multivariate analysis was performed on abundance replicates from each station. Stations 22, 24, 31, 32, 33 were excluded from the analysis, since they were not exploited during most sampling cruises, due to bad weather conditions. Moreover, stations 16 and 17 were also excluded, because the species was never caught there. Five groups of sites were defined on the basis of the dendrogram and the MDS plot (Fig. 2a & 2b). The major factors responsible for the clustering of the sites seemed to be depth (Fig. 2c) and bottom substrate (Fig. 2d). In sites of group I, where the species presented highest abundance values, depths ranged from 220 to 450 m. Sea bottom in these sites was in all cases covered by mud or by sandy mud. In addition, the synthesis of the macrobenthic fauna encountered was such that we can characterize these sites as the biotopes of the Deep Muds (DM) and Bathyal Gravels (BG) biocoenoses (AUGIER, 1982). Abundance values decreased progressively from sites of group II to sites of group V. Relatively smaller abundance values coincided with sites having depths of 100 to 220 m (e.g. stations 23, 18, 14), and of 450 to 550 m (e.g. site 2). In waters shallower than 100 m and deeper than 550 m the presence of the species was very rare. In cases, however, where bottom type was unfavorable, the influence of

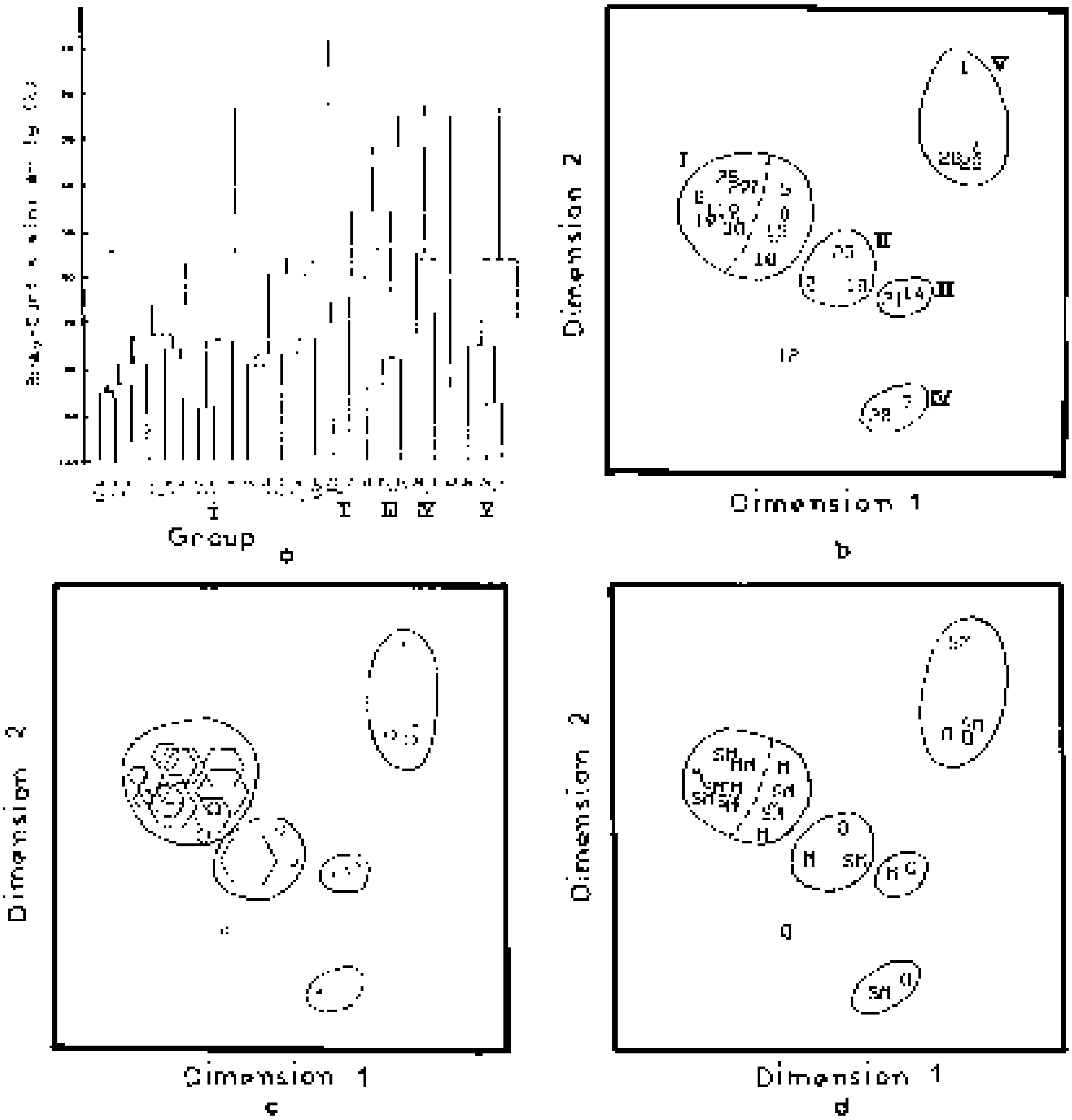

Fig. 2. – Dendrogram (a) and multidimensional scaling plot (b) of stations grouped by similarity in seasonal abundance (Nh^{-1}) of four-spotted megrim. (c) Same as in (b) with superimposed symbols of linear dimensions proportional to the depth at these stations (smallest exagon represents 66 m, largest 545 m). (d) Same as in (b) with superimposed the characterization M, SM for stations having muddy, sandy-muddy substrate, or O for stations with other type of sea bottom.

depth was obscured. This is clear for the sites of group V where the presence of the species was considered occasional. In the latter case, sites were either out of the preferred depth range, or when depth was within range, the limiting factor seemed to be bottom substrate. In particular, stations 1 and 4 had depths less than 100 m. Although stations 26 and 28 were within the preferred depth range, the first one was heavily colonized by echinoderms and the second one was covered by hard substrate. The fact that depth and bottom substrate were factors discriminating the various groups of stations derived from the classification mentioned above was corroborated by the results of discriminant analysis (Table 1).

The second-degree polynomial regressions relating abundance and depth were calculated for males, females and sexes combined (Fig. 3) as follows:

Males: $Ln(N+1)=-2.4874+0.0397D-0.0000592D^2$, $r^2=0.61$, $n=17$

Females: $Ln(N+1)=-3.0652+0.0457D-0.0000681D^2$, $r^2=0.66$, $n=17$

All fish: $Ln(N+1)=-3.1161+0.0401D-0.0000590D^2$, $r^2=0.71$, $n=28$

TABLE 1

Discriminant analysis on depth and substrate type. SDFC= standardized discriminant function coefficient.

Wilks'λ	Sign. Level	Correct assignments (%)	SDFC	
			Depth	Substrate
0.312	P=0.002	56	-0.621	0.740

The parameters of the above regressions yielded Dmax=335.3 m for males, 335.5 m for females and 339.8 m for sexes combined. ANOVA applied to the four-spotted megrim abundance data at the various depths revealed no significant differences in distribution between the sexes (F=0.32, P>0.05).

The study area was divided into four depth zones (i.e.: <200m, 200-300 m, 300-400 m, >400 m), according to the results of the megrim abundance data, and the mean total length (TL) with 95% confidence interval of males and females collected in each one was calculated (Fig. 4). At intermediate waters of 200-400 m smaller megrims were collected relative to shallower and deeper areas. ANOVA applied to the size data of megrims in the four depth zones displayed significant differences (F=7.81, P<0.001). Tukey's multiple comparison tests indicated that the mean TL of fish caught at depths 200-400 m was significantly smaller than the respective ones of fish caught in shallower and deeper areas. The comparison of the length frequency distributions in the four depth zones showed that at 300-400 m, the majority of specimens had lengths between 70 and 140 mm TL (Fig. 5). Moreover, in this depth zone young-of-the-year (50-80 mm) were recruited to the trawl fishery. At 200-300 m the bulk of the collected specimens had lengths of 100-170 mm TL. In deeper and shallower areas, however, small megrims (TL<120 mm) were rarely found among catches.

DISCUSSION

Depth and bottom type seemed to be the major factors influencing the four-spotted megrim distribution/abun-

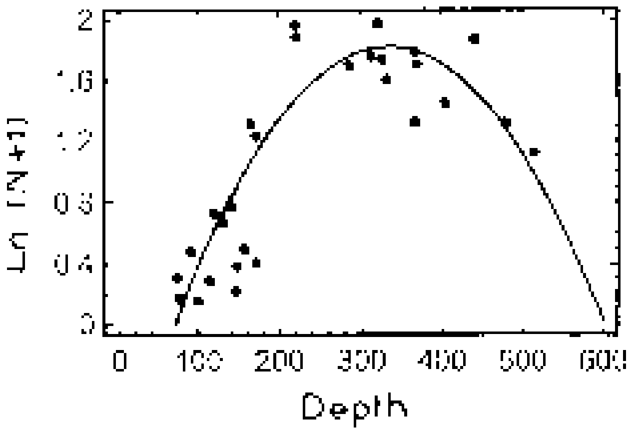

Fig. 3 (upper left). – Relationship between the natural logarithm of the mean abundance (N: n/h), Ln[(N+1], of four-spotted megrim, both sexes combined, collected from 1990 to 1992 and depth (D in m) at the stations of the study area.

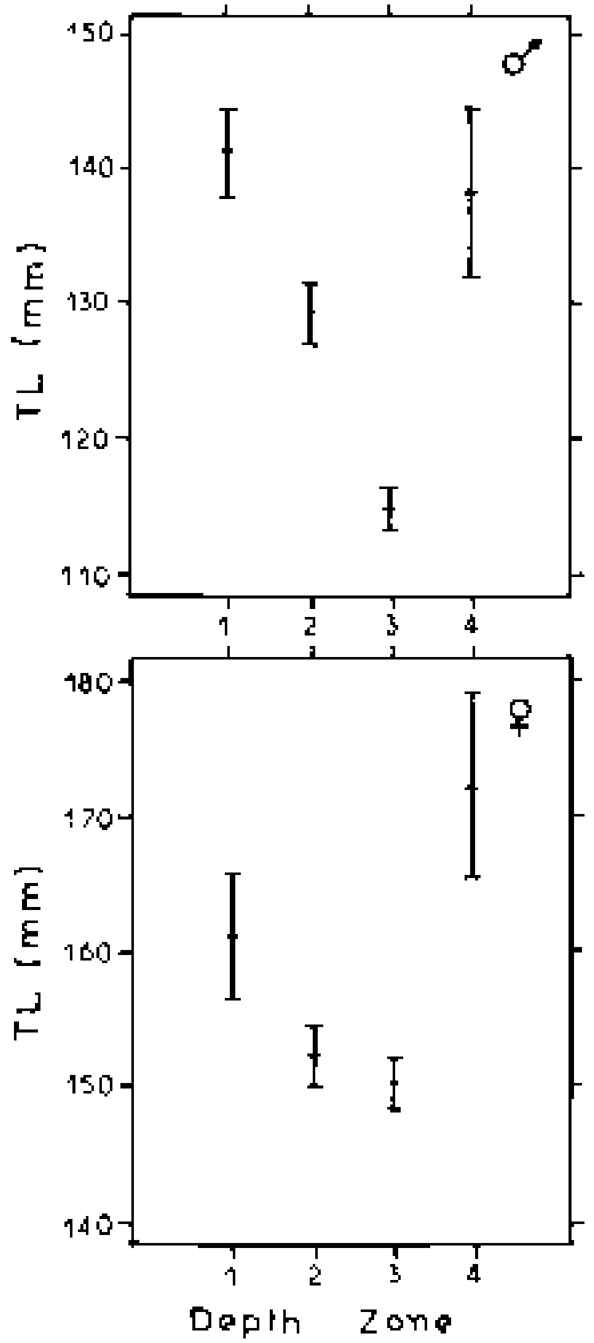

Fig. 4 (lower left). – Mean total length (TL, mm) of male and female four-spotted megrim at the four depth zones (1: <200m, 2: 200-300m, 3: 300-400m, 4: >400m) of the Aegean Sea.

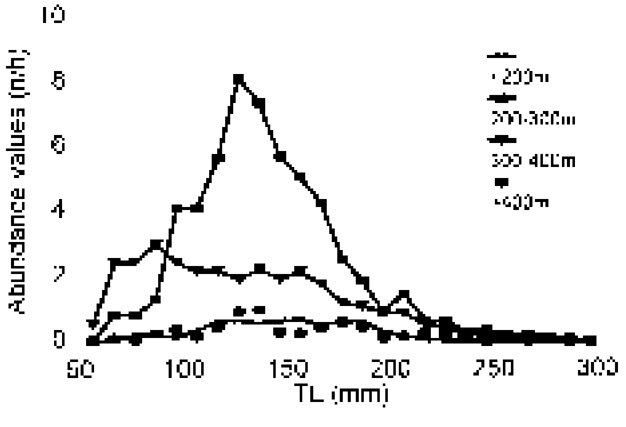

Fig. 5 (lower right). – Length frequency distribution of four-spotted megrim collected in the Aegean Sea between 1990-1992.

dance; favorable areas were found to be those with depths of 200 to 450 m, having muddy or sandy-muddy bottom substrate. The species was rare or absent from sites not in the depth range of 100 to 500 m and/or when the sea bed was covered by coarse substrate or presented high concentration of benthic organisms (i.e. echinoderms). DWIVEDI (1964) mentioned that the optimum depth range for the species was between 100 and 250 m in the Mediterranean coast of France and between 300 and 400 m in the Atlantic. BELLO et al. (1988) considered the four-spotted megrim as one of the most abundant teleosts in depths from 100 to 400 m in the Adriatic. FUERTES (1978) reported that off the Spanish coast of Galicia the species preferred areas with depths between 150 and 375 m.

FARGO & TYLER (1991), mention that flatfish off the Canadian coast, with feeding specialized for different benthic organisms, are expected to be distributed according to sediment type, as well as depth. The importance of feeding was very pronounced for the distribution of Dover sole (*Microstomus pacificus* Lockington) (FARGO & TYLER, 1991). The four-spotted megrim diet in the north Aegean Sea comprised mainly decapod crustaceans (unpubl. data, 1996). In fact, the natant *Alpheus glaber* (Olivi), which dominated the stomach contents, is known to dwell exclusively on muddy substrates (VAMVAKAS, 1971). Moreover, the natant *Processa canaliculata* (Leach) and the mysid *Lophogaster typicus* (M. Sars), which were also important components of the species diet, are usually found on muddy substrates (HOLTHUIS, 1987; HATZAKIS, 1982).

Another factor that could play a role on the distribution of the species over fine bottom sediment could be related to the foraging behavior and/or predator avoidance (VASSILOPOULOU, 1998). MARSHALL (1966) suggests that flatfish living on mud or sand, settle into the bottom, flouncing their body and fins so as they cloak themselves with a thin layer of deposit leaving no more to view than a pair of watchful eyes.

The bathymetric pattern that usually appears in demersal fish is associated with larger specimens shifting to deeper waters. This trend is so common that it has been referred to as Heincke's law (CUSHING, 1981). In the case of the Aegean sea four-spotted megrim, however, the results showed strong evidence of a dome-shaped relationship between depth and four-spotted megrim abundance. Fish abundance was maximized at intermediate depths of about 340 m, which appeared to form an "optimal environmental window" either side of the dome apex. A dome-shaped relationship was also found to exist between depth and abundance of the red bandfish (*Cepola macrophthalma* L.) in the Aegean Sea (STERGIOU, 1993). Another important feature that should be also considered regarding the four-spotted megrim bathymetric distribution is that specimens collected in areas of maximum density were usually smaller than those collected at deeper and shallower waters. The above suggest that a density-dependent pattern of depth distribution could possibly exist for the species in the Aegean Sea. In areas where stock abundance is high, a spreading out may occur, with specimens expanding their distribution into marginal habitats. The fact that larger fish are those that appear outside the areas of high concentration may be consistent with the positive effect of fish size in swimming speed and migration or dispersal rates. Bathymetric trends in demersal fish size may be linked to temperature and prey abundance in a manner that optimizes growth and maintenance (MACPHERSON & DUARTE, 1991). Thus larger megrims, with higher bioenergetic needs and increased mobility, could disperse in areas of relatively lower population density, where intraspecific competition for food should be decreased. Moreover, ontogenetic shifts appear in the diet of megrims, with mysids being the main component of the diet of small specimens, replaced by decapods and fish in the diet of larger four-spotted megrims (VASSILOPOULOU, 1998). The existing information on the distribution of these food resources in the Aegean (STERGIOU et al., 1997), does not allow determination of the role of ontogenetic diet differences in the observed bathymetric pattern. Further studies are required in order to elucidate the role of density in the distribution of fish and to clarify specific matters associated with the dispersal of specimens in different habitat types and especially under different conditions of food availability.

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Variation in body temperatures of the African Chameleon *Chamaeleo africanus* Laurenti, 1768 and the Common Chameleon *Chamaeleo chamaeleon* (Linnaeus, 1758)

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ABSTRACT. Data on the thermal ecology of the African Chameleon *Chamaeleo africanus* Laurenti, 1768 and the Common Chameleon *Chamaeleo chamaeleon* (Linnaeus, 1758) are reported from Greece.

In the field the Tb values ranged from 10.4°C to 31.6°C for *C. africanus* and 23.5°C to 31°C for *C. chamaeleon*.

There was a significant correlation between Tb and Ta in spring and summer for both species. There was also a significant correlation between Tb and Ts only in the spring and only for *C. africanus*. Cloacal temperatures differed significantly between spring and summer and so did substrate temperatures and air temperatures. As the months became hotter the animals reached higher temperatures.

In a laboratory temperature gradient, the preferred body temperatures of *C. africanus* and *C. chamaeleon* were measured and compared with field body temperatures.

The preferred body temperature in the laboratory gradient ranged from 26.0°C to 36.0°C for *C. chamaeleon* and from 25.0°C to 35.0°C for *C. africanus*.

The mean Tb for *C. africanus* in the laboratory was 31°C while for *C. chamaeleon* it was 31.6°C.

The results indicate that both chameleon species are thermoconformers.

Cloacal temperatures differed significantly between the two species in the field but not in the laboratory. There was no difference between the Tb of the two sexes, both in the field and in the laboratory.

INTRODUCTION

Few field records of Chameleon body temperatures exist and there are no data from the Mediterranean region. STEBBINS (1961) gave body temperature records of captive *C. dilepis* and *C. namaquensis*. BURRAGE (1973) has done most of the work on both field and laboratory thermoregulation of *C. pumilus* and *C. namaquensis*. AVERY (1982) referred to BURRAGE's and STEBBINS' studies.

The distribution of the African Chameleon ranges from the Red Sea to western Mali (Central Africa) (BÖHME, 1985); to the north it has reached Egypt (JOGER, 1981). The presence of the species at Ramleh, close to Alexandria in Egypt, was first recorded by Anderson (1898). The African Chameleon is a new species of the Greek and European herpetofauna (BÖHME et al., 1998 & KOSUCH et al., 1999). In Greece this species has been observed only at Divari lagoon, Gialova near Pylos, in the southwestern Peloponnese (21° 40' E, 36° 58' N).

The Common Chameleon has the broadest distribution of all chameleon species, found from Morocco and the southern Iberian Peninsula over the whole of North Africa, to the Near East, Turkey, Cyprus and Southern Arabia and – perhaps with a gap in Iran – to India and Sri Lanka (HILLENIUS, 1959, 1978). The distribution of this species in Greece includes the Aegean islands of Samos, Chios and Crete (ONDRIAS, 1968; CHONDROPOULOS, 1986).

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In the present study, data on the thermal ecology of the African Chameleon *Chamaeleo africanus* Laurenti, 1768 and the Common Chameleon *Chamaeleo chamaeleon* (Linnaeus, 1758) are reported from Greece. The present preliminary results are the first on the thermal ecology of the two European chameleon species.

The preferred body temperatures in a laboratory temperature gradient of the African Chameleon and the Common Chameleon were measured and compared with field body temperatures.

METHODS AND STUDY AREA

Fieldwork at Gialova, Pylos was carried out in August 1997, April, May, June and August 1998 and June 1999. The study area of the African Chameleon at Pylos is a coastal area with sea inlets (20 ha). The habitat in which this species has been observed consists of salt marshes, sand dunes, agricultural land, maquis and phrygana formations and some reeds.

The study area for the Common chameleon is situated on the island of Samos, which has the greatest population of this species in Greece (IOANNIDES et al., 1994).

On Samos island, fieldwork was done in May 1997, June 1998 and July 1999. At Samos the study area was a riverside with riverine vegetation, olive groves and other cultivation on the eastern part of the island.

Most of the fieldwork was carried out during the afternoon, evening and night, between 18:30-03:00, while a few data were also collected during the daytime. Most of the data were collected when it was dark, since at that time it is more effective to find the animals using flashlights (CUADRADO, 1997). The animals were first observed when they were starting to climb on plants at about 18:50, and at 21:00 when they were sleeping on them. All of the data were taken when the animals were resting. After the taking of temperature measurements the specimens were released where they were caught. Only subadult and adult individuals were measured.

Body temperatures (Tb) were obtained using a Weber quick-reading cloacal thermometer. A total of 109 cloacal temperatures were recorded in the field, 93 for the African Chameleon and 16 for the Common Chameleon. Air temperature (Ta) at 1-1.5m above the ground surface and substrate temperature (Ts) were measured at the site of each animal capture. Tb was measured within 10 seconds after capture. For analysis of the data, the study period was divided into two seasons: spring (April- May-June) and summer (July-August).

Seven adult individuals of the African Chameleon (two males and five females) and two of the Common Chameleon (males) were housed in a temperature gradient to study the preferred body temperatures. In order to create a laboratory temperature gradient a 100cm x 20cm terrarium was marked at 5cm intervals without places for animals to hide. At one side of the gradient there was a heating lamp and at the opposite side ice caps. The temperature gradient ranged from 14 to 60° C. The chameleons were placed one at a time in the terrarium and temperatures were taken every 20 minutes. Air (Ta) and substrate temperatures (Ts) in the terrarium were measured at the site of capture. Four hundred and sixteen cloacal temperatures were recorded from individuals in the laboratory, 300 for the African Chameleon and 116 for the Common Chameleon.

For statistical comparisons of temperature values t-tests and Mann-Whitney U tests were used. The latter was used for the field data where the number of measurements was small. We also used regression analysis to examine the possible correlation between Tb and Ta and between Tb and Ts.

RESULTS

The results of the field temperature measurements are shown on Table 1. Cloacal temperatures differed significantly between spring and summer for both species (U test for *C. chamaeleon* z=-2.91 p< 0.0 1, for *C. africanus* z=-5.79 p< 0.0 1) and so did substrate temperatures (U test for *C. chamaeleon* z=-2.91 p< 0.0 1, for *C. africanus* z=-7.13 p< 0.0 1) and air temperatures (U test for *C. chamaeleon* z=-2.91 p< 0.0 1, for *C. africanus* z=-6.77 p< 0.0 1). As the months became hotter the animals reached higher temperatures (Fig.1).

Correlation analysis indicated that for both species there was a significant positive correlation between Tb and Ta both in spring and summer (Table 2).

There was a significant positive correlation between Tb and Ts only in the spring and only for *C. africanus* (Tb=4.02+0.86 Ts, r=0.97).

Cloacal temperatures differed significantly between the two species in the field (U test, z=4.72 p< 0.01), as did substrate temperatures (U test, z=3.18 p< 0.01) and air temperatures (U test, z=4.60 p< 0.01). However cloacal temperatures did not differ significantly between the two species in the laboratory (t-test, t=1.43 p=0.15).

The results of the temperatures in the laboratory are shown in Table 1. The preferred body temperature (80% of the values) in the laboratory gradient ranged from 26.0° C to 36.0° C for *C. chamaeleon* and from 25.0° C to 35.0° C for *C. africanus*. The median Tb for *C. africanus* was 31° C while for *C. chamaeleon* it was 32.7° C (Fig. 2).

For both species there was no difference between the Tb values of the two sexes in the field (U test for *C. chamaeleon*, z=0.22 p> 0.05 for *C. africanus,* z=-0.07 p> 0.05) and laboratory (U test for *C. africanus* z=-. 74 p> 0.05).

TABLE 1

Body (Tb), substrate (Ts) and air temperatures (Ta) measured in the field and laboratory for *Chamaeleo africanus* and *C. chamaeleon* in the spring and summer (N= number of measurements, S.D. = standard deviation).

FIELD

Chamaeleo africanus

	Spring				**Summer**			
Variable	N	Mean	Range	S.D.	N	Mean	Range	S.D.
Tb	39	20.80	10.40-31.60	3.38	54	24.71	20.10-31.00	2.40
Ts	39	19.58	10.20-32.60	3.81	54	26.24	19.90-33.00	3.11
Ta	39	19.22	11.60-25.00	2.95	54	24.00	18.80-28.10	2.43

Chamaeleo chamaeleon

	Spring				**Summer**			
Variable	N	Mean	Range	S.D.	N	Mean	Range	S.D.
Tb	4	24.17	23.50-25.00	.66	12	28.34	27.00-31.00	1.13
Ts	4	21.85	19.60-23.80	1.84	12	29.57	27.80-31.00	.93
Ta	4	22.10	20.20-24.00	1.60	12	28.13	27.00-29.80	.90

LABORATORY

	Chamaeleo africanus				***Chamaeleo chamaeleon***			
Variable	N	Mean	Range	S.D.	N	Mean	Range	S.D.
Tb	300	30.96	15.60-38.20	3.77	116	31.56	23.00-39.60	3.89
Ts	300	29.80	14.50-50.00	5.97	116	31.71	19.70-59.90	6.92
Ta	300	25.74	15.80-31.00	3.47	116	29.43	20.00-36.00	4.32

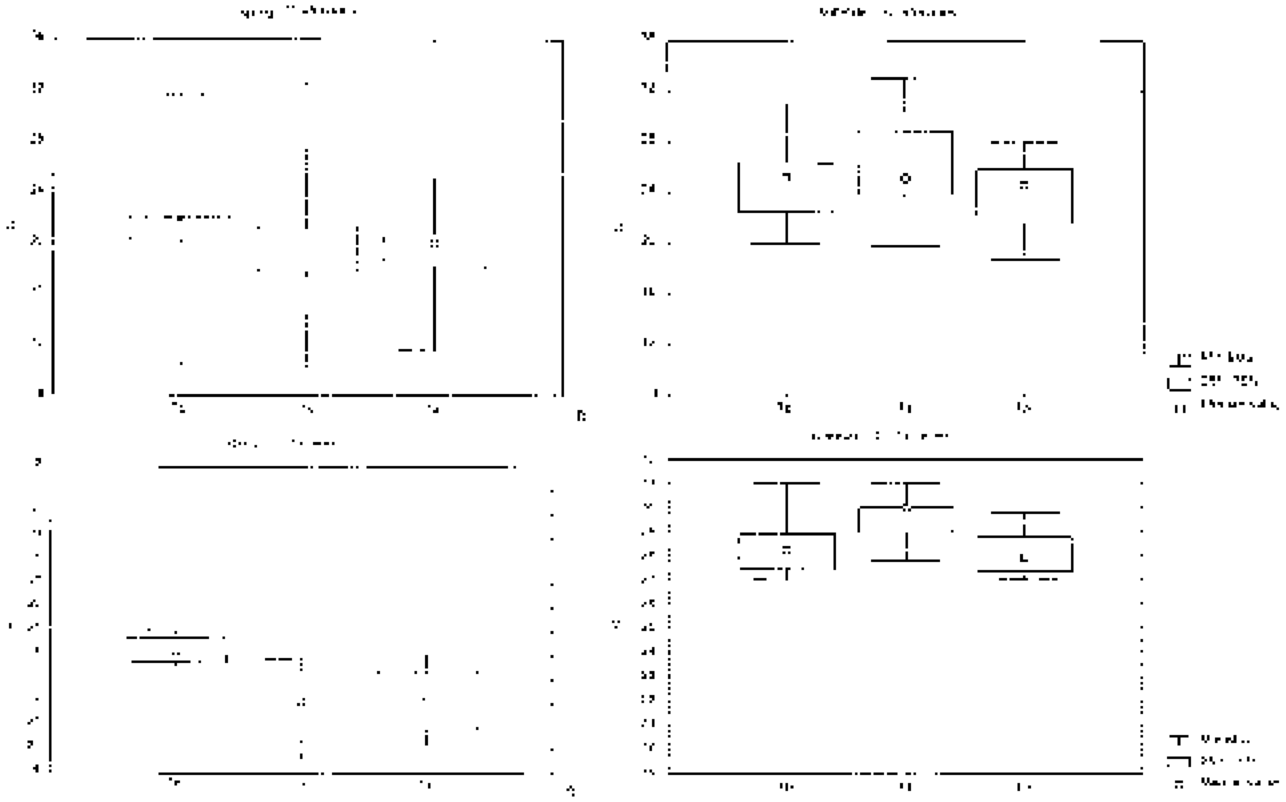

Fig. 1. – Body (Tb), substrate (Ts) and air temperature (Ta) measured in the field for *hamaeleo africanus* and *C. chamaeleon* in spring and summer.

TABLE 2

The correlation between Tb and Ta in spring and summer for the two chameleon species.

Tb vs. Ta	spring	summer
Chamaeleo africanus	Tb= 0.24+1.1 Ta, r=0.93	Tb= 1.98+0.95 Ta, r=0.96
Chamaeleo chamaeleon	Tb= 15.16+0.41 Ta, r=0.98	Tb= -1.87+1.07 Ta, r=0.85

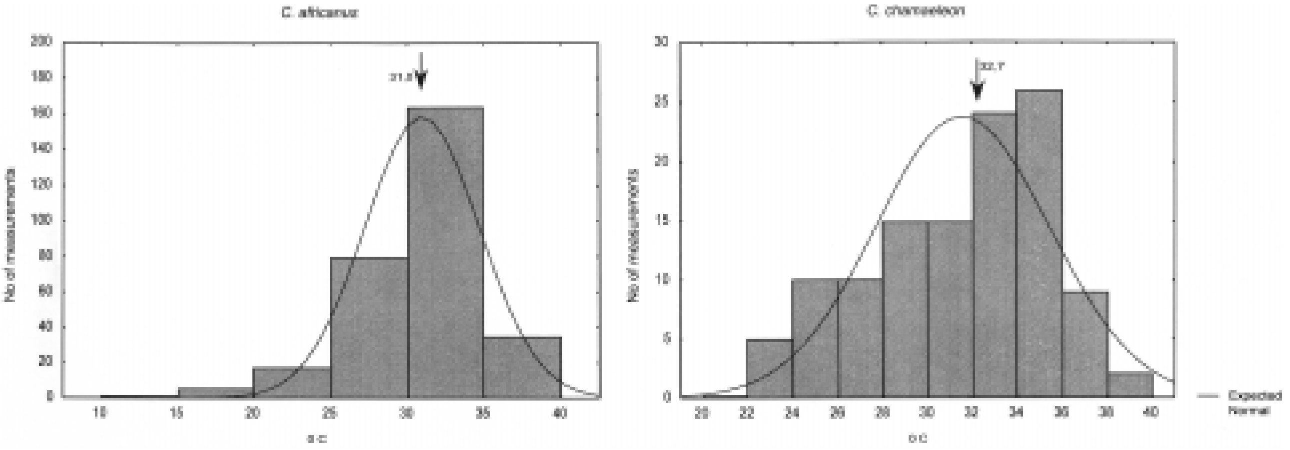

Fig. 2. – Body temperatures (Tb) for *Chamaeleo africanus* and *C. chamaeleon* measured in the laboratory. Arrows indicate the respective median values.

DISCUSSION

It should be noted that differences in the Tb between the two chameleon species studied have been found only in the field. Also Ts and Ta were different at the two different study areas. So it is possible that the difference between the Tb of the two species are due to the different environmental conditions, however, more controlled experiments are needed to clarify this possibility.

According to the range of the values of the two chameleon species studied, *C. chamaeleon* has a greater minimum value, both in the field and laboratory, while the maximum values are similar.

The preferred body temperature in the laboratory gradient is similar between the two examined species (Fig. 2).

The values of BURRAGE (1973) for *C. pumilus* and *C. namaquensis* at rest are lower than ours for *C. chamaeleon* and *C. africanus* (Table 3), although our data cover only two seasons (Fig. 1).

Body temperatures are lower when animals are at rest (Burrage, 1973) however our values are similar to the range of values for active *C. dilepis*, *C. pumilus* and *C. namaquensis* (Table 3).

The mean Tb for *C. namaquensis* at rest is much lower than for *C. chamaeleon* and *C. africanus* (Table 3). Both

TABLE 3

Body temperatures of chameleon species mentioned in the literature.

Species	Field		Laboratory		
	Range ºC	Mean ºC	Range ºC	Mean ºC	
C. dilepis	21.0-36.5	31.2			Stebbins, 1961
C. pumilus	3.5-37.0	22.4			
	(asleep 0.5-26.5)	—	7.0-30.0	25.0	Burrage, 1973
C. namaquensis	14.0-39.7 (rest 7-13 coastal, 9-16 inland)	28.7 (rest 10.6 coastal 12.3inland)	18.5-36.2	29.3	Burrage, 1973
C. namaquensis				33.5	Stebbins, 1961
C. africanus	10.4-31.6	23.1	15.6-38.2	31.0	Present study
C. chamaeleon	23.5-31.0	27.3	23.0-39.6	31.6	Present study

our values are more similar to those of active chameleon species from literature (Table 3), but our data cover only two seasons.

According to the laboratory data *C. namaquensis* is within the range of the two species we studied, while *C. pumilus* values are lower than those of the two Greek chameleon species (Table 3).

Stebbins (1961) gives a preferred body temperature range of 28.5-36.5°C for *C. namaquensis*. These values are within the range of the two studied species (Fig. 2). According to BURRAGE (1973), the mountainous species *C. bitaeniatus* and *C. hohnelii* are "active" in a similar thermal range in the laboratory, like *C. pumilus* and *C. namaquensis*.

The mean Tb values for *C. africanus* and *C. chamaeleon* in the laboratory, are similar to those for *C. namaquensis* but greater than those for *C. pumilus* (Table 3).

It seems that both chameleon species of the present study are thermoconformers because the slope of the equation Tb Vs Ta is near to 1. This is in accordance with HUEY & SLATKIN (1976). Thus, we are continuing this study because more data are needed.

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Congruence and conflict in *Albinaria* (Gastropoda, clausiliidae). A review of morphological and molecular phylogenetic approaches

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ABSTRACT. *Albinaria* is a pulmonate genus distributed around the north-eastern coasts of the Mediterranean, exhibiting a high degree of morphological and molecular differentiation, especially in southern Greece and in the external insular Hellenic arc. As a consequence, traditional taxonomists have named more than 200 taxa (species and subspecies). Three major revisions indicate the complexity of this taxon and several authors have questioned the validity of its current taxonomy.

Recently, the use of both molecular and morphological phylogenetic approaches, on similar taxa sets, showed contradictory results. In cases where the palaeogeographic history of the area occupied by the studied taxa was unambiguous and the terminal taxa studied were relatively few, morphological and molecular results almost coincided. However, in cases where landscape modifications were drastic and unstable, and the number of terminal taxa was high, these approaches came to conflicting conclusions. The above situation indicates the methodological limitations of each approach and implies that evolutionary processes within *Albinaria* do not follow a stable and uniform (spatial and temporal) pattern.

In the present contribution the above mentioned contradictory results and methodological shortcomings are discussed, and a re-evaluation of characters and is proposed. I suggest that interpretation of results must be phylogenetically consistent and only monophyletic taxa should be accepted. Additionally, rate-dependent clustering or rooting methods which resulted in the more unresolved, unstable and unjustified inter- and intra-specific relations should be avoided. Unresolved, unstable and conflicting inter- and intra- specific topologies implied that certain characters must be re-evaluated or avoided in phylogenetic analyses for *Albinaria*. Such characters are: morphometrics, genitalia, certain highly homoplasious shell characters or allozyme loci, and non-conservative or ambiguously aligned genes

KEY WORDS: *Albinaria*, phylogeny, biogeography, morphology, molecular, phenetics, cladistics.

INTRODUCTION

Albinaria is a pulmonate genus distributed around the north-eastern coasts of the Mediterranean (Fig. 1), exhibiting a high degree of morphological and molecular differentiation, especially in southern Greece and in the external insular Hellenic arc. As a consequence, traditional taxonomists have named more than 200 taxa (species and subspecies). Several major revisions indicate the complexity of this taxon (BOETTGER, 1878; WAGNER,

Fig. 1. – The distribution of the genus *Albinaria* (shaded area).

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1923, 1924; Nordsieck, 1977, 1999) and several authors questioned the validity of its taxonomy (Mylonas et al., 1987; Mylonas, 1992; Giokas, 1996; Douris et al., 1998a). Beside mere taxonomic tasks, the study of *Albinaria* differentiation can be very informative about evolutionary processes because of the high species number that does not appear to be associated with a commensurate amount of ecological differentiation (Gittenberger, 1991), and of the complex palaeogeographic and climatic history of the Hellenic region (Andel & Shackleton, 1982; Angelier et al, 1982; Bonneau, 1984; Paepe, 1986; Bertoldi et al., 1989; Anastasakis & Dermitzakis, 1990; Dermitzakis, 1990).

Until 1980 the taxonomy of *Albinaria* was based on shell and lamellae morphology through the viewpoint of traditional taxonomy. However, more recently new approaches were followed using 1) new characters, such as: a) qualitative and quantitative morphological characters, b) allozymes, c) mtDNA, d) rRNA, and 2) modern analytical methods, such as: a) phenetics and b) cladistics (see Table 1). Now, there is fair account of information available, as several studied cases regarding phylogenetic relations in *Albinaria* have been published. However, almost no approach used all *Albinaria* species; usually they are restricted to the study of taxa within certain geographical regions of Greece.

The use of both molecular and morphological phylogenetic approaches on similar taxa sets led to contradictory results. Nevertheless, all of these approaches have certain merits and drawbacks. This situation is indicative of the methodological limitations of each approach. As Hillis (1987) states "congruence between studies is strong evidence that the underlying historical pattern has been discovered, while conflict may indicate theoretical or procedural problems in one or both analyses, or it may indicate that additional data are needed to resolve the phylogenetic relationships in question".

The present study attempts to investigate a series of questions concerning the genus *Albinaria* that can be summarised as follows: a) which are the proposed phylogenetic relations between *Albinaria* species or monophyletic groups? b) are the results of phylogenetic analyses congruent with current taxonomy of the genus? c) which taxa appear to be paraphyletic or polyphyletic? d) how much effective is each approach in revealing robust phylogenetic relations? e) in what extent are the different approaches congruent?, and f) which are the evolutionary processes within *Albinaria*; is there a unique model of differentiation?

Therefore, in this study, these contradictory results and their shortcomings are presented, and the proposed evolutionary scenarios are discussed. An effective comparison of the various approaches concerning phylogenetic relations in *Albinaria* should inevitably take into consideration the fact that they dealt with sub-sets of *Albinaria* taxa, usually in the same biogeographical setting. Consequently, in order to compare the results of different studies these were divided into four categories corresponding to the following geographical regions of Greece: 1) Ionian islands, 2) Greek mainland, 3) Aegean region, 4) Crete. Finally, a last category, that is the whole of Greece, was added,. For each approach the following data were recorded (Table 1): a) character type, b) analysis methods, c) number of species/populations studied.

The following criteria were applied for evaluating the effectiveness of each approach: a) representative sample of taxa and populations, b) statistically significant sample of specimens studied, c) stability of topologies, d) degree of homoplasy, e) concordance with geography and palaeogeography, and f) consistency of methodology. Finally, the results of these studies were collated and compared according to their corroboration in the corresponding phylogenetic hypotheses.

TABLE 1

Presentation of studies on *Albinaria* phylogeny with data on: a) character type, b) analysis methods, c) number species/populations studied, for each study.

	Character type	Analysis methods	No of Species / populations studied
A. Ionian islands			
Kemperman, 1992	• genitalia	•UPGMA & DAF	4 / 12
Kemperman et al., 1992	• radula	• UPGMA & DAF	4 / 13
Kemperman & Degenaars, 1992	• allozymes	• UPGMA	8 / 45 (3 *Albinaria* & 1 *Isabellaria* species from eloponnisos & Crete)
Kemperman & Gittenberger, 1992	• shell qualitative-quantitative characters	• UPGMA & DAF	4 / 12
Giokas, 1996	• shell qualitative characters	• parsimony analysis	24 / 36 (5/8 Ionian taxa & the rest from mainland)

	Character type	**Analysis methods**	**No of Species / populations studied**
B. Greek mainland			
Schilthuizen et al., 1995	• rRNA (ITS1)	• parsimony analysi	5 / - (3 *Albinaria* & 1 *Isabellaria* species)
Giokas, 1996	• shell qualitative characters	• parsimony analysis	24 / 36 (1 *Sericata* & 3 *Isabellaria* species)
Douris et al., 1998b	• mtDNA (lrRNA) • shell qualitative characters	• neighbor-joining • parsimony analysis	10 / - (1 *Clausilia*, 1 *Sericata*, 1 *Idyla* & 3 *Isabellaria* species)
C. Aegean region			
Douris et al. 1995	• mtDNA (rest. site) • shell qualitative characters	• parsimony analysis	3 / 10 (1 mainland species: *A. grisea*)
Giokas, 1996	• shell qualitative characters	• parsimony analysis	12 / 31 (outgroup: *Sericata sericata*)
Douris, 1997	• mtDNA (COII)	• UPGMA • neighbor-joining • parsimony analysis	7 / 22 (3 mainland populations of: *A. grisea* & *A.voithii*)
Douris, 1997	• mtDNA (lrRNA)	• UPGMA • neighbor-joining • parsimony analysis	10 / 54 (12 mainland populations of: *A.grisea, butoti, discolor, puella, voithii & Isabellaria*
D. Crete			
Schilthuizen & Gittenberger, 1996	• allozymes	• UPGMA • parsimony analysis	13 / 38 (3 non-Cretan *Albinaria* & 1 *Isabellaria* species)
Giokas, 1996	• shell qualitative characters	• parsimony analysis	31 / 67 (outgroup: *Sericata sericata*)
Douris et al., 1998a	• mtDNA (lrRNA)	• neighbor-joining • parsimony analysis	18 / 27 (1 non-Cretan *Albinaria* & 1 *Isabellaria* species)
E. Greece			
Ayoutanti et al., 1993	• allozymes	• neighbor-joining	15 / 31
Ayoutanti, 1994	• allozymes	• UPGMA	15 / 31
Giokas, 1996	• shell qualitative characters	• parsimony analysis	68 / - (outgroup: *Sericata sericata*)
Douris, 1997	• mtDNA (lrRNA)	• UPGMA • neighbor-joining • parsimony analysis	27 / 39 (outgroup: *Isabellaria saxicola*)
Douris et al., 1998a	• mtDNA (lrRNA)	• neighbor-joining • parsimony analysis	23 / - (outgroup: *Isabellaria saxicola*)

COMPARATIVE PRESENTATION OF RESULTS AND DISCUSSION

Ionian islands

The study of KEMPERMAN (1992) on two Ionian islands (Kephallinia and Ithaki) was the first attempt to investigate phylogenetic relations of *Albinaria* taxa (*A. senilis*, *A. adrianae*, *A. contaminata* and *A. jonica*) in this region. Kemperman studied several types of characters; genitalia, radulae, allozymes, shell qualitative and quantitative characters (Table 1A). A series of important outcomes were revealed.

KEMPERMAN's (1992) results questioned the taxonomic importance of genitalia characters, as different groupings resulted according to the date of specimen collection (autumn, spring) and the method of analysis. Additionally, the distinction of nominal species was ambiguous (for example *A. senilis* and *A. adrianae* appeared to be polyphyletic).

KEMPERMAN et al. (1992) found strong indications for parallel evolution of radulae characters, and of correlation of teeth size with substrate. Therefore, radulae characters did not result to unambiguous nominal species distinction, as groupings of subspecies corresponded only partly with current taxonomy (only A. *contaminata* subspecies constituted a monophyletic group).

KEMPERMAN & DEGENAARS (1992) working with allozymes proposed the monophyly of the *Albinaria* species from Ionian islands, in respect to *Albinaria* species from Crete and Peloponnisos. Additionally, they found low genetic distances between *Albinaria* species from Ionian islands, as well as evidence for introgressive hybridisation. However, ambiguous species distinction resulted again, as only subspecies of *A. contaminata* constituted a monophyletic group.

KEMPERMAN & GITTENBERGER (1992) adopted the view that morphological characters represent two kind of components, viz., size and shape (HUMPREYS et al., 1981; POVEL, 1987). Size can be regarded as single scale factor and shape is composed of diverse factors. Their UPGMA analysis of dissimilarity matrices on size and shape components of morphometric characters resulted in different dendrograms according to “size” and “shape” differences.

Therefore, there were several drawbacks concerning the efficiency of these studies to reveal phylogenetic relations in *Albinaria*, as genitalia, radulae, allozymes and morphometry led to different groupings of the studied *Albinaria* taxa. However, it is surprising that when KEMPERMAN & GITTENBERGER (1992) combined (Dissimilarity matrix of All Factors-DAF) some sets of all the above characters types (genitalia, radulae, shell, and genetic distances), an unexpected distinction of nominal *Albinaria* taxa, concordant with current taxonomy, was obtained. Nevertheless, the use of rate-dependent clustering methods (UPGMA, DAF), puts into question the validity of the latter result.

GIOKAS (1996) studied all *Albinaria* nominal species from Ionian islands analysing shell qualitative characters via parsimony analysis. The results of this study confirmed the monophyly of each Ionian *Albinaria* species. Additionally, the revealed inter-specific relations did not support either NORDSIECK (1977, 1999) or KEMPERMAN & GITTENBERGER (1992), besides the fact that *A. contaminata* was found to be the more distant relative within Ionian *Albinaria* taxa. However, intra-specific topologies were rather unstable, as relatively high homoplasy was observed.

Greek mainland

There are few approaches concerning exclusively *Albinaria* taxa from the Greek mainland. Usually, with the exception of GIOKAS (1996), these studies used only a small portion of nominal *Albinaria* species. Additionally, they included certain *Isabellaria* taxa and populations, in an effort to investigate the questionable monophyly of the genus *Isabellaria* (GITTENBERGER, 1994).

All studies resulted to the same major conclusion, that *Isabellaria* is a polyphyletic genus. Certain supposedly *Isabellaria* species from Peloponnisos are placed within *Albinaria*, e.g. *I. haessleini* (SCHILTHUIZEN et al., 1995; GIOKAS, 1996; DOURIS et al., 1998b), *I. butoti* and *I. campylauchen* (GIOKAS, 1996; DOURIS et al., 1998b). There is a partial concordance between morphological and molecular results. In Douris et al. (1998b), the exclusion of certain morphological characters associated with the GCA clausilium (an homoplastic complex of lamelae (SCHILTHUIZEN et al., 1995; DOURIS et al., 1998b) from the analysis led to almost identical morphological and molecular topologies.

In some cases, however, inter- and intra-specific relations were unstable (GIOKAS, 1996; DOURIS et al., 1998b) or unresolved (GIOKAS, 1996), probably due to the homoplasy of several studied characters. This is a problem resulting from all character sets, but in the case of mtDNA and rRNA, because of the small number of taxa studied and their distant relationships, did not result to considerable drawbacks, as for example in GIOKAS (1996), where a relatively large number of populations and species of *Albinaria* was studied. However, GIOKAS (1996) succeeded to identify certain monophyletic *Albinaria* groups in this region, usually congruent with nominal taxa. Additionally, *Albinaria* taxa from Kythira and Antikythira (*A. cytherae, A. grayana, A. compressa*) constituted a monophyletic group which is a sister group to certain south-east Peloponnisian *Albinaria* taxa (*A. discolor, A. voithii, A. haessleini*).

Finally, according to GIOKAS (1996) differentiation of the genus *Albinaria* in this region could be biogeographically explicable in terms of a vicarianistic model partly consistent with the palaeogeography of this region (ANDEL & SHACKLETON, 1982), as there is a clear separation of *Albinaria* taxa from west Greece, as well as those from KYTHIRA-ANTIKYHIRA island group, and certain taxa from the formerly temporally isolated south Argolis (*A. mixta, A. profuga*) constitute a monophyletic group. Nevertheless, this pattern is modified by probable secondary dispersal phenomena. Consequently, it is obvious that more research is needed in order to resolve phylogenetic relations, especially between *Albinaria* taxa from Peloponnisos.

Aegean region

Several approaches (see Table 1 and below) have been attempted to investigate phylogenetic relations between *Albinaria* taxa distributed in this region. However, they usually use a small number of the nominal taxa of this area, probably with the exception of GIOKAS (1996).

The monophyly of certain nominal taxa (*A. discolor, A. turrita, A. puella*) is confirmed. However, there are some notable exceptions where current taxonomy is disputed. Namely, in all analyses (Ayoutanti et al., 1993; Giokas, 1996; Douris, 1997) two nominal species, *A. coerulea* and *A. brevicollis*, appear to be polyphyletic. However, when their populations were considered together they constitute a definite monophyletic group and consequently these two species are lumped. Another case of lumping concerns *A. unicolor* and *A. olivieri* (Giokas, 1996) two taxa distributed on the Karpathos-Kasos island group.

Douris et al., (1995) estimated a nucleotide divergence rate of about 5%/myr. Additionally, Douris, (1997), according to mtDNA results, supports that intra-specific differentiation is lower within island than mainland *Albinaria* species and that there is a strong indication for star phylogeny.

However, certain inter-and intra- specific relations are often unstable. There is no general agreement concerning inter- and intra-group topologies. In mtDNA analyses different topologies arise depending on the gene studied (COII or IrRNA), or on the methods of analysis (UPGMA, neighbor-joining, parsimony). Especially, phenetic methods resulted to unexpected, or biogeographically unjustified groupings (e.g. topology of *A. puella, A. lerosiensis* and *A. voithii*). Topologies resulting from different data sets (shell characters and mtDNA) conform only if the studied taxa and populations are few, as in Douris et al., (1995). Probably, instabilities are due to the insufficient number of specimens studied resulting to unknown intra-population variation. Additionally, it seems that when more than few populations are studied, the stability of the topologies is affected by the conservative nature of the studied genes, e.g. the less conservative COII yields more unstable topologies than the IrRNA. In that way the limitations of each approach are indicated.

The more coherent topology, in terms of its agreement with the palaeogeography of this area, comes from the parsimony analysis of shell qualitative characters (Giokas, 1996), besides the relatively high homoplasy observed. This topology definitely supports a vicarianistic model of differentiation of *Albinaria* in this region consistent with the sequence of the palaeogeographic events of this region (Angelier et al, 1982; Anastasakis & Dermitzakis, 1990; Noomen et al, 1996; Reilinger et al, 1997). There is a clear distinction of the monophyletic group of north-eastern Aegean taxa (*A. cristatella, A.puella, A. mitylena*), followed by the separation of one mainland species (*A. discolor*). Finally, there is a separation of southern and eastern Aegean *Albinaria* taxa (*A. munda*, *A. coerulea*, *A. turrita*, *A. olivieri*, *A. unicolor*). Within the latter there is a distinction between east and west distributed species.

Crete

Results from approaches concerning Cretan *Albinaria* taxa, have the most problematic interpretations, are the more diverse, and generally reveal strong incongruence. The monophyly of Cretan taxa is either disputed (Ayoutanti et al., 1987; Douris et al., 1998a) or favoured (Schilthuizen & Gittenberger, 1996). Paraphyletic or polyphyletic nominal species are common place, and all researchers agree that the current taxonomy does not reflect phylogeny accurately.

More specifically, Schilthuizen & Gittenberger (1996), working with allozymes, support the monophyly of Cretan taxa using parsimony analysis (using one *Isabellaria* species and 3 mainland *Albinaria* species as outgroup). Additionally, they confirm the monophyly of certain nominal species (*A. spratti, A. idaea*). However, in their UPGMA analysis, the outgroup mainland taxon *A. scopulosa* clusters within Cretan species. Moreover, the two kinds of analysis differ in the revealed inter- and intra-group topologies. Probably, this suggests that UPGMA suffers from its assumptions that evolution rates are homogenous. However, between islands and the mainland, there good reasons for rates being different. In spite of the fact that certain nominal species proved to be polyphyletic (A. *cretensis*, A. *hippolyti*), or paraphyletic (*A. corrugata,* which contains *A. moreletiana*), Schilthuizen & Gittenberger (1996) support the view that especially the latter does not consist a major taxonomic problem (as hard cladistic theory supports), and they claim that paraphyletic species can be considered as natural phenomena. These authors justify their opinion by proposing that isolated populations, resulting from the accidental colonization of single patches of suitable habitat, or being relicts of former, larger areas of distribution, may be regarded as genetically cut off from others. Such isolates, due to sufficient natural selection, genetic drift and/or founder effect, may move away from their original, balanced phenotype, and evolve into new species. Nevertheless, this argument, as the above authors admit, is weakened by the fact that the sample of *A. corrugata* to which *A. moreletiana* appears most closely related, originates from a distant part of *A. corrugata* range. However, these authors dispute the current taxonomy in the case of the polyphyletic *A. cretensis*.

Douris at al. (1998a), studying about half of the Cretan nominal species, support the non-monophyly of Cretan *Albinaria* species, as certain non-Cretan species from the Aegean region (*A. lerosiensis, A. coerulea, A. turrita*) or the Greek mainland (*A. discolor, A. grisea, A. butoti*) are placed within species from Crete. However, the revealed inter-group topologies have a low bootstrap support, and intra-group topologies arising from parsimony and neighbor-joining analysis differ in certain cases. Douris et al. (1998a) provide a biogeographic explanation for phylogenetic relations within Cretan taxa, supporting that there is a macro-geographical pattern of distribution of clusters along an east-west axis of the island. However, they either avoid to explain in biogeographic and palaeogeographic terms certain problematic topologies e.g. the position of mainland (*A. discolor*, *A. grisea* and *A. butoti*) and

Aegean taxa (*A. coerulea*, *A. turrita*) within Cretan taxa, or when they do, as their estimation of 1-1.2% differentiation rate per myr, they base their arguments (in the case of *A. lerosiensis*) on dubious palaegeographic data (see DERMITZAKIS, 1987; ANASTASAKIS & DERMITZAKIS, 1990; WESTEWAY, 1994), such as former connections of Dodekanissa with Crete. Finally, these authors identify certain monophyletic (*A. hippolyti, A. spratti, A. corrugata, A. torticollis*) and paraphyletic (*A. praeclara, A. eburnea*) nominal species.

AYOUTANTI et al. (1993), working on allozymes, provide intermediate results concerning the monophyly of Cretan *Albinaria* taxa. In their mid-point rooted neighbor-joining tree, *A. lerosiensis* is also placed within Cretan taxa, while *A. grisea, A. discolor, A. coerulea* and *A. turrita* are not.

GIOKAS (1996), determined certain monophyletic (*A. hippolyti, A. corrugata, A. eburnea, A. sublamelosa, A. violacea, A. manselli, A.torticollis*), paraphyletic (*A. teres*) and polyphyletic (*A. cretensis, A. idaea*) nominal *Albinaria* species, questioning, in several cases, the current taxonomy and the monophyly of Cretan taxa. However, the highest levels of homoplasy and many unresolved (hard or soft polytomies) and unstable topologies were observed in Cretan taxa. Probably, this was due to the large number of populations studied, or it may reflect more complex evolutionary phenomena. GIOKAS (1996) provided a partial geographic (East-West axis distinction of *Albinaria* groups in Crete), and palaegeographic interpretation (proposal for relict species). However, in central Crete, where accurate palaegeographic data are poor, and ecological and landscape alterations are frequent and drastic, it seems that secondary dispersal phenomena can modify unpredictably this evolutionary scenario, resulting to “noisy” information.

Greece

Analogous extreme discordance is apparent among analyses containing taxa from all over Greece. The neighbor-joining results of AYOUTANTI et al. (1993) suggest the distinction of mainland, island and Cretan species, with the exception of the insular species *A. lerosiensis* (Kos island) and *A. cytherae* (Antikythira island) that are clustered together with Cretan species. UPGMA results of AYOUTNANTI (1994) showed more complex groupings. Results of DOURIS (1997) support the non-monophyly of taxa distributed to adjacent regions and even though groupings from prior analyses are retained, high instability arises (low bootstrap values), and different analysis methods (UPGMA, neighbor-joining, parsimony) produce different inter- and intra-group topologies. DOURIS (1997) explains the low bootstrap values near the roots of the dendrograms by suggesting that at the first stages of the *Albinaria* evolution, differentiation was rapid (star phylogeny), and he does not discuss the option of methodological shortcomings. On the contrary, GIOKAS (1996) disputes the validity even of his own results, and suggests that in front of extreme unstable topologies phylogenetic interpretations are doubtful.

CONCLUSIONS

Both morphological and molecular systematic approaches have certain advantages for phylogenetic reconstruction. Thereby, studies that combine them can increase information content and utility. However, it is important to choose analysis methods that are as possible assumption-free and responsive to combination of data sets. This requires rate-independent methods of tree construction and rooting, as well as use of character-state data rather than distance summaries whenever possible (HILLIS, 1987). Such combinations of molecular and morphological studies could provide a comprehensive view of biotic evolution.

Nevertheless, all of these approaches on *Albinaria* phylogeny appear to have certain merits and drawbacks. The use of both molecular and morphological approaches on similar taxa sets showed contradictory results. The above situation is indicative of the methodological limitations of each approach and implies that evolutionary processes within *Albinaria* do not follow a stable and uniform (spatial and temporal) pattern. In cases where the palaeogeographic history of the area occupied by the studied taxa was unambiguous and the terminal taxa studied were relatively few, morphological and molecular results coincided. However, in cases where landscape modifications were drastic and unstable, and the number of terminal taxa was high, these approaches came to conflicting conclusions.

More specifically I suggest that: (1) Phylogenetic relations within mainland and Ionian *Albinaria* taxa are partly congruent with the palaeogeography of this area. Dispersal, or secondary contact phenomena have a useful supplemental explanatory role. Phylogenetic analyses result in conflicting results. However the validity of UPGMA results should be treated with caution. (2) The monophyly of most nominal species in the Aegean region, and the partial concordance (in most analyses) of their phylogenetic relations with area’s geography and palaeogeography, favours the hypothesis of a vicarianistic differentiation as the most probable evolutionary scenario for Aegean *Albinaria* taxa. (3) Different analyses resulted in significantly different topologies concerning Cretan *Albinaria* taxa. Questionable and unstable inter- and intra-group polyphyletic or paraphyletic topologies among Cretan *Albinaria* taxa imply complex evolutionary processes, resulting partly from vicarianistic phenomena, followed by secondary contact and introgression, as well as by species or populations range expansion and contraction. (4) *Isabellaria* is a polyphyletic genus, as in all analyses several former *Isabellaria* taxa are grouped within certain *Albinaria* species.

However, an overall resolution of the phylogenetic relations within *Albinaria* has not been achieved yet due to: 1) high homoplasy in certain cases, 2) certain morphological character incompatibility, 3) uncertainty in homologizing allozyme variants, 4) insufficient calibration and drawbacks of molecular techniques, 5) limitations of computing algorithms, 6) fragmented palaeogeographic and palaeoecological information.

Moreover, I support that interpretation of the results must be phylogenetically consistent. Consequently, lumping of *Albinaria* species forming robust monophyletic groups, and splitting polyphyletic nominal species should be favoured. As far as it concerns paraphyletic "species" several authors suggest that paraphyletic "species" may be a general phenomenon in nature (Hafner et al, 1987; De Queiroz & Donoghue, 1988; Schilthuizen & Gittenberger, 1996). However, I do not agree with the viewpoint of Schilthuizen & Gittenberger, (1996) that paraphyletic species are not evidence for incorrect taxonomy. Nominal species must not be accepted a priori. Robust parsimony analyses can identify paraphyletic "species". That means that formerly named species found to be placed within other species should not retain their former species status because in cladistic terms a species must be monophyletic.

Finally, rate-dependent clustering or rooting methods result in the more unresolved, unstable and unjustified inter- and intra-specific relations and should be avoided in phylogenetic analyses. Unresolved, unstable and conflicting inter- and intra- specific topologies imply that certain characters must be re-evaluated or avoided in phylogenetic analyses for *Albinaria*. Such characters are: morphometrics, genitalia, certain highly homoplasious shell characters or allozyme loci, and non-conservative or ambiguously aligned genes.

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Interannual changes in the composition of the macrobenthic fauna of Drana lagoon (Evros Delta, n. Aegean Sea): preliminary note

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ABSTRACT. The qualitative and quantitative composition of macrobenthic fauna of Drana lagoon, Evros Delta, was investigated during summer of 1981, 1997 and 1998. Since 1987 Drana lagoon has been isolated from the sea due to the obstruction of its narrow opening. In September 1998, samples were collected in an emerged and in a submerged site, since the major part of the lagoon was temporarily drained due to evaporation. An alteration in the faunal composition characterized by the vanishing of marine species and an increase of the dominance of the most resistant estuarine species (*Hydrobia* sp., *Hediste diversicolor*, *Gammarus aequicauda*), was recorded during July 1997 in relation to September 1981. This alteration may be the result of the lagoon's isolation from the sea. Furthermore, this isolation resulted in the temporary drainage of the major part of the lagoon during September 1998, when *Hydrobia* sp. showed a 98% dominance at the submerged site and was the unique species found at the emerged one. Number of species and total density together with biomass had comparatively higher values in September 1981 and in July 1997 samples, respectively. Species diversity and evenness showed significantly lower values in September 1998 samples while evenness had significantly higher values in July 1997 samples.

KEY WORDS: Interannual changes, Macrozoobenthos, Lagoon, Aegean Sea.

INTRODUCTION

Lagoons and estuaries of the Mediterranean have been the subject of extensive studies. However, our knowledge on the temporal changes of the qualitative and quantitative composition of their macrobenthic fauna is very restricted. Some relatively recent publications refer mainly to seasonal variation (e.g. GUELORGET & MICHEL, 1979a; 1979b; REIZOPOULOU et al., 1996; GOUVIS et al., 1997; KEVREKIDIS, 1997; ARVANITIDIS et al., 1999). Changes in the composition of the macrobenthic fauna occurring several years after the first records are of special interest, particularly if an alteration in the hydrological regime of a lagoon has been taken place during this period. It is known that hydrological factors must be responsible for the biological organization of Mediterranean lagoons rather than hydrochemical or sedimentological ones (FRISONI et al., 1984).

We studied the qualitative and quantitative composition of the macrobenthic fauna in Drana lagoon (Evros delta, N. Aegean Sea) during summer of 1981, 1997 and 1998. Since 1987 Drana lagoon has been isolated from the sea due to the obstruction of its narrow opening.

MATERIAL AND METHODS

The Evros Delta is located at the NE end of the Aegean Sea (Fig. 1). The Drana lagoon, one of the four lagoons of the delta (Fig. 1), is biologically significant since, among others, a large number of avifauna species inhabits the area. Fresh water occasionally enters the lagoon area mainly from the stream Mikri Maritsa and from drainage channels (Fig. 1). In the past the lagoon communicated with the sea through a narrow opening 4m wide. In order for the Drana lagoon to be drained, its opening was

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obstructed in 1987 and since then there has been no direct communication with the sea. However permanent drainage of the lagoon has not occurred until today. In September 1998, due to evaporation, the largest part of the lagoon was drained for a period of 3 weeks except for a small area (about 1/5 of the lagoon) in the site DA_1 (Fig.1), which remained submerged.

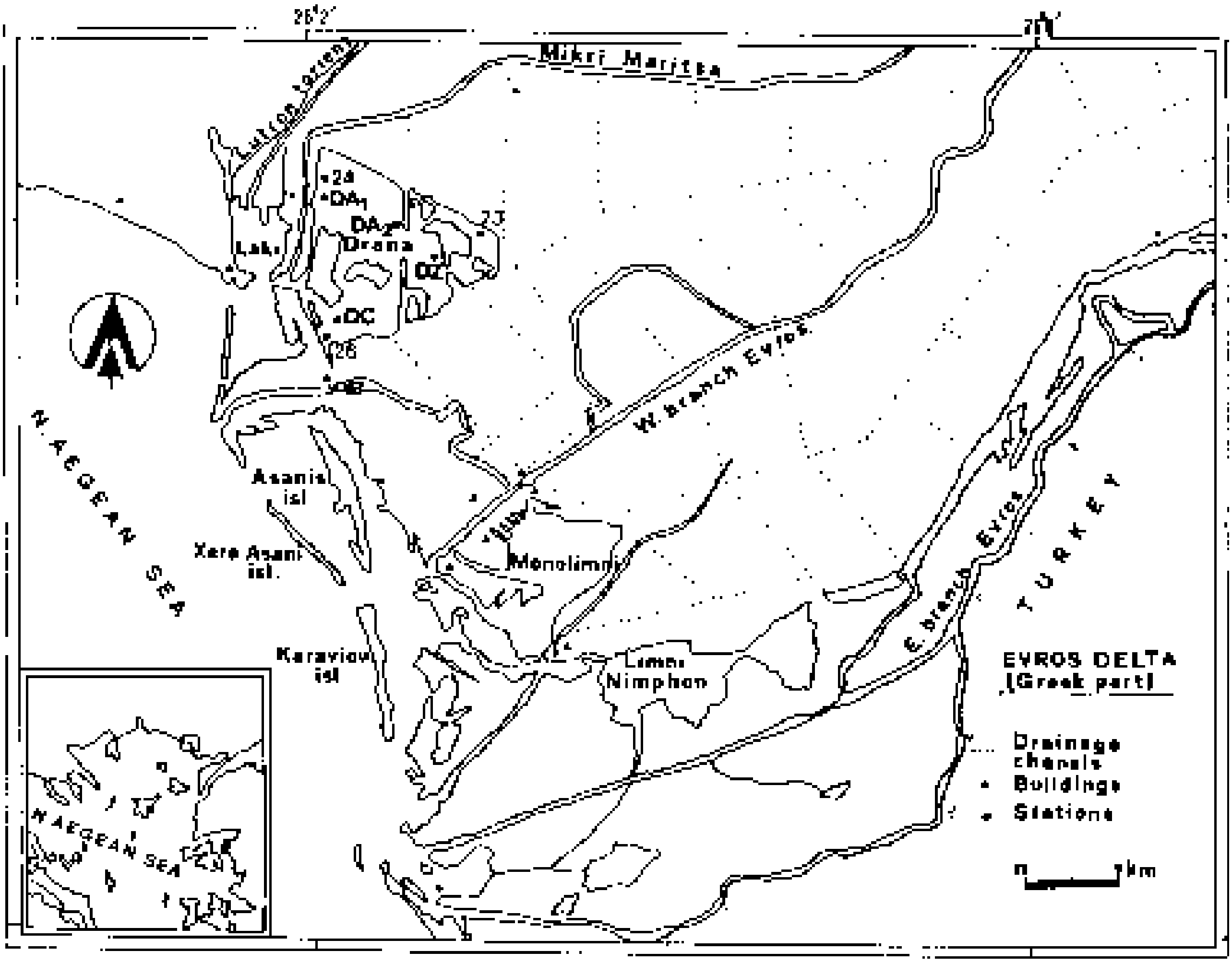

Fig. 1. – Map of Evros Delta showing the sampling stations.

Samples of the macrobenthic fauna were taken from Drana Lagoon at the sites 23, 24 and 26 in September 1981, DA_1, DA_2, DC and DZ in July 1997 and DA_1 and DC in September 1998 (Fig.1). Each sample was made up of 4 random replicates at each site in September 1981 and at DA_1 site in September 1998 and of 2 random replicates at each site in July 1997 taken by a modified Van Veen grab; the grab covered a surface of 400 cm^2 (20 cm x 20 cm) and penetrated to a depth of 20 cm. In September 1998, 2 random replicates were taken at the emerged DC site with the use of a corer having a 20 cm diameter and penetrating to a depth of 20 cm. The samples were sieved in the field through a 0.5 mm screen. In each sampling site, depth, salinity and the temperature of the water near the bottom, as well as the temperature of the sediment at a depth of 1 cm were also measured. These water physicochemical parameters were also measured at DA_1 and DC sites in September 1997. All animals were identified, counted and the wet weight was measured after drying on laboratory tissue. Bivalve and gastropod shells were not included in weight determinations.

In order to determine the similarity among species, cluster analysis was performed on faunal abundance data (R-analysis). The Bray-Curtis similarity coefficient was used on log transformed data [$x = \ln(x+1)$]. Hierarchical clustering of species was achieved using the group-average sorting strategy (Lance and Williams, 1967).

Species diversity (HY') was estimated using Shannon's formula: $HY' = \sum_{i=1}^{S} pi \log_2 pi$

Evenness (J) was measured by the formula $HY'/\log_2 S$, where S = total number of species. Statistical analyses [one-way analysis of variance, least-significant difference method (L.S.D.)] were performed on log transformed data ($x = \ln x$).

RESULTS

The values of the physicochemical parameters in the sampling sites are given in Table 1. The water salinity exceeded 40‰ in September 1981, varied between 14.8‰ and 17.0‰ in July 1997 and between 50.3‰ and 53.7‰ in September 1997 and had a value of 11.2‰ at the submerged site in September 1998.

TABLE 1

Water and sediment data of sampling sites

Date	Stations	Water Depth (cm)	Water Salinity (‰)	Water Temp. (°C)	Sediment Temp. (°C)
Sept. 1981	23	50	>40.0	22.0	21.7
Sept. 1981	24	15	>40.0	17.0	18.6
Sept. 1981	26	40	>40.0	21.2	20.6
July 1997	DA1	30	15.6	32.7	32.6
July 1997	DA2	55	14.8.	30.7	30.0
July 1997	DC	25	15.9	27.2	27.3
July 1997	DZ	40	17.0	29.3	28.0
Sept. 1997	DA1	20	50.3	20.2	-
Sept. 1997	DC	20	53.7	20.0	-
Sept. 1998	DA1	25	11.2	23.3	22.3
Sept. 1998	DC	-	-	-	-

A total of 12 macrobenthic species were collected during the three different sampling periods. The abundance, dominance and wet weight biomass of the species for each sample are given in Table 2.

Hierarchical classification of the species, on the basis of similarity in their distribution in the samples, is shown in a dendrogram (Fig. 2). At the 16.5% similarity level, 3 species groups were distinguished: group A, which includes the polychaetes *Capitella giardi* and *Scolelepis tridentata*, the bivalves *Abra ovata* and *Cerastoderma glaucum* and the mysid *Mesopodopsis slabberi*; group B which includes the gastropod *Hydrobia* sp., the polychaete *Hediste diversicolor* and the amphipod *Gammarus aequicauda* and group C, which includes the polychaete *Streblospio shrubsolii* and the amphipod *Corophium orientale*. The polychaete *Spio decoratus* and the decapod *Crangon crangon* were not included in any species group. The number of individuals, dominance and biomass of the species groups in each sampling period (Sept. 1981, July 1997, Sept. 1998) are given in Table 3.

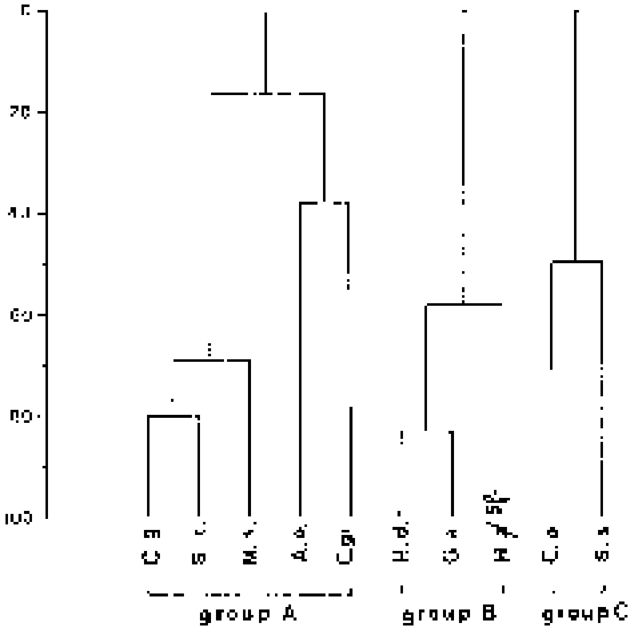

Fig. 2. – Dendrogram of hierarchical classification of species data.

A total of 10 species were collected during September 1981 (Table 2). B species group showed a high mean dominance, A species group had a remarkable mean dominance, while the cumulative mean dominance of *S. decoratus* and *C. crangon* was 0.6% (Table 3). *Hydrobia* sp. having a mean abundance of 270 ind./0.1m^2, a mean biomass of 128.3 mg/0.1m^2 and a mean dominance of 71%, was the only species of B group found in all samples (Table 2). >From the species of A group, *A. ovata* was found in all samples having a mean abundance of 186.3 ind./0.1m^2, a mean biomass of 62.0 mg/0.1m^2 and a mean dominance of 21.8% (Table 2). Moreover, *C. giardi* had a remarkable mean abundance of 9 ind./0.1m^2 and mean dominance of 4.5% (Table 2).

Only the three species of B group and one species of C group (*S. shrubsolii*) were found during July 1997 (Table 2). The gastropod *Hydrobia* sp., the sizable polychaete *H. diversicolor* and the amphipod *G. aequicauda* were recorded in all stations, having a mean abundance of 741 ind./0.1m^2, 126 ind./0.1m^2 and 127 ind./0.1m^2, a mean biomass of 280.0 mg/0.1m^2, 2356.0 mg/0.1m^2, and 327.0 mg/0.1m^2 and a mean dominance of 73.2%, 16.8% and 9.9%, respectively (Table 2). Consequently, B species group had a very high mean dominance (Table 3). *S. shrubsolii* (C species group) was only collected in DC site and had an extremely low mean abundance, mean biomass and mean dominance (Tables 2 and 3).

The above four species, together with *A. ovata* and *C. glaucum* (A species group) and *C. orientale* (C species group), were also found at the site DA$_1$ in September 1998 having, except *Hydrobia* sp., very low values of abundance and dominance (Table 2). *Hydrobia* sp. had a high abundance, biomass and dominance at this site, while it was the only species found at the emerged site DC (Table 2). B species group had a dominance of 99.5% in site DA$_1$, while the corresponding value of both A and C species groups was 0.25% (Table 2).

The major biological parameters (total density, total biomass, number of species, species

TABLE 2

Species abundance (A, ind./0.1m^2), dominance (D, %) and wet weight biomass (B, mg/0.1m^2) in the samples

	September 1981									July 1997									September 1998					
	23			24			26			DA$_1$			DA$_2$			DC			DZ			DA$_1$			DC		
Species	**A**	**D**	**B**	**A**	**D**	**B**	**A**	**D**	**B**	**A**	**D**	**B**	**A**	**D**	**B**	**A**	**D**	**B**	**A**	**D**	**B**	**A**	**D**	**B**	**A**	**D**	**B**
Gastropoda																											
Hydrobia sp.	151	83.9	65.6	347	38.0	182.5	312	90.9	136.5	234	65.4	122.4	851	83.3	550.4	660	71.7	148	1219	72.6	298.6	683	98.0	1201.9	33	100	40.5
Bivalvia	4	2.2	12.1	542	59.5	171.5	13	3.8	2.3													1	0.2	68.1			
Abra ovata (PHILIPPI, 1836)																											
Cerastoderma glaucum (POIRET, 1789)				3	0.3	215.3																1	0.2	300.8			
Polychaeta																											
Capitella giardi (MENSIL, 1897)	24	13.3	27.1	3	0.3	1.1																					
Hediste diversicolor (MULER, 1776)				3	0.3	10.9	3	0.9	202.3	108	30.2	4458.3	155	15.2	1258	150	16.3	278.5	91	5.4	3427.4	6	0.8	309.6			
Scolelepis tridentata SOUTHERN, 1914)				3	0.3	3																					
Spio decoratus BEBRETZSKY, 1870							5	1.5	0.6							3	0.3	0.3									
Streblopsio shrubsolii (BUCHANAN, 1890)																						1	0.2	.1			
Mysidacea																											
Mesopodopsis slabberi (VAN BENEDEN, 1861)				8	0.8	4.4																					
Amphipoda																											
Corophium orientale (SCHELLENBERG, 1928)																						1	0.2	0.2			
Gammarus aequicauda (MARTYNOV, 1931)				5	0.5	20.6	10	2.9	11.3	16	4.4	182.8	15	1.5	59.1	108	11.7	303.3	369	22.0	763	3	0.4	60.2			
Decapoda																											
Crangon crangon (LINNAEUS, 1758)	1	0.6	39																								

TABLE 3

Number of individuals (A, ind./0.1 m^2), dominance (D, %) and wet weight biomass (B, mg/0.1 m^2) of species groups, as well as the major biological parameters of the benthic ecosystem in each sampling period (mean values ± SE).

		Date Sites	**Sept. 1981** 23, 24, 26	**July 1997** DA$_1$, DA$_2$, DC, DZ	**Sept. 1998** DA$_1$, DC
S. decoratus	**A**		1.7±1.7		
	D		0.4±0.4		
	B		0.2±0.2		
C. crangon	**A**		0.3±0.3		
	D		0.2±0.2		
	B		13.0±13.0		
Group A	**A**		200.0±179.5		1.0±1.0
	D		26.8±17.5		0.2±0.2
	B		145.9±125.0		184.0±184.0
Group B	**A**		277.0±267.0	994.0±270.8	362.5±329.5
	D		72.6±17.1	99.9±0.1	99.6±0.3
	B		210.0±82.0	2963.0±990.0	806.0±766.0
Group C	**A**			0.8±0.8	1.0±1.0
	D			0.1±0.1	0.2±0.2
	B			0.1±0.1	0.2±0.2
Total Nr. Species			10	4	7
Nr. species/0.1 m^2			5.7±1.2	3.3±0.3	4.0±3.0
Nr. individuals/0.1 m^2			479.0±222.5	995.0±270.8	364.5±331.5
Wet weight biomass mg/0.1m^2			369±135	2935±1011	1414±1373
HY'			0.848±0.171	1.013±0.100	0.086±0.086
J			0.345±0.043	0.601±0.056	0.030±0.030
Description			The lagoon communicated with the sea	The lagoon was isolated from the sea	The lagoon was isolated from the sea and its major part was drained (DC site)

TABLE 4

Comparison between two and three sampling periods (Fisher LSD coefficient and factor-F correspondingly) concerning number of species, total number of individuals, total biomass, species diversity (HY') and evenness (J).

Sample group	**Nr. species**	**Nr. individuals**	**Biomass**	**HY'**	**J**
Sept. 81 - July 97	1.133	2.053	2.682	0.538	0.415*
Sept. 81 - Sept. 98	1.355	2.453	3.205	0.813*	0.628*
July 97 - Sept. 98	1.285	2.327	3.041	0.788*	0.608*
Sept. 81 - July 97 - Sept. 98	1.008	1.727	2.105	16.714**	46.515***
		where :	*P<0.05	**P<0.01	***P<0.001

diversity and evenness) of each sampling period are summarized in Table 3. Total number of species and mean number of species had comparatively higher values during September 1981 (Table 3). Total density and biomass had comparatively higher mean values during July 1997 (Table 3). Species diversity and evenness showed significantly lower values in September 1998 samples while evenness had significantly higher values in July 1997 samples (Fisher LSD coefficient, P<0.05) (Tables 3 and 4). The statistics of the differences in the number of species, total density, total biomass, species diversity and evenness, concerning the 3 sampling periods, are given in Table 4.

DISCUSSION

Species group B showed increased numbers of individuals and dominance in July 1997 and September 1998 samples, in relation to September 1981 ones. The 3 species of this group are resistant estuarine species. *Hydrobia* sp. was the only species that appeared in all samples collected from Drana lagoon during September 1981, July 1997 and September 1998. Moreover, it was the most dominant species in all sampling periods. *Hydrobia* species, being characteristic inhabitants of brackish waters, tolerate extreme salinity values and usually prefer areas isolated from the sea (e.g. MARAZANOF, 1969; KEVREKIDIS et al., 1996). *H. diversicolor* and *G. aequicauda* showed a remarkable abundance and dominance only in July 1997 samples. These species are considered characteristic inhabitants of brackish waters, showing a very euryhaline and resistant character and having usually higher abundance in areas isolated from the sea (e.g. KEVREKIDIS & KOUKOURAS, 1988; GOUVIS et al., 1998).

Species group A, including 2 estuarine species (*A. ovata* and *C. glaucum*) and 3 marine species (*C. giardi, S. tridentata* and *M. slabberi*), showed a remarkable dominance only in September 1981 samples. *A. ovata* and *C. glaucum* have been considered characteristic inhabitants of brackish waters preferring areas with a greater degree of contact with the sea, than *Hydrobia* species (e.g. FRISONI et al., 1984; KEVREKIDIS et al., 1996). *C . giardi, S. tridentata* and *M. slabberi*, together with *S. decoratus* and *C. crangon*, were not found in July 1997 and September 1998 samples. All these species are marine ones occurring more or less often in estuaries and lagoons (e.g. BELLAN, 1971; MAKINGS, 1977; HATZAKIS, 1982; HOLTHUIS, 1987; GRAVINA et al., 1988; ARVANITIDIS, 1994; GOUVIS et al., 1998).

Species group C including 2 estuarine species (*S. shrubsolii* and *C. orientale*) was found only in July 1997 and September 1998 samples, having a very low dominance. *S. shrubsolii* has been reported in enclosed, brackish European zones (e.g. SARDA & MARTIN, 1993). *C. orientale* is an exclusive inhabitant of lagoons being very euryhaline, but showing a less resistant character than *G. aequicauda* (e.g. KEVREKIDIS & KOUKOURAS, 1988).

The alteration in the faunal composition in July 1997 and September 1998 in relation to September 1981 characterized by the vanishing of marine species and the increase both in dominance and number of individuals of the most resistant estuarine species (*Hydrobia* sp., *H. diversicolor*, *G. aequicauda*) may mainly be the result of Drana lagoon's isolation from the sea. FRISONI et al. (1984) reported that hydrological factors must be responsible for the biological organization of the Mediterranean lagoons rather than hydrochemical or sedimentological factors, mainly because they control the rate of exchange with the open sea and consequently the renewal rhythm of certain marine elements necessary for the organisms (vitamins, trace elements, etc.). The small_difference in sampling times during the summer period of 1981 and of 1997 (September and July respectively) should not play a noticeable role in the alteration in the faunal composition since the species composition of an estuarine assemblage remains generally homogeneous throughout the year (BOESCH et al., 1976; GOUVIS et al., 1997; KEVREKIDIS, 1997).

Furthermore, the lagoon's isolation from the sea resulted in the temporary drainage of the major part of the lagoon during September 1998, when *Hydrobia* sp. showed a 98% dominance and an increased abundance at the submerged site and was the only species found at the emerged one. All *Hydrobia sp.p.* possess the ability to survive periods of emergence by burrowing into substrate and closing the operculum (BRITTON, 1985). Moreover, BRITTON (1985) reported that *H. acuta* is able to survive up to four months in a state of inactivity within damp clay or sand.

Species diversity and evenness showed significantly lower mean values in September 1998 samples while evenness had a significantly higher mean value in July 1997. Moreover, evenness mean values of September 1981 and 1998 samples being lower than 0.6 indicate, according to ODUM (1980) scale, that the assemblage was under pressure mainly during these periods.

Finally the development of the macrobenthic fauna of Drana lagoon after its reflooding is of a special interest and is the subject of research that is in progress.

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Adaptations of some amphibian species to Mediterranean environmental conditions

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ABSTRACT. Recent morphological and electrophoretic studies on the amphibian species *Rana ridibunda, Bufo viridis, Hyla arborea* and *Triturus alpestris* from northern and central Greece revealed a high degree of morphological and genetic variation. Morphological variation was assessed by colour patterns and several morphometric characters. Genetic variation was estimated from values of the parameters P (proportion of polymorphic loci) and H (mean heterozygosity), which were found to increase in the order: *Triturus alpestris* (H = 0.122) < *Hyla arborea* (H = 0.136) < *Bufo viridis* (H = 0.139) < *Rana ridibunda* (H = 0.149).

Furthermore, our studies on the reproductive biology of *R. ridibunda, B. viridis* and *H. arborea* revealed some differences concerning their reproductive patterns.

The results of our studies provide evidence that the morphological and genetic variability as well as the differentiation of reproductive patterns are partly adaptations to the ecological factors of the area.

KEY WORDS: Amphibia, reproductive period, morphometrics, genetic variation.

INTRODUCTION

The premises of ecological genetics are that natural populations are adapted to their physical and biological environments, and that the genetic mechanisms respond to environmental change. There are many studies on the phenotypic differences among species of amphibians in different environments, but the genetic factors responsible for these differences are known only in a few cases (DUELLMAN & TRUEB, 1994). The patterns of genetic variation within and among populations of a species may be related either to natural selection, random genetic drift, migration or breeding structure. Many amphibian species are adapted to a variety of environments and these species have been used to investigate the possible relationships between population genetic structure and habitat (AVISE & AQUADRO, 1982; NEVO, 1988; NEVO & BEILES, 1991).

Recent studies carried out on the Greek amphibian species *Rana ridibunda* Pallas 1771, *Bufo viridis* Laurenti, 1768, *Hyla arborea* L., 1758 and *Triturus alpestris* Laurenti, 1768, revealed a high degree of morphological and genetic variation (KYRIAKOPOULOU-SKLAVOUNOU,1980; KYRIAKOPOULOU-SKLAVOUNOU et al., 1991, 1992, 1997; KARAKOUSIS & KYRIAKOPOULOU-SKLAVOUNOU, 1995). This paper is an overview of the above reports, and an attempt is made to relate this morphological and genetic variability to the environmental conditions prevailing in this part of the Mediterranean region .

The reproductive patterns of the amphibians that breed in this region seem to be affected by the environmental conditions. Studies on the reproductive biology of *Rana ridibunda, Rana dalmatina* Bonaparte, 1840 and *Bufo viridis* (KYRIAKOPOULOU-SKLAVOUNOU, 1983; SOFIANIDOU & KYRIAKOPOULOU-SKLAVOUNOU, 1983; KYRIAKOPOULOU-SKLAVOUNOU & KATTOULAS, 1990; KYRIAKOPOULOU-SKLAVOUNOU & LOUMBOURDIS, 1990; KYRIAKOPOULOU-SKLAVOUNOU & ALEXIOU, 1994) showed that these species in Greece exhibit some differences in several components of their reproductive strategies, particularly the length of the breeding period, compared to those reported for the same species in northern Europe (e.g. HEYM, 1974; OBERT, 1975; TESTER, 1990; NOLLERT & NOLLERT, 1992). Besides, it is also known that patterns of reproduction in amphibians are modified by natural selection so as to produce strategies with high fitness, and they reflect a compromise among many selective pressures (DUELLMAN & TRUEB, 1994).

Since almost all the above species are geographically widespread in central and northern Europe, in this paper

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we compare our data concerning the Greek populations with those reported for the northern European ones, in order to point out the differences. In several cases comparisons were also made with conspecific populations from other Mediterranean countries. Some data, particularly those concerning the length of the reproductive periods of *B. viridis* and *H. arborea* are reported for the first time for this region.

MATERIAL AND METHODS

Reproductive period

The data on the reproductive activity and the duration of the breeding seasons of the species *R. ridibunda, H. arborea* and *B. viridis* are based on field observations that were made during the period 1979-1999. Most of our observations have been made in ponds situated close to the city of Thessaloniki, Gallikos river, Chalkidiki (Nikiti & Kassandra) and Lake Kerkini. Occasional observations have been made in many other localities of the country in north and south Greece. All localities situated around the city of Thessaloniki at a distance of 10 to 100 km were visited repeatedly during the breeding season. Our visits were more frequent at the beginning, the end, and the peaks of reproductive activity, and rarely during the remainder of the reproductive period. The temperature in °C of the water in ponds was measured during every visit.

Morphometric and Isozyme analysis

The materials and methods concerning the numbers of populations and specimens, and the morphometric and isozyme analyses have already been described in detail (Kyriakopoulou-Sklavounou et al., 1991, 1992, 1997; Karakousis & Kyriakopoulou-Sklavounou, 1995). All the examined populations are derived from northern and central Greece: three *R. ridibunda* populations from Lake Vistonis, River Gallikos and Lake Ioannina; two *H. arborea* populations from Kerkini and Halkidiki; five *B. viridis* populations from Thessaloniki, Nestos, Halkidiki (Nikiti & Kalandra) and Elassona; four populations *T. alpestris* from Agia Paraskevi, Metsovo, Pertouli and Kapenissi. The collected specimens were transported alive to the laboratory. Morphological variability was estimated by the description of coloration patterns and the measurement of several morphometric characters selected in each case.

Five morphometric characters widely used for discrimination between different taxa in the water frogs were measured in *R. ridibunda:* length of body (L), tibia (T), femur (F), digitus primus (DP), and callus internus (CI). From these characters ten ratios were calculated. Four morphometric characters were measured on the left and right sides of all specimens of *H. arborea:* snout-vent length (SVL), tibia (T) and maximum length (LI) and width (WI) of the inguinal loop. Seven morphometric characters were measured in all specimens of *B. viridis*: lengths of body (L), femur (F), tibia (T), digitus primus (DP) and callus internus (CI), distance between paratoid glands (LPG), and head width (HW). Mean values and standard deviations were computed. Differentiation among populations was investigated using the discriminant analysis (PROCs UNIVARIATE, STEPDISC; SAS 1988). Based on the morphometric characters Mahalanobis distances between the populations were calculated.

Horizontal starch electrophoresis was used for the enzymes and Hb). For the analysis of the serum transferrins and albumins, acrylamide gel electrophoresis was used. Blood samples were taken by cardiac puncture (using EDTA as an anticoagulant), and centrifuged. Skeletal muscle from each frog was homogenized in 0°C phosphate-phenoxyethanol-sucrose buffer using standard procedures (Nakanishi et al. 1969). Homogenates were stored at -25°C. For the electrophoretic analysis, 10% starch gels were used. Three different buffer systems were used. N-(-3-aminopropyl) morpholine/citrate buffer pH 6.1, described by Clayton & Tretiak (1972); for the enzymes, aspartate aminotransferase, malate dehydrogenase and phosphomannose isomerase (AAT E.C. 2.6.1.1, MDH E.C. 1.1.1.37 and PMI E.C. 5.3.1.8). Tris-citrate buffer pH 8,2 described by Ridgway et al. (1970); for the enzymes phosphoglucomutase and lactate dehydrogenase (PGM E.C. 2.7.5.1, LDH E.C. 1.1.1.27, and CK E.C. 2.7.3.2). Tris-LiOH-Boric buffer pH 8.2, described by Ashton & Braden (1961), for the esterases (EST E.C. 3.1.1.-), superoxide dismutase (SOD E.C. 1.15.1.1) and for the haemoglobin (Hb).

For the analysis of the transferrins and albumins, acrylamide gel electrophoresis was used. Conditions were as follows: electrode buffer=0.065 M Tris, 0.031 M Boric acid pH = 8.8; gel buffer=0.35M Tris, 0.056M HCL pH = 8.9. Running gel consisted of 10 ml acrylamide (30% acrylamide- 0.8% bis), 22.5 ml buffer 40 µl TEMED, 20 mg Ammonium persulfate (APS) and stacking gel of 1.5 ml acrylamide 9 ml buffer 10 µl TEMED and 10 mg APS. For the analysis of transferrins, the serum samples were treated overnight with 0.25% Ferrum ammonium sulfate at 4°C.

Electrophoretic data were analysed using the BIOSYS-1 computer package (Swofford & Selander, 1989). Allele nomenclature follows that of Allendorf & Utter (1979).

RESULTS

Reproductive period

The results on the duration of the reproductive periods of the three anuran species studied are shown in Table 1. In this table we present the extreme dates of beginning and end of the reproductive periods that we observed mainly during 1979-1989. Usually, the spawning season

TABLE 1

Maximum length of the reproductive periods of some anuran species in Greece and Central Europe. In parentheses the duration of these periods in months.

Species	Reproductive period Greece	 Central Europe
Bufo viridis	15 February-5July (4.5)	April-June (3)
Hyla arborea	26 February-5 July (4)	April-June (3)
*Rana ridibunda**	23 March-8 July (3.5)	May-June (2)

*The data on *R. ridibunda* are reported in Kyriakopoulou-Sklavounou, P. & M.E. Kattoulas (1990).

of *B. viridis* and *H. arborea* starts at the end of February or the first week of March and lasts until the first week of June, always depending upon the weather conditions. The spawning season of *R. ridibunda* starts about fifteen days later and lasts until the end of June or the beginning of July. It is obvious that all examined species are long breeders. The length of their spawning season ranged from about 3.5 to 4.5 months in northern Greece. In middle European countries their reproductive periods are much shorter (e.g. Heym, 1974; Obert, 1975; Tester, 1990; Nollert & Nollert, 1992). It should be noticed that in mountain habitats with altitude more than 500 m the reproductive activity of the same species can be delayed from fifteen days to one month.

We observed that temperature is the major factor that affects the initiation and the duration of reproductive activity of the three anuran species. In all cases, when the water temperature suddenly drops below the lowest limit for oviposition, the oviposition is interrupted and starts again when temperature rises. As the weather is unstable in spring, such interruptions may happen many times during the reproductive period. Rainfall, is mostly related with initiation of the breeding activity, particularly of the species *B. viridis* and *H. arborea*. Oviposition occurs when the water temperature ranges from 10°C to 25°C for *B. viridis* and *H. arborea,* and 15°C to 25°C for *R. ridibunda*. The optimal water temperatures for spawning of *B. viridis*, and *H. arborea* ranged between 16°C-20°C and 18°C-22°C, for *R. ridibunda*.

Genetic and morphological variation

Five polymorphic enzyme systems encoding eight loci (AAT-1, AAT-2, LDH-1, LDH-2, MDH-1, MDH-2, PGM, PMI) were found in *R. ridibunda* (Kyriakopoulou-Sklavounou et al., 1991). From the nine protein systems examined in *H. arborea* that represent 20 gene loci, four were found to be monomorphic (AAT, CK, SOD and Hb) and five polymorphic (ALB, EST, LDH, MDH and TRF) (Kyriakopoulou-Sklavounou et al., 1992). The six enzyme and three non-enzyme protein systems investigated in *B. viridis* encode 14 gene loci. Five of these loci were found monomorphic in all populations (AAT-1, EST-1, LDH-1, CK-1,Hb-1), and the other nine polymorphic (ALB-1, TRF-1, AAT-2, MDH-1, MDH-2, SOD-1, EST-2, EST-3, LDH-2) (Karakousis & Kyriakopoulou-Sklavounou, 1995). Finally, the ten enzyme systems investigated in the alpine newt *T. alpestris*, allowed the scoring of 14 gene loci. Six of them were monomorphic in all examined populations (SOD-1, SOD-2, ME-1, IDH-1, MDH-1, GPD-1) and the remaining eight were polymorphic (AAT-1, EST-1, EST-2, IDH-2, LDH-1, MDH-2, PGI-1, PGM-1) (Kyriakopoulou- Sklavounou et al., 1997).

The results on the genetic variation of the four amphibian species are shown in Table 2. The mean heterozygosity (H) among the four amphibian species studied ranges from 0.122 to 0.149 and the proportion of polymorphic loci (P) from 37.5% to 50 %. It is evident that the water frog *R. ridibunda* shows the greatest variability for both of the above genetic parameters.

TABLE 2

Genetic diversity of some Greek amphibian species. N: number of specimens (in parentheses the number of populations sampled). H: mean heterozygosity; P: proportion of polymorphic loci.

Species	N	H	P
(Urodela)			
Triturus alpestris	95 (4)	0.122	37.52
(Anura)			
Bufo viridis	112 (5)	0.139	47.12
Hyla arborea	51 (2)	0.136	38.46
Rana ridibunda	110 (3)	0.149	50.00

The results on morphological variation should be separated in two phases: (A) The description of pattern of external morphology and colouration observed in studied populations: (B) The morphometric data and their analysis.

The three populations of *R. ridibunda* differ in colouration, being bright green with a white belly at Ioannina, whereas *R. ridibunda* from Lake Vistonis are pale green with a yellow wash in the groin. The colouration of the Gallikos frogs resembles more that of Ioannina frogs. In morphometric analysis all populations showed a significant degree of morphological differentiation from one another. Of the ten morphometric ratios three, DP/CI, L/T and T/DP were selected in order of precedence as contributing significantly to the discrimination between the populations. The most distinct population was that from Ioannina (Kyriakopoulou- Sklavounou et al., 1991).

Tree frogs in northern Greece show colour and morphological variability. Colouration varies from bright green to dark brown and many individuals have a green-grey colour with dark spots on their backs. The most striking morphological variation of *H. arborea* in this region is the very narrow inguinal loop that many individuals have;

in some, it is completely absent. This variation is more apparent in populations inhabiting the sea shore zones. Morphometric analysis showed that the two populations differ only in body length, which was larger in the population of Halkidiki; this was the most significant discriminator between populations; when its effect was removed, no other variable differed significantly between populations (KYRIAKOPOULOU-SKLAVOUNOU et al., 1992).

External polymorphism is also great in the toad *B. viridis*. The colour of the dorsal pattern is usually grey or greenish to olive with defined greenish markings, often with dark edges. But in several animals these markings are very pale and in some others they are faint or even absent. Phyletic external polymorphism has also been observed. The females have red spots scattered on all the dorsal surface of their body. External polymorphism in *B. viridis* was greater in the population of Halkidiki, as in *H. arborea*. Discriminant analysis of morphometric characters indicated that the population from Elassona differed significantly from the others. The two variables that contribute most to the differentiation are tibia length (T) and body length (KARAKOUSIS & KYRIAKOPOULOU- SKLAVOUNOU, 1995).

Finally, it should noticed that for the species *R. ridibunda* and *B. viridis* it was found that morphological differentiation is related to altitude. Our studies also showed that morphological and genetic variability are not directly correlated.

DISCUSSION

In Table 1 it is shown that *B. viridis*, *H. arborea* and *R. ridibunda* in this region have very prolonged breeding periods compared to those of central Europe. In particular, it was found that *R. ridibunda* oviposits twice within this prolonged period (KYRIAKOPOULOU-SKLAVOUNOU & LOUMBOURDIS, 1990). According to our observations, temperature seems to be the most important environmental factor that affects the length of the reproductive period of the above species in this Mediterranean region; rainfall is the second one, mainly in relation to the initiation of breeding. Dependence of breeding activity upon temperature was also reported for the species *R. ridibunda* in Germany (e.g. HEYM,1974; OBERT,1975) and for *H. arborea* in Switzerland (TESTER,1990). Consequently, in central and northern European regions where temperature is usually lower, the reproductive period of the above species starts about one month later, and it is shorter. On the contrary, in Italy where climatic conditions are similar to those in Greece, the toad *B. viridis* breeds in March and April (GIAKOMA et al. 1997); *Rana esculenta* L., 1758, a water frog closely related to *R. ridibunda* breeds from late March to early July, very much like *R. ridibunda* in Greece (RASTOGI et al. 1983). From the above, it is concluded that the reproductive period of Greek amphibians is well adapted to the climatic conditions of this region.

Environmental conditions of this region are probably responsible for the genetic and morphological variation that was found in some Greek amphibian species. From the values of the mean heterozygosity (H) it is evident that intraspecific genetic variation increased in the following order: *T. alpestris* (H=0.122) < *H. arborea* (H=0.136) < *Bufo viridis* (H=0.139) < *Rana ridibunda* (H=0.146). These values are much higher than the average values of H reported for Salamandridae (0.058), Hylidae (0.051), Ranidae (0.075) and Bufonidae (0.105) (NEVO & BEILES, 1991). The mean heterozygosity found in Greek populations of *T. alpestris* (H=0.122) is higher than that reported by ARANO et al. (1991) (H_e = 0.109) for *T. a. alpestris* from west and central Europe, and lower than those of KALEZIC & HEDGECOCK (1980) (H_e=0.154) for *T. a. alpestris* in Yugoslavia. High genetic variation was also reported for the same species of anuran in Israel where the average heterozygosity was 0.088, 0.088 and 0.169 respectively for *Rana, Hyla* and *Bufo* (NEVO & BEILES, 1991). According to the above authors, the degree of variation is correlated with the species habitat. Species living under broader and unpredictable ecological spectra, such as *B. viridis* and *H. arborea,* generally have higher values of genetic variation than species living in a more stable environment such as *R. ridibunda.* However, our results do not coincide with those of (NEVO & BEILES, 1991). In Greece, *R. ridibunda* seems to have the highest genetic variation, which was also high in *H. arborea*. Generally, because heterozygosity estimates can be influenced by several evolutionary factors as well as by the choice of loci studied and laboratory conditions, differences in heterozygosity level should be interpreted with caution (AVISE, 1977). However, we will attempt to make some speculations about the factors responsible for this high genetic variation found in our studies. All the examined populations inhabit the same geographical region and face the same environmental factors. Such factors are the climatic conditions and the great heterogeneity of the environment. However, for *R. ridibunda* the average heterozygosity was 0.200 for the Vistonis population, 0.088 for Gallikos, and 0.159 for Ioannina. Thus, other factors should be examined. It is known that at Ioannina lake *R. ridibunda* lives in sympatry and hybridizes with *Rana epeirotica* Schneider et al., 1984 (SCHNEIDER et al. 1984; KYRIAKOPOULOU-SKLAVOUNOU et al., 1991); therefore, the high proportion of polymorphic loci suggests a gene flow from *R. epeirotica* into *R. ridibunda*. Similar reasoning could be adopted for the high heterozygosity at Vistonis lake where probably two species of water frogs, *R. ridibunda* and *Rana balcanica** live also in sympatry. Recently, it was reported that only the lake frogs of Thrace (the most north-eastern part of Greece including Lake Vistonis), belong to *R. ridibunda* while most of Greece is inhabited by a second species named *Rana balcanica* Schneider et al., 1993 (former *R. ridibunda*) (SCHNEIDER et al., 1993; SOFIANIDOU e*t al*., 1994). Another explanation is that the region of Vistonis represents a hybrid zone between *R. ridibunda* and *Rana bedriagae* Camerano, 1882 from Anatolia; it also suggests there is a gene flow from *R. bedriagae* to *R.*

ridibunda. From the above it is evident that more investigation is needed in order to obtain a complete data set.

Finally, it should be noted that the morphological variability revealed in all the examined amphibian species does not correspond to genetic variation. It seems more probable that these variations in colouration patterns in southern European populations could be considered as different ecotypes. The altitude seems to be the major factor that influences the differentiation of morphometric characters in *R. ridibunda* and *B. viridis,* and probably the temperature and aridity in *H. arborea*.

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Strategies in open field behaviour of *Mus spicilegus* and *Mus musculus musculus*

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ABSTRACT. The behaviour of *Mus spicilegus* and *Mus musculus musculus* was studied in a 15-minute open field test. A total of 12 behavioural events were recognized for both species. The dynamics and patterns of association of these behavioural events were analyzed. Significant sex-specific and species-specific differences were found in the dynamics of exploratory activity and also in some events of emotionality. Male and female *M. spicilegus* and *M. m. musculus* showed different patterns of behaviour in the experiment. Males demonstrated lower emotionality and appeared to explore the environment more actively than females. *M. m. musculus* showed higher exploratory activity than *M. spicilegus*.

KEY WORDS: exploratory behaviour, emotionality, behavioural strategies, *Mus spicilegus* and *Mus musculus musculus*.

INTRODUCTION

Mus spicilegus Petenyi 1882 and *Mus musculus musculus* Linnaeus 1758 are closely related species, which belong to different groups: outdoor mice and synanthropic mice. The distribution of *Mus spicilegus* and *M. m. musculus* in Bulgaria is restricted to the north of the Stara Planina Mountains. *M. m. musculus* is semi-synanthropic. *M. spicilegus* inhabits mainly agroecosystems. The populations of both species are sympatric during the spring-autumn period. At the end of autumn *M. m. musculus* comes back to houses, where it spends the winter. In autumn, *M. spicilegus* builds mounds of seeds and a complex of underground tunnels and nest cameras, which it uses in winter.

The differences in open field behaviour of many rodent species are interpreted on the basis of their ecological strategies and adaptation (FRYNTA, 1994). The behaviour of *M. spicilegus* has not yet been studied well. Only a few studies on the exploratory behaviour of *M. spicilegus* and *M. m. musculus* have been known so far (MESHKOVA et al., 1986; SOKOLOV et al., 1990; KOTENKOVA et al., 1994). According to KOTENKOVA et al. (1994) the behaviour of outdoor *musculus* is similar to that of outdoor forms of *spretus* and *spicilegus*, but SOKOLOV et al. (1990) showed that *M. spicilegus* is not as ecologically flexible as the synanthropic species. Therefore, more comparative data are needed to make a more precise comparison between the species of genus *Mus*.

In this context, the aim of the present paper is to study and compare the patterns of behaviour of *M. spicilegus* and *M. m. musculus* in an open field test.

MATERIAL AND METHODS

A total of 85 individuals of *M. spicilegus* and 58 individuals of *M. m. musculus* were tested. Adult and sexually mature animals were used in the test. Females were neither pregnant nor lactating. The mice tested had an average weight of 14.3±0.2 g for *M. spicilegus* and 14.8±0.2g for *M. m. musculus*. Both species were captured from wild populations in the same region of Northern Bulgaria (latitude 43° 22' N, longitude 24° 23' E). *M. spicilegus* and *M. m. musculus* were regularly captured and studied from spring to autumn for the period 1990-1993. The animals captured were individually housed in standard laboratory rodent cages for at least one week before the beginning of the experiments. The mice were kept in the laboratory at a temperature of 20°C±2°C, and in natural daylight. The animals were fed a mixed seed diet supplemented with carrots, apples and provided with water a*d libitum.*

Each animal was individually tested for 15 min (three series of 5 min intervals each) in the open field experi-

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ment. The experiments were performed in a glass terrarium (50x50x40 cm). The bottom was demarcated into 10 squares of equal dimensions (10x10cm) by painted dark lines. Mice were tested during their active phase in the morning and in the evening (METCHEVA & GERASIMOV, 1994). The data from the open field test were registered in protocols by shorthand.

The skulls of mice tested were measured craniometrically for species identification by special keys for craniometrical determination (GERASIMOV et al. 1990).

A total of 12 behavioural events were established in the experimental groups of *M. spicilegus* and *M. m. musculus* tested by the open field method: ambulation (the animals move actively within the experimental arena), rearing (the animals rear up on hind legs), jumping (the animals perform jumps), self-grooming (self-grooming is predominantly demonstrated as washing), defecation (self-explanatory), climbing (the animals attempted to climb the walls), gnawing (the animals gnaw at the walls, corners and the floor of the experimental arena), digging (the animals perform digging movements with hind legs), touching and moving of feces (the animals touch or move feces with their muzzles), urination (self-explanatory), standing still (the animals stop moving for a short time) and turning (the animals turn around themselves).

Horizontal activity (ambulation) was measured by the number of squares visited. Significance of differences between the mean scores of the observed behavioural events was estimated by the Mann-Whitney test. Principal coordinate analysis based on matrices of Pearson's product moment correlation was used to study the patterns of association among events of behaviour in male and female animals (LEGENDRE & LEGENDRE, 1983; JONGMAN et al., 1987). Then the correlation matrices were used in factor analysis with varimax rotation.

RESULTS

Ambulation of females of both species remained lower than that of males (Table 1, Table 2). Ambulation of male *M. m. musculus* was higher than that of male *M. spicilegus* during the first two time intervals ($P<0.001$). At the 1st and 3rd min interval the ambulation of female *M. m. musculus* was also significantly higher than that of female *M. spicilegus* ($P<0.05$) and ($P<0.01$), respectively. The rearing of male and female *M. spicilegus* was significantly higher than that of male and female *M. m. musculus* during 2nd and 3rd min intervals ($P<0.01$). The mean scores of self-grooming decreased significantly ($P<0.05$) both in male and female *M. spicilegus*, but increased significantly ($P<0.01$) both in male and female *M. m. musculus*.

TABLE 1

Mean scores (M), standard error (SE), significance of difference between sexes (P) for the behavioural events observed in *M.spicilegus* and proportion (%) of individuals performing the act.

		males (n=54)			females (n=31)			
Events	**code**	**M**	**SE**	**%**	**M**	**SE**	**%**	**P**
ambulation 5 min	Am	75.3	4.3	100	76.9	6.1	100	
ambulation 10 min		79.8	3.8		67.5	4.1		<0.05
ambulation 15 min		65.4	3.6		48.8	3.7		<0.01
rearing 5 min	Re	29.4	2.2	100	30.1	2.4	100	
rearing 10 min		40.4	2.3		36.7	1.9		
rearing 15 min		35.3	2.3		29.4	2.4		
jumping 5 min	Jm	0.1	0.1	43	0.0	0.0	29	
jumping 10 min		0.7	0.2		0.3	0.1		
jumping 15 min		1.8	0.6		0.7	0.3		
self-grooming 5 min	Gr	2.9	0.2	100	2.4	0.2		
self-grooming 10 min		1.4	0.1		1.5	0.1		
self-grooming 15 min		1.2	0.1		0.9	0.2		<0.05
gnawing	Gn	2.5	0.4	69	0.9	0.2	41	<0.01
standing still	St	1.1	0.2	48	1.2	0.3	58	
climbing	Cl	0.9	0.2	39	1.1	0.2	54	
defecation	Df	3.5	0.2	100	3.5	0.3	100	
touching	Tch	0.6	0.2	27	0.7	0.2	35	
digging	Dg	0.04	0.03	14	0.4	0.1	32	<0.05
urination	Ur	0.4	0.1	30	0.8	0.2	48	<0.05
turning	Tr	0.3	0.1	24	0.7	0.1	48	<0.05

TABLE 2

Mean scores (M), standard error (SE), significance of difference between sexes (P) for the behavioural events observed in *M.m.musculus* and proportion (%) of individuals performing the act.

		males (n=54)			**females (n=31)**			
Events	**code**	**M**	**SE**	**%**	**M**	**SE**	**%**	**P**
ambulation 5 min	Am	126.9	7.6	100	97.0	7.5	100	
ambulation 10 min		111.5	5.0		80.2	6.1		
ambulation 15 min		83.2	8.3		71.5	6.4		<0.001
rearing 5 min	Re	30.3	2.4	100	31.1	2.5	100	
rearing 10 min		24.5	2.0		20.1	1.0		
rearing 15 min		17.8	1.6		16.1	1.8		
jumping 5 min	Jm	12.0	4.2	90.3	2.8	0.7	74	
jumping 10 min		10.1	3.3		6.4	2.0		
jumping 15 min		9.6	3.8		4.4	1.7		
self-grooming 5 min	Gr	1.0	0.2	100	1.0	0.2	100	
self-grooming 10 min		1.7	0.2		2.0	0.2		
self-grooming 15 min		2.9	0.2		1.4	0.2		
gnawing	Gn	10.1	1.2	100	6.4	0.7	100	<0.01
standing still	St	3.1	0.3	100	5.6	0.5	100	<0.001
climbing	Cl	11.3	1.3	100	7.3	1.2	99.5	<0.01
defecation	Df	3.6	0.5	90.3	3.3	0.3	96.2	
touching	Tch	0.4	0.2	16.1	0.1	0.1	11.1	
digging	Dg	5.0	0.6	93.5	2.1	0.5	77.7	<0.001
urination	Ur	0.3	0.1	25.8	0.1	0.1	11.1	
turning	Tr	0.6	0.1	54.8	0.7	0.3	40.7	

Jumping was performed by almost all male and female *M. m. musculus* during the test, while it seemed to be a rear behavioural act performed by *M. spicilegus.* The pooled mean score (for 15 min) of this behavioural event was significantly ($P<0.05$) greater in males of *M. spicilegus* than in females of the same species. The counts of jumping of male and female *M. m. musculus* remained significantly higher than those of *M. spicilegus* ($P<0.01$).

Significant differences between male and female *M. spicilegus* were established in the mean scores of gnawing, digging, turning and urination (Table 1). A significantly ($P<0.05$) larger proportion of males than females performed gnawing during the experiment, while significantly ($P<0.05$) fewer males than females performed turning. Significant differences between male and female *M. m. musculus* were established in the mean scores of standing still, climbing and digging (Table 2). The counts of gnawing, standing still, climbing and digging of male and female *M. m. musculus* were significantly higher than those of both male and female *M. spicilegus* ($P<0.001$). Only urination had significantly higher mean scores ($P<0.01$) in female *M. spicilegus* in comparison with female *M. m. musculus*.

The above-mentioned sex-specific and species-specific differences in some of the behavioural events suggested that more general differences existed between the open field behaviour of *M. spicilegus* and that of *M. m. musculus*. To check this hypothesis a principal coordinate analysis, based on Pearson's product moment correlation coefficient of behaviour events, was used. Ambulation and rearing in male *M. spicilegus* are strongly correlated (Fig.1 A). A gradient from events associated with emotionality (left part of the diagram) towards exploratory behaviour (right part of the diagram) can be traced. Such events as self-grooming, standing still, and events representing mixed behavioural activity, tend to cluster near to the origin of the diagram. A different pattern of association between the different events is demonstrated in female *M. spicilegus* (Fig. 1 B). In this case behavioural events seem to be more diffusely associated.

Ordination diagrams of male *M. m. musculus* (Fig. 2 A) show that ambulation and rearing are connected with the 1st factor. The cluster between climbing-gnawing-digging is well expressed, therefore, the exploratory behaviour is related to the escape behaviour. The events: urination and touching and moving of feces are located at the top end of the diagram. A diffusion of behavioural events of female *M. m. musculus* is observed (Fig.2 B), with the exception of the group of events associated with exploratory and escape behaviour (lower right part of diagram).

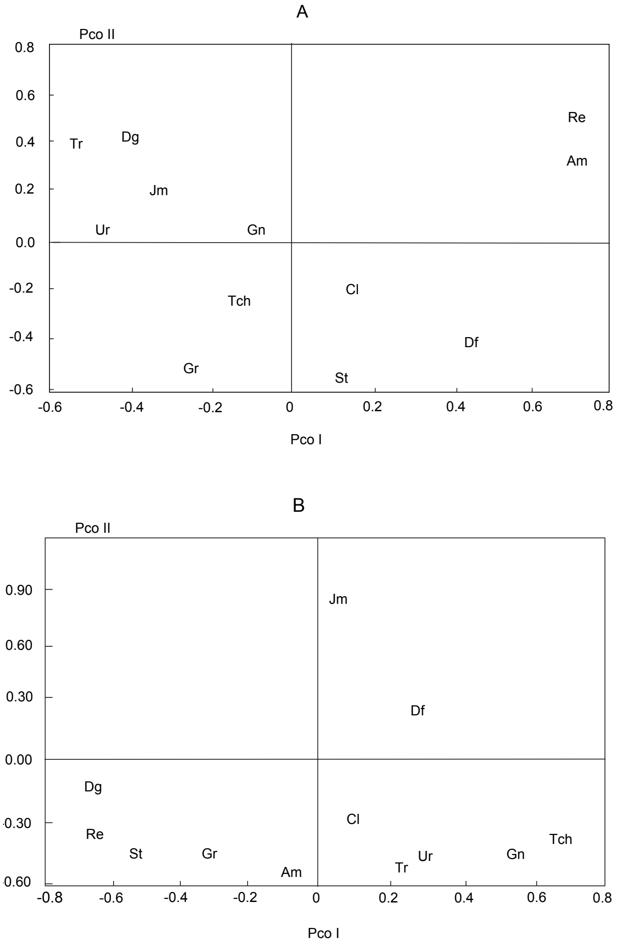

Fig. 1. – Strategies in open field behaviour of Mus spicilegus and Mus musculus musculus.

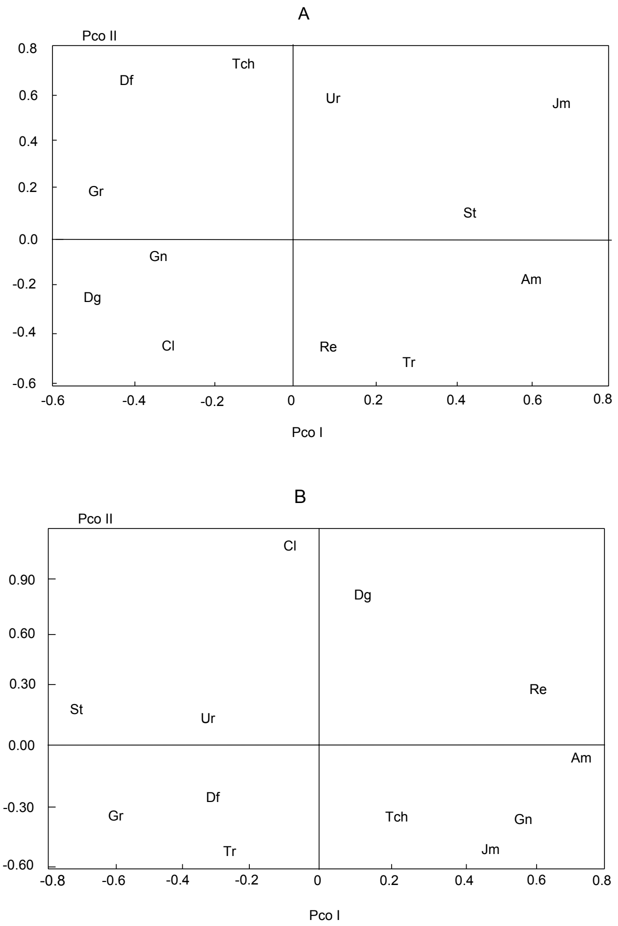

Fig. 2. – Strategies in open field behaviour of Mus spicilegus and Mus musculus musculus.

TABLE 3

Varimax factor analysis, based of Pearson's correlation coefficient of the behavioural events in males and females of *Mus spicilegus*. (*Events contributing to at least 25% from the variance in each factor).

	males				**females**			
factor	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
variances %	19.3	15.4	12.1	10.9	25.6	16.1	11.6	11.1
cumulative %	19.3	34.7	46.7	57.6	25.6	42.2	53.3	64.9
ambulation	0.8*	-0.2	0.08	-0.05	0.4	0.6*	0.01	0.5*
rearing	0.9*	-0.07	-0.02	-0.005	0.2	0.4	0.6*	0.4
jumping	-0.02	0.1	0.3	0.7*	-0.4	-0.5*	0.04	0.3
self-grooming	-0.5	-0.3	0.4	0.2	-0.2	0.8*	0.04	0.3
gnawing	-0.04	-0.03	-0.1	0.8*	0.2	0.4	-0.7*	0.4
standing still	-0.3	-0.3	-0.03	-0.3	-0.07	0.6*	0.3	0.03
climbing	-0.2	0.07	-0.4	-0.1	0.6*	-0.02	0.07	0.2
defecation	0.02	-0.6*	0.2	-0.2	0.07	-0.2	-0.1	0.8*
touching	-0.1	-0.1	0.7*	-0.3	0.4	-0.02	-0.6*	-0.09
digging	-0.01	0.8*	0.04	-0.1	0.2	0.1	0.7*	-0.07
turning	-0.04	0.7*	0.5	0.1	0.9*	0.07	0.04	-0.02
urination	-0.09	0.2	0.7*	0.2	0.7*	0.2	-0.09	0.08

Moreover, a factor analysis on the same correlation matrices was carried out in order to analyze further the pattern of association between behavioural events. This will help in the search of the mechanism that is responsible for sex- and species-specific differences in behaviour. The results of the varimax rotation of the factor matrices for male and female *M. spicilegus* showed that the first factor of male *M. spicilegus* was well correlated with ambulation and rearing. Thus, it was associated with exploratory activity (Table 3). The second factor seemed to be associated with emotionality. The third factor could be associated with marking activity and the last factor with escape behaviour. The factor solution of female *M. spicilegus* was clearly different from that of males and that was difficult to interpret. Ambulation, rearing, jumping and touching and moving feces had a high contribution to several factors. Only, the fourth factor seemed to be associated with defecation.

The first factor of male *M. m. musculus* seemed to be associated with exploratory behaviour and tendency to escape (Table 4). The events associated with the second factor showed that it could be linked to a greater extent with exploratory activity. The third and the last factors were probably associated with emotionality. The factor solution of female *M. m.musculus* showed that ambulation, rearing, jumping and gnawing were related to exploratory behaviour and closely associated with the 1st factor. Climbing and digging had high contributions to the 2nd factor, therefore, it was most likely associated with exploratory behaviour and tendency to escape. The 3rd factor was probably associated with emotionality.

TABLE 4

Varimax factor analysis, based of Pearson's correlation coefficient of the behavioural events in males and females of *Mus musculus musculus*. (*Events contributing to at least 25% from the variance in each factor).

	males			**females**			
factor	**1**	**2**	**3**	**4**	**1**	**2**	**3**
variances %	27.6	24.7	18.7	14.9	50.7	20.3	14.0
cumulative %	27.6	52.3	71.0	85.8	50.7	71.0	85.0
ambulation	-0.02	0.8	-0.2	0.3	0.9*	0.1	0.07
rearing	0.5*	0.4*	0.2	0.1	0.7*	0.3	-0.001
jumping	-0.6*	0.3	-0.06	-0.04	0.7*	-0.1	-0.001
self-grooming	0.1	-0.6*	0.2	0.6*	-0.3	-0.4*	0.7*
gnawing	0.8*	0.07	-0.1	-0.3	0.8*	-0.06	0.08
standing still	-0.02	0.002	0.6*	-0.02	-0.8*	-0.1	0.3
climbing	0.5*	-0.3	0.4*	0.3	-0.2	0.8*	-0.04
defecation	0.1	-0.3	-0.2	-0.08	-0.1	-0.08	-0.3
touching	0.008	-0.2	-0.08	-0.4*	0.3	-0.04	-0.2
digging	0.6*	-0.06	-0.1	0.1	-0.04	0.6*	0.4*
turning	0.1	0.2	0.9*	0.3	-0.08	-0.2	0.04
urination	-0.007	-0.02	0.002	-0.3	-0.08	0.005	0.09

DISCUSSION

The sex and species differences in occurrence and patterns of association between behavioural events suggested that there were differences in the general strategies of exploring a new environment used by males and females of *M. spicilegus* and *M. m. musculus.* Males of *M. spicilegus* and *M. m. musculus* demonstrated higher horizontal activity (ambulation) than females in the experiment. According to Mota (1987) high ambulation scores were usually seen as indicative of reduced fear in the animals and active environment exploration. Pooled mean scores of some events as jumping and gnawing were also significantly greater in males. On the other hand, events such as urination, turning, standing, usually associated with emotionality were significantly more frequent in females. Thus, males of *M. spicilegus* and *M. m. musculus* seemed to explore the environment more actively and maybe more efficiently than females. Exploratory activity in females, especially in females of *M. spicilegus*, appeared to be affected by emotionality to a higher degree. Ambulation and rearing were correlated well in male *M. spicilegus* as well as in female *M. m. musculus*. So it was neither a sex-specific nor species-specific correlation of these behavioural patterns. However, the behaviour of female *M. spicilegus* in the test seemed to be more influenced by emotionality in comparison with that of female *M. m. musculus*. The mean scores of urination and turning were greater in female *M. spicilegus* and the correlation between defecation and ambulation was better expressed. An emotional reactivity as reflected by plasma corticosteron was found to be higher in feral populations of *M. domesticus* compared with commensal populations of this species (Frynta, 1994).

M. m. musculus demonstrated higher exploratory activity than *M. spicilegus*. Moreover, *M. m. musculus* performed significantly more jumping, climbing and gnawing compared with *M. spicilegus*. The results showed that these actions were directed to active exploration of the surroundings and tendency to escape and probably they were connected with the commensal way of life. The relatively high proportion of climbing and jumping behaviour distinguished *M. m. domesticus* from *M. spretus* (Mota, 1987). Similar differences in exploratory behaviour were recorded between *Mus macedonicus* and *Mus musculus* (Frynta et al., 1994). According to Kotenkova et al. (1994) the differences in exploratory behaviour of outdoor and synanthropic forms of mice demonstrated the importance of living conditions in the formation of stereotypes of exploratory behaviour during evolution. Therefore, presumably the commensal way of life of *M. m. musculus* contributed to the faster orientation and adaptation to new and unknown environments and situations.

Rearing was significantly higher in *M. spicilegus* than in *M. m. musculus*, a behaviour most likely related to the open field habitat in which they lived. Bruell (1969) noted that the urination was an act of emotionality, but also connected with marking behaviour of mice in natural conditions. According to Archer (1973) defecation in new surroundings contributed to easier orientation of the animals on the basis of olfactory stimuli. The higher scores of urination of female *M. spicilegus* and the correlation between urination and touching and moving feces established for male *M. spicilegus*, suggested that marking activity had significant importance in orientation and exploration of the surroundings of this species. Sokolov et al. (1990) also established difference in marking activity between *M. spicilegus* and *M. m. musculus*. According to Sokolov et al. (1990) the marking activity was higher in *M. spicilegus*.

The presence of sex-specific and species-specific differences in the patterns of open field behaviour probably is connected with the different adaptation and ecological strategies of *M. spicilegus* and *M. m. musculus*, resulting from the way of life of each sex and species in the populations. However, some further studies on movements, migrations and social interactions between both species are needed to improve the knowledge of their ecological adaptation and relationships in populations.

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Growth variation of *Eunicella singularis* (Esper, 1794) (Gorgonacea, anthozoa)

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ABSTRACT. Gorgonian colonies of *Eunicella singularis* were collected randomly from Arethoussa and Phidonissi at Kavala bay (North Aegean Sea, Greece) using SCUBA diving. The following biometric macro-features were measured after stabilisation of colony dry weight (DW): maximum height, maximum width, total branch length and rectangular surface area. The allometric or isometric relationships were examined using the equation: log(Y) = log(b)+a*log(X) or Y=b*Xa (Y: Dry Weight and X: the four macro-features) The investigation of the relationships between the dry weight and the other four macro-features demonstrates the presence of two groups of parameters. The first group, which includes the height and the rectangular surface area, exhibits a variation of growth depending on the sampling sites. The second one includes the width and the total branch length and does not exhibit significant variations depending on the hydrodynamical characteristics of the site. These results indicate that the most appropriate parameters for estimating the growth of the *Eunicella singularis* gorgonian colonies during a population dynamic survey were width and total branch length.

INTRODUCTION

While the number of studies concerning the growth and the secondary production of gorgonian colonies increases (MISTRI & CECCHERELLI, 1994) there are only a few studies on gorgonian biometry and morphology (VELIMIROV, 1976; RUSSO, 1985; MISTRI, 1995). The choice of which macro-feature best describes the growth depends on the gorgonian species, and different authors use various macro-features. Thus, comparison of the results obtained by the use of too many different macro-features was not possible. *Eunicella* (*stricta*)*singularis* is a very common Mediterranean gorgonian but there are not many studies concerning the morphology and biometry of this species (THEODOR, 1963; WEINBERG & WEINBERG, 1979; SKOUFAS et al., 1996). The aim of the present paper was to give, for the first time, the most appropriate macro-feature that best describes the growth of *E. singularis*. In addition, we examine the relationships between dry weight and four biometric parameters, and the hydrodynamical conditions in the sample sites. Macro-features were chosen as candidates for the estimation of Dry Weight on the basis of their ability to be measured using SCUBA diving and without destruction of the gorgonian colonies.

MATERIAL AND METHODS

Eunicella singularis colonies were collected randomly using SCUBA diving in Kavala bay (North Aegean sea, Greece) at Arethoussa and Phidonissi. The depth range of the samples was 5-8 m and 9-13 m at Arethoussa (AR 5-8 and AR 9-13) and 5-10 m and 11-13 m at Phidonissi site (PH 5-10 and PH 11-13). Evaluation of the effect of hydrodynamics according to KAANDORP (1986) was based on the corrosion of plaster in the water. Comparison of the two sampling sites indicated that the Arethoussa site is more exposed than the Phidonissi one. The number of collected colonies at each sampling site was: AR 5-8 m: 31 colonies, AR 9-13 m: 39 colonies, PH 5-10 m: 45 colonies, PH 11-13 m: 48 colonies. The following macro-features were measured after stabilisation of the colonies: Dry Weight: (DW, mg) Height (H, mm) defined as the greatest height of the colony from the base to the apex, Width (W, mm) defined as the greatest width of the colony perpendicular to the axis, Rectangular surface area (HxW, mm^2) and Total Branch Length (TBL, mm) defined as sum of the length of all the branches of the colony. Allometric growth was examined using the relationships between the dry weight and the four macro-features. The equation of the simple allometry applied was $y=b*x^a$ and the logarithmic transformation logy=logb+

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a*logx (Gould, 1966). The isometric hypothesis (a=3 or a=3/2) was investigated using the t-test as suggested by Dagnellie (1975) and applied by Guillaume (1988). Comparison of the linear regression was examined using Bartlett's test as proposed by Snedecor & Cochran (1967).

RESULTS

The results from the measurements of dry weight and macro-features are given in Table 1. It was interesting to note that the colonies from the shallow water of the exposed site (AR 5-8) exhibited the higher values.

TABLE 1

Macro-feature values (mean ± standard error) of *Eunicella singularis colonies* at Arethoussa (AR 5-8 and AR 9-13) and Phidonissi (PH 5-10 and PH 11-13) sampling sites.

	Dry weight (mg)	**Height (mm)**	**Width (mm)**	**H x W (mm^2)**	**Total branches length (mm)**
AR 5-8 n=31	1373.744±181.107	162.161±6.758	96.355±7.027	16463.290±1656.301	1135.419±135.196
AR 9-13 n=39	1007.872±152.164	154.949±6.801	74.308±7.086	12727.179±1451.504	810.949±93.246
PH 5-10 n=45	802.711±77.733	129.822±4.550	68.467±5.107	9546.400±914.046	710.489±62.784
PH 11-13	1016.479±109.799	151.521±6.040	76.167±6.471	13068.729±1424.336	917.917±93.305

Relationships between DW and the four macro-features (H, W, HxW and TBL) were examined and two groups were distinguished. The first group included H and HxW, which exhibited a variability of relationships with DW that is related to sampling site. The second group included W and TBL; the relationships of these parameters with DW did not differ significantly in relation to the sampling sites.

$DW=b*H^a$

The linear regressions between log(DW) and log(H) at the four sampling (Fig. 1) sites show significantly different variances (Bartlett's test, d.f.=3, χ^2=33.752, p>0.05) and the four samples of gorgonians were considered as different populations. The investigation of those four relationships demonstrates that H was related to DW by a negative allometric relationship (a<3) (AR 5-8: t-test, d.f.=29, t=10.059, p>0.05; AR 9-13: t-test, d.f.=37, t=11.274, p>0.05; PH 5-10: t-test, d.f.=43, t=12.848, p>0.05; PH 11-13: t-test, d.f.=46, t=10.059, p>0.05). The ecological significance of this observation was that H increased more than DW.

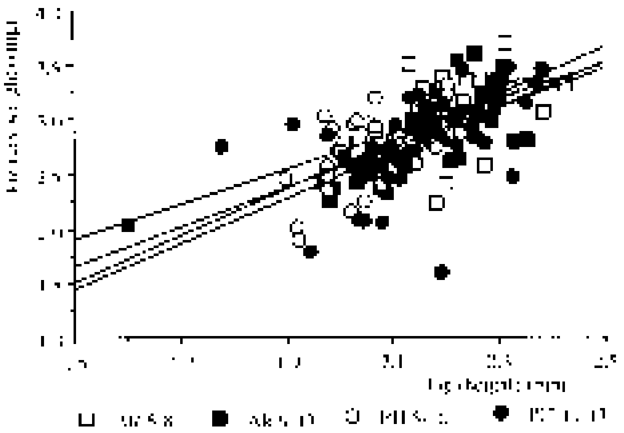

Fig. 1. – Linear regression between log(dry weight) and log(height) of *Eunicella singularis* colonies at Arethoussa (AR 5-8 and AR 9-13) and Phidonissi (PH 5-10 and PH 11-13) sampling sites. AR 5-8: $DW=0.486*H^{-1.595}$, n=31, r=0.441, p<0.05; AR 9-13: $DW=1.690*H^{-2.087}$, n=39, r=0.784, p<0.05; PH 5-10 $DW=1.742*H^{-2.162}$, n=45, r=0.671, p<0.05; PH 11-13: $DW=1.071*H^{-1.814}$, n=48, r=0.541, p<0.05.

$DW=b*(HxW)^a$

The relationships between log(DW) and the rectangular surface area log(HxW) (Fig. 2) were significantly different at the four sampling sites (Bartlett's tets, d.f.=3,

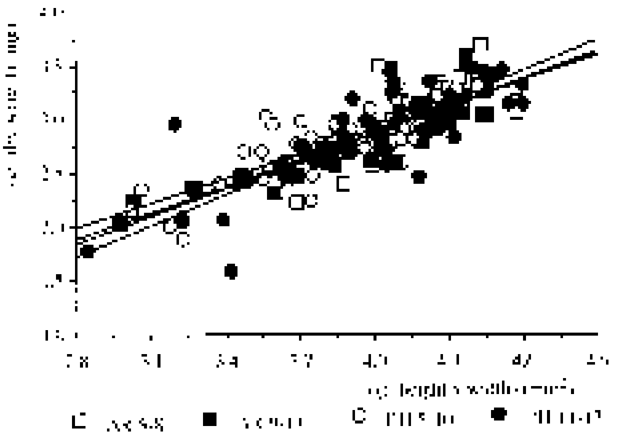

Fig. 2. – Linear regression between log(dry weight) and log(height x width) of *Eunicella singularis* colonies at Arethoussa (AR 5-8 and AR 9-13) and Phidonissi (PH 5-10 and PH 11-13) sampling sites. AR 5-8: $DW=0.961*(HxW)^{-0.961}$, n=31, r=0.755, p<0.05; AR 9-13: $DW=0.394*(HxW)^{-0.817}$, n=39, r=0.868, p<0.05; PH 5-10 $DW=0.145*(HxW)^{-0.762}$, n=45, r=0.814, p<0.05; PH 11-13: $DW=0.564*(HxW)^{-0.860}$, n=48, r=0.806, p<0.05.

χ^2=30.136, $p>0.05$) and the gorgonian colonies were considered as different populations. The isometric hypothesis (a=3/2) was also examined and accepted for the gorgonian samples from AR 9-13 and the two sites of Phidonissi (AR 9-13: t-test, d.f.=37, t=0.740, $p<0.05$; PH 5-10: t-test, d.f.=43, t=0.740, $p<0.05$; PH 11-13: t-test, d.f.=46, t=0.751, $p<0.05$). However, (HxW) was related to DW in the AR 5-8 gorgonian colonies, with a negative allometric relationship (a<3/2) (t-test, d.f.=29, t=2.003, $p>0.05$).

DW=b*(W)^a

Comparison of the linear regressions between log(DW) and log(W) (Fig. 3) did not exhibit significant differences (Bartlett's test, χ^2: 1.945, d.f.: 3, $p<0.05$) at the four sampling sites, and all the gorgonian samples were considered as one population. W was related to DW with a negative allometric relationship (t-test, d.f.=161, t=5.474, $p>0.05$).

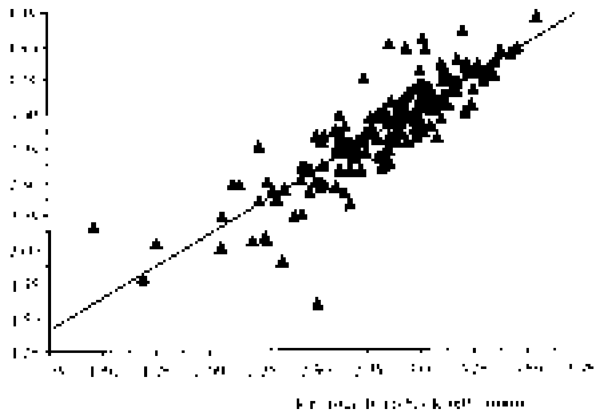

Fig. 3. – Linear regression between log(dry weight) and log(width) of *Eunicella singularis* colonies. The gorgonian colonies from the sampling sites of Arethoussa and Phidonissi were considered as one population. DW=0.984*W$^{1.039}$, n=163, r=0.808, $p<0.05$.

4DW=b*(TBL)^a

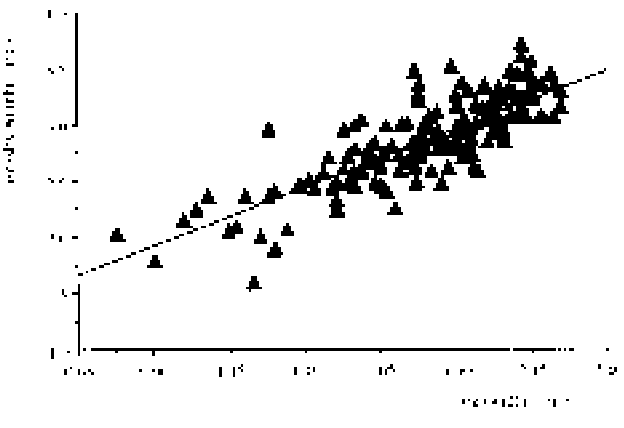

Fig. 4. – Linear regression between log(dry weight) and log(total branch length) of *Eunicella singularis* colonies. The gorgonian colonies from the sampling sites of Arethoussa and Phidonissi were considered as one population. DW=0.236*TBL$^{0.931}$, n=163, r=0.859, $p<0.05$.

The linear regression between log(DW) and log(TBL) (Fig. 4) did not exhibit significant differences (Bartlett's test, χ^2: 2.854, d.f.: 3, $p<0.05$) among the colonies from the four sampling sites. Therefore, for this relationship these colonies could be considered as one population. In this case the relationship between dry weight and TBL was a negative allometric one (isometry: a=3) (t-test, d.f.=161, t=1.988, $p>0.05$).

DISCUSSION

Gorgonian growth is a compromise between current intensity and feeding ability. Some species of gorgonian colonies, as for example *Eunicella cavolinii* (VELIMIROV, 1976), have reduced surface area which enables them to resist the water intensity. However, other authors (JEYASURIA & LEWIS, 1987; LOWENSTAM & WEINER, 1989; LEWIS et al., 1992) have demonstrated the ability of some gorgonians to increase their flexibility in order to increase their resistance to the water intensity. Our investigation proved that the *Eunicella singularis* colonies belong to the second group, and we have observed that the significantly higher colonies were located in the exposed site (Tab. 1). The question that arose when we observed the previous results was whether growth and the relationships between DW and the macro-features were affected by the hydrodynamic properties of the site. The present investigation demonstrates the presence of two groups of macro-features related to DW: the first group (H and HxW) exhibits a variability depending on the sampling site, and in the second one (W and TBL) the relationships between the DW and the biometric parameters are independent from the sampling site and are still constants.

The height of colonies is an easy parameter to measure, but is not the most appropriate parameter to describe the gorgonian growth. The linear regression growth curve exhibits significant differences among the colonies from the four sampling sites. Moreover, the observations of WEINBERG & WEINBERG (1979), also in *Eunicella singularis*, prove that the use of colony height is not appropriate because this biometric parameter could be affected by predation on the colony from other animals.

The growth curve obtained by the use of rectangular surface area as suggested by RUSSO (1985) also exhibits significant differences that depend on the hydrodynamic properties of the sampling sites. In addition to our observations, application of the same biometric parameter in the growth study of *Paramuricea clavata* gorgonians (MISTRI, 1995) did not give satisfactory results. Interesting were also the results in the present study obtained by allometric investigation. The isometry hypothesis relating DW to HxW was accepted in all the three sampling sites, except in the gorgonian samples from the shallow water of the exposed site (AR 5-8), which exhibit a negative allometric relationship. That difference between the colonies from AR 5- 8 and the other three sites could be explained by two hypotheses. The first one concerns circulation of the more important nutrients at the AR 5-8 site and a consequently higher feeding capacity of the gorgonian colonies. The second, and preferable, hypothesis suggests that this growth mode of the shallow water colonies at the exposed site is an adaptation to the strong spatial competition between gorgonians and the sponges *Aplysina aerophoba*. In situ observations indicate a high density of this sponge species within the hard substratum area covered by the gorgonian colonies.

The second, and more interesting, group of biometric parameters includes width and total branch length of the colonies. The linear regression between those macro-features and the DW did not exhibit significant differences that depended on the hydrodynamic characteristics of the environment. Thus, concerning those relationships, all the *Eunicella singularis* colonies can be considered as one population. According to the fractal growth process proposed by several authors (MANDELBROT, 1982; BURLANDO et al., 1991 ; KAANDORP & DE KLUIJVER, 1992; MISTRI & CECCHERELLI, 1993), the gorgonian width increases more regularly than the other biometric parameters. In addition, the use of the total branch length was also proposed by other authors (VELIMIROV, 1976; WEINBERG & WEINBERG, 1979) as a very interesting biometric parameter for the growth study.

In conclusion, in the present study we have indicated that the growth of *Eunicella singularis* followed a negative allometric relationship ($a<3$ and $a<3/2$) as has also been demonstrated in other cnidarian colonies (MIGNE & DAVOULT, 1993). The measurements of all the four macro-features that have been investigated did not involve the destruction of colonies, and they could be used for monitoring of the gorgonian population. However, the most appropriate biometric parameters applied to the gorgonian growth study seems to be W and TBL. The relationships between those parameters and the dry weight did not exhibit significant differences that depended on the hydrodynamic characteristics of the sampling areas.

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Multispecies Ichthyoplankton associations in epipelagic species: is there any intrinsic adaptive function?

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ABSTRACT. Species composition, distribution patterns and abundance of larvae of epipelagic fishes during June 1995 and June 1996, are described in waters of the northeastern Aegean Sea (eastern Mediterranean). Significant interannual differences were found that appeared to be associated with differences in environmental conditions. In June 1996 waters were colder, less saline and richer in zooplankton, which indicated a delay in the spring-to-summer transition during that year. Concurrently, larvae of the small-sized anchovy (*Engraulis encrasicolus*) were significantly more abundant in 1996 than in 1995. This contrasted with larvae of the middle-sized pelagics: *Sardinella aurita, Trachurus mediterraneus, Scomber japonicus* and *Auxis rochei*, which were most abundant in 1995. These results indicate differences in reproductive strategies among pelagic fishes, which we discuss in terms of 'income' versus 'capital' breeding, as well as inter-specific differences in the performance abilities of the larvae. The multispecies larval association of middle-sized pelagics may be adaptive and result from similar responses among species to the pelagic environment.

KEY WORDS: Engraulis encrasicolus, Sardinella aurita, Trachurus mediterraneus, Scomber japonicus, Auxis rochei, reproduction, early life history.

INTRODUCTION

Most marine teleost fishes have external fertilization and complex life cycles, characterized by an early meroplanktonic larval phase. During this relatively short period of the life cycle, individuals are extremely vulnerable to starvation, predation and unfavorable transport, and it has long been realized that the larval phase may have a potentially disproportionate influence on the local abundance of adult populations (HEATH, 1992; LEGGETT & DEBLOIS, 1994).

The timing and intensity of spawning in fishes is believed to be adaptive, reflecting the phase of the mean seasonal cycle of the environment favorable for offspring survival. Hypotheses proposed to explain the need for precise timing of reproduction have emphasized the relative importance of temporal variability in either food chain processes (CUSHING, 1990), predation pressure (HEATH, 1992), physical dispersive patterns of an area (SINCLAIR, 1988) or the interaction between physical forcing and conditions favorable for larval growth (CURY & ROY, 1989). Despite fluctuations in recruitment caused by environmental variability, reproductive strategies adapted to long-term average conditions of an area will result in maintenance of the local populations.

A well-known description of an adapted reproductive strategy can be found in capelin in Newfoundland coastal waters (FRANK & LEGGETT, 1982). Interestingly, the results of a subsequent study (FRANK & LEGGETT, 1983) indicated a synchronous emergence and resulting co-occurrence of capelin larvae and larvae of other demersal spawning species that led the authors to propose that multispecies larval fish associations are adaptive and result from similar responses among species to the pelagic environment. Under the general framework of multispecies approaches to the study of fish populations, 'an important consideration in larval assemblage studies is whether they have an intrinsic adaptive function' (MOSER & SMITH, 1993), as suggested by FRANK & LEGGETT (1983).

In the Mediterranean, the period of late spring-early summer, characterized by the development of the seasonal thermocline, is a transition period in the spawning of

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fishes (SABATES, 1990; SABATES & MASO, 1992; SABATES & OLIVAR, 1996). It coincides with the onset of the spawning season for those species that spawn in summer. The intent of this study was directed towards an understanding of the factors that influence the timing and intensity of spawning of the eastern Mediterranean epipelagic fishes, during the period of early summer. We followed a comparative approach, which examined co-variation in their larval distribution and abundance. Interannual variability in the assemblage of epipelagic fish larvae might trace variability in physical processes and be particularly useful in highlighting shared or contrasting adaptations of species to the pelagic environment.

MATERIAL AND METHODS

Larval fishes were collected during two surveys carried out in 15-22 June 1995 and in 6-14 June 1996 in the northeastern Aegean Sea (eastern Mediterranean). Sampling was based on transects running parallel to lines of longitude and spaced 10 nautical miles apart (Fig. 1). Sampling stations were located at approximately 5 or 10 nautical mile intervals on each transect in 1995 and 1996, respectively. In 1996, five additional offshore stations were sampled. At each station, a vertical profile of salinity and temperature was taken using a Seabird 19 CTD (conductivity/temperature/depth meter).

A 60-cm bongo-net sampler was used on both cruises. Mesh sizes on the sampler were 335 and 250 microns. Tows were double-oblique from within 5 m of the bottom to the surface or from 120 m depth to the surface at deep stations. Volume filtered was determined from a calibrated flowmeter in the mouth of each net. Maximum tow depth and volume of water filtered were subsequently used to standardize catches to numbers per m^2 (SMITH & RICHARDSON, 1977). More details are provided in SOMARAKIS ET AL. (1998). In the laboratory, all samples were sorted and larvae were identified to the lowest possible taxonomic level.

To provide a more powerful test for among years differences in the abundance of species, we bootstrapped 95% confidence intervals on resulting means (THORROLD & MCKINNON, 1995). We calculated 1000 bootstrapped estimates. A preliminary analysis showed that interannual differences in sampling intensity did not affect the results. Hence, results using all data are presented here.

RESULTS

Environmental conditions

The water column in the northern Aegean Sea presented typical early summer conditions in both 1995 and 1996. It was generally well stratified (Fig. 2) and characterized by a warmer, less saline, mixed surface layer, which was occasionally very shallow (e.g. transect No 8), separated by a thermo-halocline from the cold and more saline deeper layer. The mean depth of the thermocline, which was generally above 40m of depth, was 33.32m in 1995 and 25.18m in 1996. In general, the upper water column was colder and less saline in 1996 than in 1995 (Fig. 3A). Mean zooplankton displacement volume (ZDV, ml/m^2), which is a rough index of zooplankton production (SMITH & RICHARDSON, 1977), was twice as much in 1996 than in 1995 (Fig. 3A).

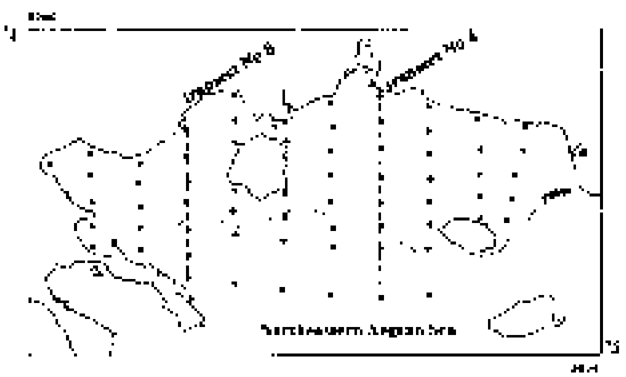
Distribution and abundance of epipelagic fish larvae

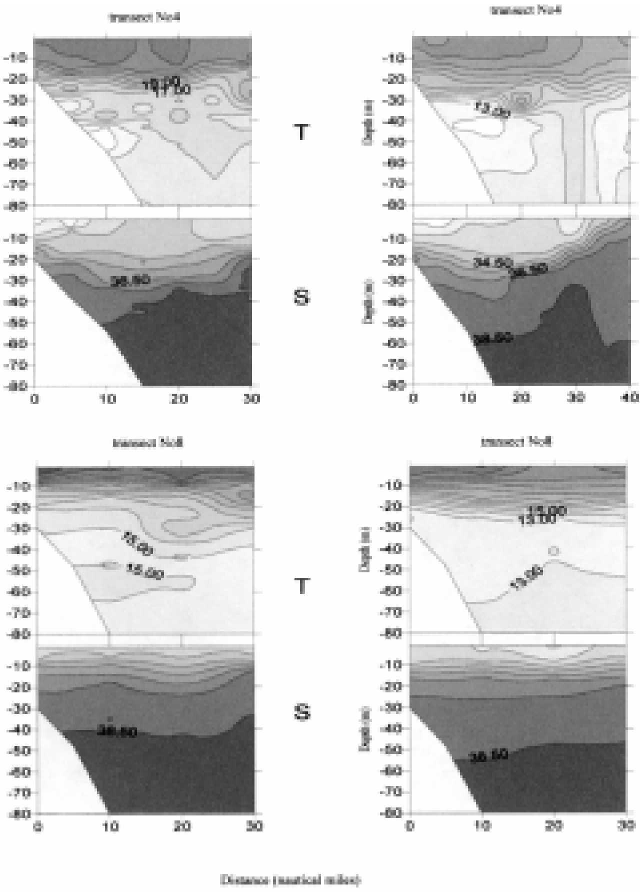
A total of seven epipelagic species were captured during the surveys (Table 1). Larvae of the winter/spring spawning *Trachurus trachurus* were very rare in the collections, and we did not consider this species in further analysis, since early summer marks the end of its spawning period (KARLOU-RIGA & ECONOMIDES, 1997). The scombrid *Euthynnus alletteratus* was captured at a single station, in 1995. The distribution and abundance of larval anchovy, *Engraulis encrasicolus*, and larvae of *Sardinella aurita*, *Scomber japonicus*, *Auxis rochei* and *Trachurus mediterraneus* are presented in Fig. 4. In general, their distribution was wide, particularly over the outer continental shelf, but there were marked differences in both frequency of occurrence (Fig.4, Table 1) and abundance (Fig. 3B, Fig. 4) between 1995 and 1996. Larvae of *S.*

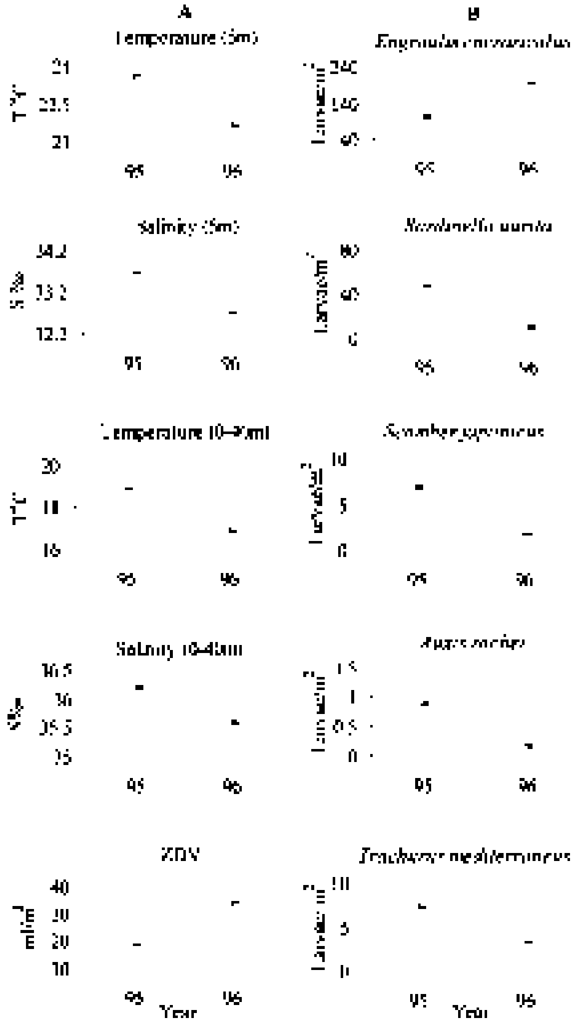
TABLE 1

Epipelagic larval species collected during June 1995 and June 1996 in the northeastern Aegean Sea. %P = % of positive stations. A = mean abundance (larvae/m^2).

Species	1995		1996	
	%P	A	%P	A
Sardinella aurita Valenciennes, 1847	93.44	43.03	56.10	7.91
Engraulis encrasicolus (Linnaeus, 1758)	100	99.65	100	186.54
Trachurus trachurus (Linnaeus, 1758)	11.48	0.16	12.20	0.15
Trachurus mediterraneus (Steindachner, 1868)	86.89	7.07	65.85	3.17
Auxis rochei (Risso, 1810)	44.26	0.82	4.88	0.13
Euthynnus alletteratus (Rafinesque, 1810)	1.64	0.03	-	-
Scomber japonicus Houttuyn, 1782	65.57	6.59	34.15	1.45







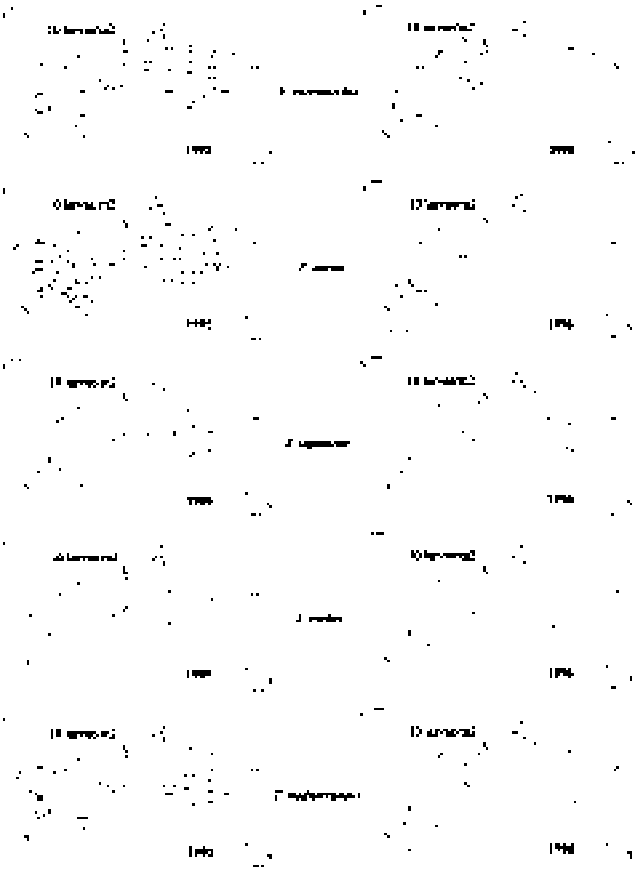


Fig. 1 (upper left). – Map of the study area showing the location of sampling stations. Black cycles = stations sampled both in 1995 and 1996. Open cycles = stations sampled only in 1995. Crosses = offshore stations sampled only in 1996.

Fig. 2 (upper right). – Vertical sections (transect No 4 and No 8 in Fig. 1) of temperature (T) and salinity (S). Isolines are contoured for every 1ºC temperature and 1 psu salinity.

Fig. 3 (lower left). – Means and 95% confidence intervals for 1995 and 1996. (A) Surface (5m) and upper water column (0-40m) temperature and salinity. Zooplankton displacement volumes (ZDV). (B) Abundance of epipelagic fish larvae.

Fig. 4 (lower right). – Distribution and abundance of epipelagic fish larvae. Diameters of disks are proportional to the logarithms of abundance.

aurita, *S. japonicus*, *A. rochei* and *T. mediterraneus* were more frequent and their abundance was significantly higher in 1995 than in 1996. On the contrary, the abundance of *E. encrasicolus* was higher in 1996 (Fig. 3B).

DISCUSSION

Substantial interannual differences were found in this study: In June 1996, waters were colder, less saline and richer in zooplankton, a fact indicating a delay in the spring-to-summer transition. Concurrently, larvae of the small-sized anchovy (*E. encrasicolus*) were significantly more abundant in 1996 than in 1995. In contrast, the larvae of the middle-sized pelagic fishes (*S. aurita, T. mediterraneus, S. japonicus* and *A. rochei)* were most abundant in 1995.

Studies in the Western Mediterranean (SABATES, 1990; SABATES & MASO, 1992; SABATES & OLIVAR, 1996) have shown that early summer coincides with the onset of the reproductive season of summer spawning fishes. These include the middle-sized pelagic species: *S. aurita*, *T. mediterraneus*, *S. japonicus* and *A. rochei.* The same period is characterized by the maximum spawning intensity for anchovy (PALOMERA, 1992; SOMARAKIS & TSIMENIDES, 1997).

According to FRANK & LEGGETT (1983), temporal and/or spatial covariation in the occurrence of larvae of different species in the plankton might reflect co-adapted reproductive strategies. For multiple spawning species, such as the Mediterranean pelagic fishes, covariation in spawning intensity, i.e. synchronous increases or decreases in batch fecundity and/or spawning frequency, resulting in concerted variations in the abundance of their larvae in the plankton, might also be adaptive, whereas contrasted variations might indicate alternative reproductive tactics. We hypothesize that small-sized pelagics have different reproductive tactics to middle-sized pelagics, which might be associated with differences in the performance abilities of their larvae.

Applying the terminology of life history evolution (STEARNS, 1992), we may label planktivorous short-lived small pelagic species as ‘income breeder’, spawning soon after energy for egg production becomes available. These species are characterized by substantial, ration-related short-term variations in batch fecundity and spawning frequency (PEEBLES ET AL., 1996). For example, reproductive studies of the Greek anchovy stocks (SOMARAKIS & TSIMENIDES, 1997 ; SOMARAKIS ET AL., 1997, unpublished data) indicate marked spatio-temporal variations in batch fecundity and spawning frequency, resulting in higher egg and larval abundance values when zooplankton biovolumes are much higher. Presumably, adult and/or larval prey densities are also higher. Because of the inherent interdependence between copepod life stages, income breeding may be seen as a seasonal fine-tuning of reproductive effort according to offspring survival probabilities (PEEBLES ET AL., 1996).

Longer-lived middle-size pelagics may be labeled as ‘capital breeders’, exhibiting a longer delay in their fecundity response, i.e., they use increased plankton production to acquire energy reserves and subsequently reproduce, thus their larvae are more likely to encounter lower prey fields.

If our hypothesis is reasonable, larvae of the middle-sized pelagics are expected to have higher performance abilities than larvae of anchovy. We provide some evidence of this in the next paragraphs.

The larvae of clupeoids (such as *E. encrasicolus* and *S. aurita)* are characterized by an elongate body, small fins, and a small terminal mouth. They are dependent on small sized, abundant and patchy prey, their swimming abilities are poorly developed, and they are capable of searching a small volume of water for food (HEWITT, 1981; HUNTER, 1981; BLAXTER & HUNTER, 1982). The size of prey eaten, as well as swimming speeds increase with size. WALLINE (1987) has estimated similar growth rates for *E. encrasicolus* and *S. aurita* larvae in the coastal waters of Israel. However, the latter are much bigger than anchovy (e.g. yolksac larvae of *S. aurita* are 40% bigger than *E.encrasicolus*, unpublished data) and presumably enjoy a substantial advantage in food searching and predator avoidance (MILLER ET AL., 1988).

In comparison to clupeoid species, larvae of scombrids (e.g. *Scomber japonicus*, *Auxis* spp.) and carangids (e.g. *Trachurus* spp.) share different morphologies and life history traits. Their body rapidly changes from an elongate shape to a deep body form with jaws and large eyes. They have fast growth rates (e.g. HUNTER & KIMBRELL, 1980; USHIDA, 1981; unpublished data), well developed swimming abilities, feed on increasingly large-sized, rare prey, and are capable of searching a large water volume for food (HUNTER & KIMBRELL, 1980; HEWITT, 1981; MATSUURA & HEWITT, 1995).

To sum up, larvae of the middle-sized pelagics have potentially superior performance abilities to larvae of the anchovy, the latter most probably increasing their chances of survival in low prey density - high temperature waters.

The existence of differences in the reproductive and early life history characteristics of epipelagic species has ecological but also management implications. From the fisheries perspective, factors affecting recruitment of the small-sized pelagic fishes might be substantially different from those affecting the middle-sized ones. Thus, there might be differences in the variability of the respective recruitment and subsequent catch rates, entailing separate management perceptions.

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Community structure and dynamics of the Molluscan Fauna in a Mediterranean lagoon (Gialova lagoon, SW Greece)

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ABSTRACT. The molluscan community of a shallow Mediterranean lagoon (Gialova lagoon, SW Greece, Ionian Sea) was studied on a seasonal basis during 1995-1996. A total of 23 species were recorded from the lagoon. Two of them namely: *Placida viridis* and *Polycerella emertoni* are reported for the first time from the Greek seas and the Eastern Mediterranean respectively. The dominant species were the gastropods *Bittium reticulatum, Cerithium vulgatum, Pirenella conica, Hydrobia acuta, Cyclope neritea* and the bivalves *Cerastoderma glaucum, Abra ovata*. Uni- and multivariate methods were employed to study the community structure and dynamics. On the basis of the multivariate pattern of the molluscan community a *coenocline* is observed which is strongly related with the degree of isolation. Two main zones with different faunal composition can be distinguished: one narrow zone close to the channel of communication with the sea and another in the innermost part of the lagoon. Following the *confinement scale* suggested for lagoonal ecosystems by GUELORGET & PERTHUISOT (1992) the first zone can be assigned to the "zone III" and the second to "zones IV-V". The distribution pattern of the molluscan community is governed by a different set of environmental variables in each season, discussed in detail. Although no disturbance due to anthropogenic impact was revealed, this narrow lagoonal habitat was proved to suffer severe "dystrophic episodes", during late summer and the beginning of autumn, but successfully recovered, demonstrating a seasonal community pattern.

KEY WORDS: Molluscs, coastal lagoons, population structure and dynamics, environmental impact studies, Ionian Sea.

INTRODUCTION

The distribution and dynamics of macrobenthic populations living in coastal lagoons are strongly influenced by fluctuations in the physicochemical factors, induced by the mixing of freshwater and sea inflows (AMANIEU & LASSERRE, 1982). Anthropogenic pollutants (domestic, industrial, oil discharges, etc.) transported by rivers and sea waters, interacting with the unstable environmental conditions also contribute to perturbations of the ecosystem (LARDICCI et al., 1997). In such highly variable environments, complementary long and short term studies, carried out in a range of different biotopes, are required to achieve a better understanding of the synergistic or specific actions of natural or man-made factors on the spatial and temporal benthic community patterns (STORA et al., 1995). Molluscs are either the dominant group or an important component of the macrobenthic fauna of European lagoons (BARNES, 1980; GUELORGET & PERTHUISOT, 1992). Biological and ecological aspects of mollusc species distributed in lagoons have been studied in detail (e.g. IVELL, 1979; BARNES, 1980; GUELORGET & MAYERE, 1981). The role of certain abiotic factors (mainly salinity and sediment type) in the distribution pattern of the molluscan communities has also been investigated (e.g. ZAOUALI, 1975; BARASH & DANIN, 1982; BOURGOUTZANI & ZENETOS, 1983). However, mechanisms structuring lagoonal animals and in particular molluscs have only recently received considerable attention (e.g. NICOLAIDOU et al., 1988; MCARTHUR, 1998), but still we are no nearer to putting them in rank order of importance (BARNES, 1994). Furthermore, assessment of disturbance (natural or anthropogenic) in this type of ecosystem is

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generally unreported (Reizopoulou et al., 1996; Lardicci et al., 1997).

Gialova lagoon is one of the 10 major lagoons of Greece with an important fisheries value and of great naturalistic interest, as it has been characterised as one of the important bird areas in Europe (Koutsoubas et al., 1999 and references therein). Recently, this coastal ecosytem suffered an oil spill impact in October 1993 leading to the extensive mortality of fish when the oil tanker “Iliad” hit bottom in the entrance of the neighboring Navarino bay (Dounas et al., 1998 and references therein). The oil spill incident was the reason for a multi-disciplinary study investigating the structure and functioning of Gialova lagoon along with the long-term impact of the oil spill on the biota.

The present contribution aims: (a) to analyse in detail the molluscan distribution pattern in the lagoon, (b) to investigate the key environmental factors affecting the molluscan community structure and (c): to assess the level of disturbance in the ecosystem through the molluscan biota.

MATERIAL AND METHODS

Study area

Gialova lagoon is a poly-hyperaline basin in SW Greece and lies between Navarino bay and Voidokilia embayment adjacent to the Ionian Sea (Eastern Mediterranean) (Fig. 1). It covers an area of 2.5 km^2, with a maximum depth of one metre and is connected with the adjoining Navarino bay via a small channel (100m long, 10m wide and 1.2m deep). Fluvial input is by two small inlets to the eastern part of the lagoon. Generally the sediment is muddy-sand, being covered in most areas with green algae and the eel grass *Cymodocea nodosa*. In small parts of the lagoon mud is mixed with dead shells of the bivalve *Cerastoderma glaucum*.

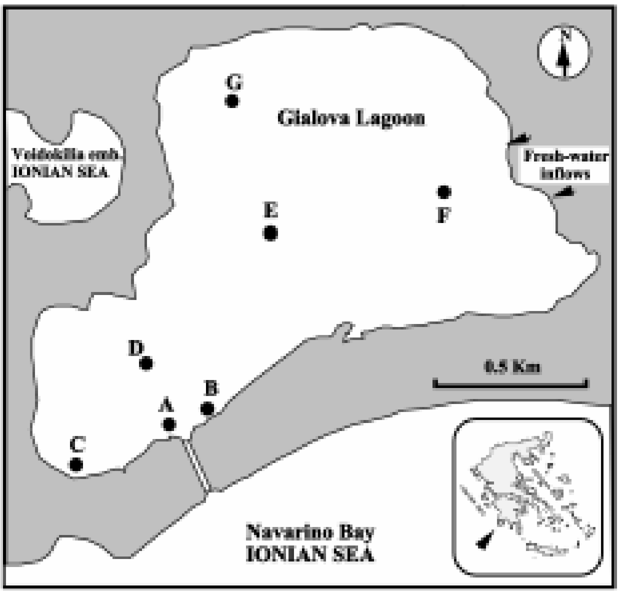

Fig. 1. – Map of Gialova lagoon indicating the seasonal sampling stations for molluscan community analysis.

Field sampling and processing

Seven stations were sampled to cover the entire body of the lagoon. Five replicate samples (total surface area of 0.25 m^2) were taken at each station in June 1995, September 1995, December 1995 and March 1996 with the use of a hand-operated van-Veen grab. The samples were sieved through a 0.5 mm mesh fixed in 5% neutralized formalin and preserved in ethyl alcohol (70%). Additional sediment and water samples were taken at each station for analysis of abiotic parameters: temperature, Redox potential, sediment particulate organic carbon (POC), chlorophyll-a, phaeopigments, mean diameter of sediment particles, silt-clay percentage, salinity (Practical Salinity Scale), dissolved oxygen, pH, ammonia, nitrates, nitrites, phosphates, and silicates. Finally sediment and bivalve samples for hydrocarbons were taken with the use of the van-Veen grab. In the laboratory, the living molluscs were sorted, identified to species level, counted and weighed after drying at 80°C for 48h. Estimations of the concentrations of the abiotic parameters listed above and hydrocarbons were obtained according to standard procedures (Koutsoubas et al., 1999 and references therein).

Data analysis

After identification to species level, molluscs were classified in relation to their environment, organismic assemblages distributed, life-mode, feeding type and zoogeographical categories (summarised in Table 1) based on information derived from Graham, 1955; Pérès & Picard (1964); Barnes (1980, 1994); Augier, 1982; Guelorget & Perthuisot (1983) and Koutsoubas (1992).

Molluscan community structure and dynamics were analysed by means of total number of species (S), average density (mean number of individuals/m^2), Shannon-Wiener diversity (H’, log2 basis) and Margalef’s species richness (d) indices. Biological data (mean of the five replicates from each sampling station) were also analysed by multivariate analyses after species’ average densities per station had been transformed to the fourth root. Faunal similarities among the stations sampled were investigated using Cluster analysis (group average) and non-metric multidimensional scaling (MDS) (Kruskall & Wish, 1978) based on the Bray-Curtis similarity index of species composition between stations (Clarke & Green, 1988). Species maximally contributing to the dissimilarity among the station groups were investigated using the SIMPER percentages procedure (Clarke, 1993). Environmental variables best correlated with the multivariate pattern of the molluscan community were identified by means of harmonic Spearman coefficient (BIO-ENV analysis) as proposed by Clarke & Ainsworth (1993). Spearmann’s rank correlation coefficient (ρ) was applied to identify any significant correlation between the number of mollusc species, density and biomass and the environmental vari-

TABLE 1

Autoecological and zoogeographical attributes of mollusc species found in Gialova lagoon (taxa listed in phylogenetic order). Abbreviations are: MAR/EST=*Marine/Estuarine species*; SVMC= Assemblage of Surficial Muddy Sands in Sheltered Areas; HP=Assemblage of *Posidonia* meadows; AP=Assembalge of Photophilic Soft Algae; LEE=Euryhaline and eutythermal assemblages in brackish waters; SFHN=Assemblage of Fine Sands in very Shallow Waters; SGCF=Assemblage of Coarse Sand and Fine Gravels under Bottom Currents; EPI=vagile epifaunal; INF=vagile infaunal; SCRAP=scraper herbivore; BROW=browser herbivore; CARN=carnivore; SUSP=suspension feeder; OCCDEP=occasionally deposit feeder; E=Mediterranean endemic; AM=Atlanto-Mediterranean; B=Boreal; C=Cosmopolitan.

Mollusc Species	Stations	Environment	Organismic Assemblage	Life mode	Feeding type	ZC
GASTROPODA						
Trochidae						
Gibbula adansonii Payraudeau, 1826	A, C, D	MAR/EST	SVMC, HP	EPI	SCRAP	E
Gibbula divaricata (Linnaeus, 1758)	C	MAR/EST	AP, HP	EPI	SCRAP	AM
Jujubinus montagui (Wood W., 1828)	C	MAR/EST	AP, HP	EPI	SCRAP	AM
Cerithiidae						
Cerithium rupestre Risso, 1826	A, B, C	MAR/EST	AP, HP	EPI	BROW	AM
Cerithium vulgatum (Bruguiere, 1792)	A, B, C, D	MAR/EST	AP, HP	EPI	BROW	AM
Bittium reticulatum (Da Costa, 1778)	A, B, C, D	MAR/EST	AP, HP	EPI	BROW	B
Potamididae						
Pirenella conica (Blainville, 1826)	A, B, C, D, E, F, G	MAR/EST	LEE	EPI	SCRAP	E
Rissoidae						
Alvania beani (Hanley in Thorpe, 1844)	C	MAR/EST	AP	EPI	BROW	B
Rissoa aartseni Verduin, 1985	A, B, C	MAR/EST	SVMC, SFHN, HP		BROW	E
Rissoa violacea Desmarest, 1814	D	MAR/EST	HP		BROW	E
Rissoa variabilis (Von Muehlfeldt, 1824)	A, D	MAR/EST	HP		BROW	E
Pusillina radiata (Philippi, 1836)	A, B	MAR/EST	AP	EPI	BROW	E
Hydrobiidae						
Hydrobia acuta (Draparnaud, 1805)	A, B, C, D, E, F, G	MAR/EST	LEE	EPI	BROW	AM
Nassariidae						
Cyclope neritea (Linnaeus, 1758)	A, B, C, D	MAR/EST	SVMC, LEE	INF	CARN	AM
Nassarius incrassatus (Stroem, 1768)	A	MAR/EST	AP, SVMC	INF	CARN	B
Aplysiidae						
Aplysia depilans Gmelin, 1791	A, B, D	MAR/EST	AP, HP	EPI	BROW	AM
Stiligeridae						
Placida viridis(Trinhese, 1871)	D	MAR/EST	HP	EPI	BROW	E
Polyceridae						
Polycerella emertoni Verrill, 1881	C	MAR/EST	AP	EPI	CARN	C
BIVALVIA						
Lucinidae						
Loripes lacteus (Linnaeus, 1758)	A	MAR/EST	SVMC	INF	SUSP	AM
Cardiidae						
Cerastoderma glaucum (Poiret, 1789)	A, B, C, D, E, F, G	MAR/EST	LEE	INF	SUSP	AM
Semelidae						
Abra ovata (Philippi, 1836)	A, B, C, D, E, F, G	MAR/EST	LEE	INF	SUSP/ OCCDEP	E
Veneridae						
Clausinella brogniarti (Payraudeau, 1826)	A	MAR/EST	SGCF	INF	SUSP	E
Tapes decussatus (Linnaeus, 1758)	A, B	MAR/EST	SVMC	INF	SUSP	AM

ables. The PRIMER package, developed in Plymouth Marine Laboratory was used.

RESULTS

Abiotic data

The environmental data showed large variations, however, some distinct temporal and spatial trends were seen. The variables showing the clearest temporal trends were: temperature (ranging from 14°C in winter to 24°C in summer), salinity (from 13 psu in spring to 60 psu in autumn), dissolved oxygen (lowest in the autumn-3.5 mgl^{-1} and highest in the spring-9.1 mgl^{-1}), redox potential in the surface sediment (0 cm) [from oxic (398 mV) in winter to anoxic (-150 mV) in autumn]. A range of values for POC (1-8.2 mg/lt and 1.5-50.3 mg/g) and chloroplastic pigments (Chl-a 0.2-18.5 μg/lt and 1-62.4 μg/g; phaeopigments 0.2-17.6 μg/lt and 1-77 μg/g) were recorded from the water column and the sediment correspondingly. Nitrates (0.3-7.49 μM), nitrites (0.04-0.64

μM) and ammonia (0.18-9.45 μM) concentrations also exhibited significant temporal fluctuations, with the maximum values reached in the spring. Spatial trends could be seen for sediment particulate organic carbon, chlorophyll-a and phaeopigments, which had a tendency to be much higher in the innermost stations C, D, E, F and G (20.95 to 38.03 mg/g; 5.86 to 64.07 μg/g; 18.68 to 59.84 μg/g for POC, Chl-a and phaeopigments correspondingly) than stations A and B located closer to the communication channel with the sea (1.58 to 16.54 mg/g; 1.51 to 4.99 μg/g; 3.00 to 11.89 μg/g). A gradient similar to this could be seen in the silt-clay distribution with C, E, F and G having the highest percentage (62.25 to 78.41%) followed by D (43.56%), with A and B consistently the lowest. (10.07 and 39.80% correspondingly). Detailed information on the environmental parameters referred to above has been given by DOUNAS & KOUTSOUBAS (1996). Hydrocarbon concentrations in the surface sediments ranged from 7.9 to 20 μg/g for the n-alkanes and from 60 to 250 ng/g for the polynuclear aromatic hydrocarbons, with lower values obtained from the innermost stations (st. D, E, F and G). Hydrocarbon concentrations in tissue of individuals of the bivalve species *Tapes decussatus* were lower than 1.5 μg/g for the n-alkanes and 60 ng/g for the polynuclear aromatic hydrocarbons.

Faunal composition

A total of 23 gastropod and bivalve mollusc species, composed of over 16.628 individuals, were identified from Gialova lagoon during the four sampling periods over summer 1995 to spring 1996 (Table 1). All the species are *marine/estuarine* species (Table 1) as identified by BARNES (1994) while specifically *lagoonal* species of marine ancestry are lacking.

Special reference should be made to two of the mollusc species found in Gialova lagoon namely: *Placida viridis* (Trinchese, 1871) a tiny ceratiform sacoglossan opisthobranch belonging to the family Stiligeridae found in station D and *Polycerella emertoni* Verill, 1881 a nudibranch opisthobranch of the Doridacean family Polyceridae found in station C, which are reported for the first time as elements of the molluscan fauna of the Greek seas and the Eastern Mediterranean respectively. Six more species [*Jujubinus montagui* (Wood W., 1928); *Rissoa aartseni* Verduin, 1985; *Alvania beani* (Hanley in Thorpe, 1844); *Pusillina radiata* (Philippi, 1836); *Hydrobia acuta* (Draparnaud, 1805) and *Aplysia depilans* Gmelin, 1791] are reported for the first time from the Ionian Sea.

Zoogeographical categories to which the mollusc species are assigned are presented in Table 1. The dominant components of the molluscan fauna, in terms of number of species, are the Atlanto-Mediterranean and the Mediterranean endemics accounting for 43.5% and 39% respectively, followed by the Boreal (13%) and the Cosmopolitan ones (4.5%).

Molluscs were among the most abundant taxa, accounting for 28% of the total number of macrofaunal species (polychaetes accounted for 39% and crustaceans 26% – DOUNAS & KOUTSOUBAS, 1996). Mean density, estimated from the four sampling periods, was 2.969 individuals/m^2, while mean dry biomass was 0.3 g/m^2. Total species number ranged from 14 in st. A (June) to 1 in st. G (September). June was the sampling period with the highest total number of species and density (19 and 6.584 individuals/m^2 correspondingly) and September with the lowest with 8 species and 1.013 individuals/m^2 (Table 2).

Among molluscs, gastropods dominate in species number (18 *vs* 5 of bivalve species), and average density (1.524 individuals/m^2 *vs* 1.446 individuals/m^2), while mean dry weight biomass was equally distributed in the two taxa (0.15 g/m^2). Nine of the species (*Bittium reticulatum, Cerithium vulgatum, Cerithium rupestre, Pirenella conica, Hydrobia acuta, Cyclope neritea, Cerastoderma glaucum, Abra ovata* and *Tapes decussatus* representing 39% of the total number of species, were present throughout the year. However, it should be mentioned that the first three of the species referred to above, along with *Cyclope neritea* and *Tapes decussatus*, are represented by very few individuals (less than 10-100 per species depending on the season) and are not distributed in the innermost stations E, F and G.

Considering feeding types, the herbivores (scrapers and browsers) dominate in terms of number of species (Table 1). However, when only the dominant species, in terms of density and presence throughout the year, are taken into consideration, suspension feeders and herbivores are almost equally represented.

Structural analysis

Total number of species (s), mean density (D), species richness (d) and diversity (H') calculated for the entire lagoon in the four sampling periods (Table 2) show the same seasonal pattern: an abrupt decrease from the summer (18 species) to the autumn (9 species) being followed by a gradual increase observed in the winter and spring sampling periods. As far as the spatial pattern of the sampling stations is concerned, higher values of H' and d were obtained in stations near the sea inlet (2.08 by 1.11) and lower values in the innermost stations (0 by 0).

TABLE 2

Seasonal changes in molluscan diversity and density over the sampling period in Gialova lagoon.

	Tot. No of Species	**Average density**	**Richness (d)**	**Shannon (H' log2)**
Summer	19	6.584	1.16	2.35
Autumn	8	1.013	0.54	0.73
Winter	10	1.169	0.69	1.27
Spring	12	3.110	0.76	1.57

Multidimensional Scaling ordinations and cluster analysis of the stations applied to each sampling period (Fig. 2) defined two main groups of stations: A first group composed of st. A and B and a second one composed of st. E, F and G. Stations C and D are clustered either in the first or second group depending on the season. ANOSIM tests showed that the aforementioned groups are significantly different (global R superior to 0.8 – except spring, 0.6 – at a significance level less than 5%).

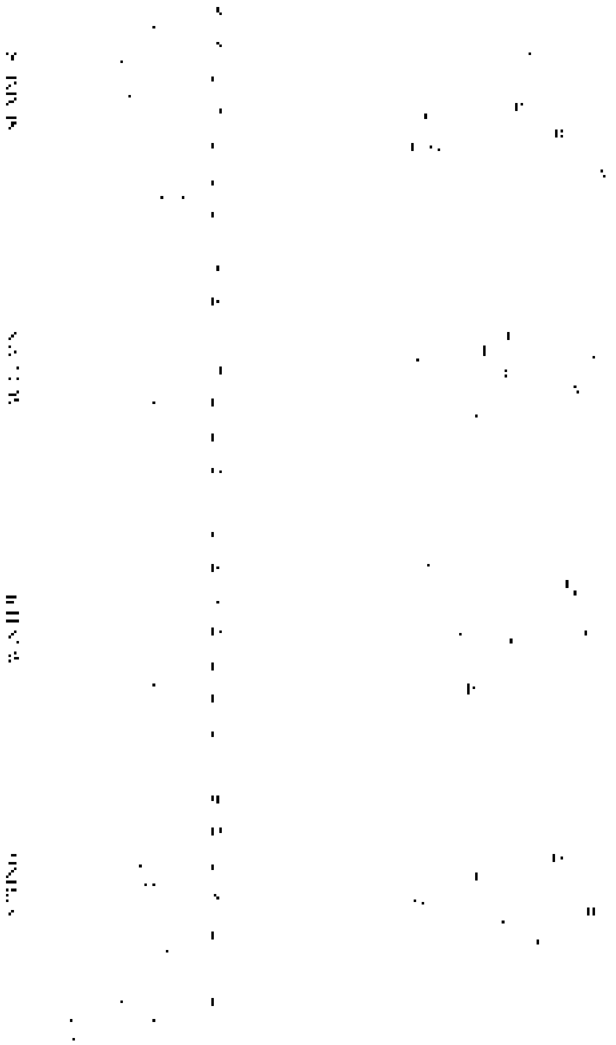

Fig. 2. – Similarity dendrograms and MDS ordination plots of stations in Gialova lagoon.

The two main groups of the above-mentioned stations showed different species composition: *Bittium reticulatum, Cerithium vulgatum* and *Cyclope neritea*, were the most abundant species in stations linked mainly with the sea inlet (A, B); *Pirenella conica*, and *Cerastoderma glaucum* were the most abundant species in the innermost stations (E, F, G).

Environment-Community interactions

The highest values of the harmonic Spearman coefficient between abiotic variables and the similarity matrices of biotic data (BIO-ENV analysis) are summarised in Table 3. BIO-ENV values exceeded 0.8 (except in spring - 0.4) thus indicating positive relationships between the multivariate pattern of the molluscan faunal density and the measured environmental variables. Different combinations of environmental variables were found associated with the multivariate pattern of the molluscan community in the four sampling periods. Only distance from the channel was among those found to be correlated with the distribution pattern throughout the year except during winter.

TABLE 3

Environmental variables best correlated with the molluscan community during the four sampling periods in Gialova lagoon (D: dist. mar. chan; T: temp.; S: salin.; O: diss. oxyg.; Eh: redox potential; MD: med. diam.; sc: silt-clay %; C: POC; F: phaeopigments; N: nitrates; N1: nitrites; ρw: weghed Spearman rank coefficient).

	D	T	S	O	Eh	pH	MD	sc	C	F	N	N1	ρw
Sum	+	+			+			+		+			0.87
Aut	+		+				+		+				0.85
Win					+	+						+	0.80
Spr	+	+		+		+	+				+	+	0.40

DISCUSSION

Despite its small size and average depth Gialova supports a rather rich, in terms of number of species, molluscan community when compared with other Mediterranean lagoons [e.g. Étang de Prevost (23 species) – Guelorget & Michel, 1979; Mer des Bihans (47) – Zaouali, 1975; Marsala (23) – Cattaneo-Vietti & Chemello, 1991; Mesologhi (46) – Nicolaidou et al., 1988; Bardawil (14) – Barash & Danin, 1982].

Taking into consideration Bianchi's (1988) classification of Italian lagoons, in relation to their climatological, hydrographic and biological features, Gialova lagoon could be assigned to the category of the "eumediterranean lagoons" as far as the mollusc composition is concerned, since Atlanto-Mediterranean and Mediterranean endemic species with a temperate and sub-tropical affinity dominate over those with a Boreal affinity.

Molluscs from brackish lagoonal waters in the Mediterranean are known from several studies (e.g. Amanieu et al., 1977; Barash & Danin, 1982; Bourgoutzani & Zenetos, 1983; Torelli, 1983; Nicolaidou et al., 1988). However, certain families or even orders of molluscs such as opisthobranch gastropod molluscs, are often ignored and there have been rather few systematic investigations of these animals in this habitat (Cattaneo-Vietti & Chemello, 1991 and references therein); the list of molluscs that can indeed live in this peculiar habitat can be considered as far from complete. The opisthobranch species *Placida viridis* and *Polycerella emertoni* prior to being found in Gialova lagoon had once again been reported as lagoonal inhabitants (Fusaro lagoon – SCHMEKEL, 1968). The presence of the aforementioned species in Gialova lagoon confirms the opinion of CATTANEO-VIETTI & CHEMELLO' (1991) – based on studies conducted in the western part of the Mediterranean – that these species can withstand the considerable fluctuations of the environmental variables, often occurring in these coastal environments, thus including lagoons in their ecological distribution.

Placida viridis and *Polycerella emertoni* are reported for the first time as elements of the molluscan fauna of the Greek seas and the Eastern Mediterranean respectively. Another six gastropod species found in Gialova lagoon represent new records for the Ionian molluscan fauna. These findings support previous authors' claims (e.g. KOUTSOUBAS et al., 1997; ZENETOS, 1997) that the marine biodiversity in Greek waters will become even more rich when studies are extended to cover neglected geographical locations or habitats and contribute to the overthrow of the "impoverished Eastern Mediterranean theory" expressed earlier in this century (e.g. PÉRÈS, 1967).

Most of the mollusc species found in Gialova have been also recorded in various substrate types and assemblages, such as soft substrate without or with the phanerogames *Posidonia* and *Cymodocea* (SGCF, SVMC, SFHN, HP assemblages), on hard substrate with photophilic algae or associated with sponges, anthozoans and other organisms (AP assemblage). However, the dominant species in this lagoonal ecosystem can be characterised as typical of brackish lagoonal waters (Euryhaline and eurythermal assemblages in brackish waters – PÉRÈS, 1967; AUGIER, 1982). These species are distributed over the major part of the lagoon throughout the year. Species in this second category have also been recorded in estuaries (McLUSKY, 1981; KEVREKIDIS et al., 1996) and other marine environments (KOUTSOUBAS et al., 1997; ZENETOS, 1997).

On the basis of the multivariate pattern of the molluscan density, two main zones can be distinguished: a first narrow zone represented mainly by stations A and B and influenced mostly by the sea and a second one in the innermost part of the lagoon, represented by stations E, F and G. A third transitional zone is clearly apparent only during summer. Following the *confinement scale* proposed by GUELORGET & PERTHUISOT (1992) the first zone can be assigned to the "zone III" and the second to "zones IV-V".

Number of species, density, diversity indices and multivariate analysis reveal the same seasonal distribution pattern of the molluscan community, ranging from a well stratified and rich community in summer to an impoverished one in autumn, apparently due to a "dystrophic crisis" episode occurring in the lagoon during late summer (DOUNAS & KOUTSOUBAS, 1996). These crises, however have been considered as a natural reaction of the lagoonal habitat to extreme environmental conditions (mainly anoxia) and are of great importance in the re-establishment of the ecological balance (CAMMETE, 1992; GUELORGET & PERTHUISOT, 1992). Similar seasonal patterns have also been observed in other Mediterranean lagoons (AMANIEU et al., 1977; GRAVINA et al., 1989; ARIAS & DRAKE, 1994; LARDICCI et al., 1997). In the subsequent seasons, mollusc species in the Gialova lagoon gradually approached the previous summer levels.

Many environmental variables have been reported to be correlated with the temporal and spatial distribution pattern of the molluscan communities in lagoonal systems. These are either clearly physical ones (e.g. salinity – BARNES, 1980) or variables involved with food supply (e.g. nutrients, organic material – GRAY, 1981; NIXON, 1982; GRAVINA et al., 1989). Based on the molluscan zonation and on the results of the BIO-ENV analysis the Gialova lagoon illustrates the role of the *confinement*, which is determined by the time of renewal with elements of marine origin at a given point of the lagoon (GUELORGET & PERTHUISOT, 1983). The innermost part of the lagoon has always a different faunal composition in comparison with the areas close to the sea inlet. Additionally the stations with the least contact with the sea support higher abundance and biomass values due to the large number of individuals mainly of the gastropod species *Hydrobia acuta, Pirenella conica* and the presence of large sized individuals of the bivalve species *Cerastoderma glaucum* and *Abra ovata*. A similar *coenocline* has been observed in Gialova lagoon for other animal groups (e.g. polychaetes – ARVANITIDIS et al., 1999) and for the whole macrobenthic community (KOUTSOUBAS et al., 1999). However, when the succession of the *coenocline* of the molluscan community over the four seasons is compared with the ones derived either from the polychaete or the whole macrobenthic community, different aspects are revealed. The molluscan community appears to recover from the effects of the "dystrophic crisis" episode earlier than the rest of the macrobenthic faunal community, i.e. in winter instead of spring. This should be probably attributed to the fact that, despite the molluscan community being less speciose in comparison with other taxocommunities (e.g. polychaete community), it is better adapted for living in this type of coastal ecosystem. Hence, at least some of the dominant mollusc species in Gialova lagoon can better withstand the "natural stress" during the "dystrophic crisis" and take advantage of food availability aided by their bioturbation activities (e.g. *Cerastoderma glaucum* – PELEGRI & BLACKBURN, 1995). This is further supported by the fact that food supply (e.g. organic material) has been found to be correlated with the molluscan community pattern even during summer and autumn, when other environmental variables (e.g. temperature, salinity, oxygen availability in the sediment) produce strong gradient along the lagoon and act as thresholds to the distribution of species belonging to other taxa (KOUTSOUBAS et al., 1999).

The hydrocarbon concentration values for both sediments and bivalve molluscs found in Gialova lagoon during 1995-96 fall within the range of those reported from other non-polluted coastal areas of the Mediterranean (UNEP, 1988). Therefore, it could be concluded that the molluscan community in Gialova lagoon does not seem to have suffered the effects of any unnatural long term disturbance due to oil pollution from the "Iliad" incident, as opposed to what has been reported in similar incidents in open coastal ecosystems in other areas of the world (ELMGREN et al., 1983). The short period of recovery in Gialova lagoon from the impact of anthropogenic activities, is further supported not only by the rich molluscan diversity revealed in this study but also by the high overall macrofaunal diversity (DOUNAS et al., 1998).

Results from this study along with the results of the multi-disciplinary study investigating the structure and functioning of Gialova lagoon (DOUNAS & KOUTSOUBAS, 1996), have revealed that, despite suffering various anthropogenic activities in the past, this lagoon still constitutes a wetland of particular value in many respects (cultural, fisheries, etc.) and acts as a "biological reservoir", which should be conserved and managed in the future so as to continue to provide significant habitats for organisms in the area.

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Preliminary results from the establishment of experimental artificial reefs in the N. Aegean Sea (Chalkidiki, Greece)

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ABSTRACT. Twenty-four artificial reef units were designed and set in the coastal waters off Neos Marmaras (Chalkidiki, North Aegean Sea, Greece) in July 1998, at depths ranging between 7 and 22 m. The volume of each unit was between 0.8 and 2.25 m^3 and the maximum weight was up to one t. Three different types of material were used for the construction of the units, namely cement, ceramic and car tyres. The units were set along a main axis and along secondary axes that were perpendicular to the main one. The artificial reef units were monitored both directly, by diving, as well as through underwater photos and experimental sampling. Our results indicated that there was a tendency: (a) of sessile organisms to inhabit the reef units and (b) for fish to find refuge in the reef units with higher number of species generally recorded after the deployment of the reef units.

KEY WORDS: Artificial reefs, fish communities, N. Aegean Sea, Mediterranean.

INTRODUCTION

The establishment of artificial reefs ranks among the most important measures for the enhancement and protection and management of fisheries resources, especially so for the highly oligotrophic environment of the SE Mediterranean Sea (e.g. SPANIER et al., 1990; BOMBACE et al., 1993). Most of the available information on artificial reefs has been published during the last ten years (e.g. BAYNES & SZMANT, 1989; BEETS 1989; POLOVINA, 1989, 1991; POLOVINA & SAKAI, 1989; ODY & HARMELIN, 1994). Recent examples of artificial reef establishment (e.g. RELINI et al. 1995; SANTOS et al. 1996) clearly indicate that fish abundance and diversity both increase considerably after the deployment. The present study is the first attempt to evaluate the effect of the deployment of artificial reefs on the local fish communities in Greek waters (i.e. North Aegean Sea, Chalkidiki).

MATERIAL AND METHODS

The artificial reefs

Artificial reef units were designed using different shapes, sizes and types of materials. The shapes of the units were selected in terms of time required to inhabit the reef units and the ultimate protection of the fisheries resources that will eventually find refuge there**.** The settlement area was selected after scuba-diving observations undertaken in the wider study area. Artificial units were set in *Posidonia* beds because their dense rhizomes could withstand the weight of the units and their leaves could reduce the overall sedimentation rate.

Overall, 24 artificial reef units were deployed in the coastal waters off Neos Marmaras (North Aegean Sea, Greece), at depths ranging between 7 and 22 m, on 19th July 1998. Three different types of material were used for the construction of the units, namely cement, ceramic and car tyres, and the reef units belonged to eight different types:

1. Three units each measuring 1x1x1.2 m, made of cement.
2. Three units each measuring 0.9x0.9x1 m, made of cemented square grids.
3. Three units each measuring 1.2x1.2x1.2 m, made of cemented plates.
4. Three units each measuring 1.5x1.05x1.05 m, made of ceramic bricks.
5. Three units each measuring 1x1x1 m, made of cemented tubes with a diameter of 20cm.

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6. Three units each measuring 1.5x1.5x1 m, made of cemented pipes with a diameter of 50cm.
7. Three cemented pipes 1 m long and with a diameter of 80 cm each.
8. Three units each measuring 1.2x0.9x0.9 m, made of car tyres.

The volume of each unit was, depending on the type used, between 0.8 and 2.25 m^3 and the maximum weight was up to one t. The units were set along a main axis and along secondary axes that were perpendicular to the main one (Fig. 1).

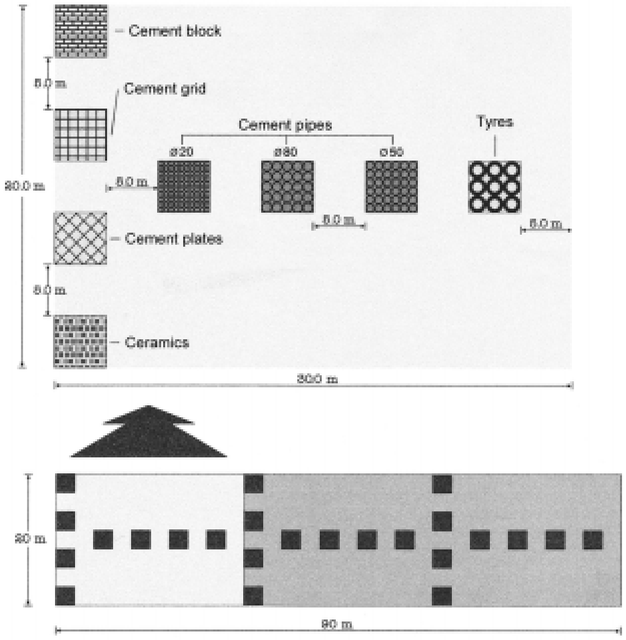


Fig. 1. – Spatial arrangement of artificial reefs.

Data collection

The abundance of benthic organisms and fisheries resources was monitored both before (in November 1996, and January, April and July 1997) and after the deployment of the reefs (August and November 1998). Biological monitoring was conducted: (a) by scuba diving; (b) through underwater photos; (c) by taking parts of the reef units to the laboratory for further analysis; and (d) by experimental fishing using gill nets of different mesh sizes (18, 22 and 28 mm bar length, each net having a length of 219 m and a height of 1, 5.5 and 3.7 m respectively) in order to sample efficiently the local fisheries communities. After the gill nets were hauled, total catch was removed and separated into species.

Data analysis

For the analysis of the fish catches per mesh size and sampling period, a matrix comprising the numbers of each species from each mesh size and each season was constructed. Numbers were expressed per 250 m of gill nets. From this matrix, a triangular matrix of similarities between all pairs of gear was computed using the Bray-Curtis coefficient (Bray & Curtis, 1957). Prior to this computation, data were transformed using the double square root transformation in order to reduce the weighting of abundant species (Field et al., 1982). Subsequently, the similarity matrices were subjected to both clustering (employing group-average linking) and ordination (employing non-metric multidimensional scaling, MDS) analysis techniques. The adequacy of the representation in two, rather than more dimensions is expressed by a “stress coefficient” (Field et al., 1982). In general, stress values <0.1 imply good representation, and discontinuities between mesh/season combinations may be accepted as real when the results of the two methods agree (Field et al., 1982; Clarke & Green, 1988).

RESULTS AND DISCUSSION

The analysis of the benthic samples collected from the study area revealed two different communities, one dominated by organisms abundant in *Posidonia oceanica* beds, and another one, which included organisms that are typical of the semi-dark grottoes and open-sea rocks. These two communities have been previously described by Peres & Pickard (1964) and Peres (1967). The typical species included *Petrosia dura* (Nardo, 1833), *Agelas oroides* (Schmidt, 1864), *Axinella cannabina* (Esper,1794), *Axinella verrucosa* (Esper, 1794), *Caryophyllia smithii* Stokes & Broderip, 1828, *Parazoanthus axinellae* (Schmidt, 1862), *Cladocora caespitosa* (Linnaeus, 1767*), Echinus melo* Lamarck, 1816*, Echinaste sepositus* (Retzius, 1783), and *Halocynthia papillosa* (Linnaeus, 1767), all of which were found in the study area in relatively high abundance.

Visual censuses and the comparison of photos taken before and after the set of the units revealed a continuous increase in the settlement rate of various benthic organisms as well as in the presence of various fish species. Overall, 38 taxa were caught with experimental fishing before the deployment of the units (Table 1). The comparison of the results of experimental fishing four months after the deployment of the reefs with those for the corresponding month before the deployment revealed eight new species (19 and 11 species respectively). When comparisons were pooled across all months of experimental fishing the number of new species fell to six (*Dentex dentex, Labrus merula, Pagrus pagrus, Sphyraena sphyraena, Spondyliosoma cantharus, Umbrina cirrosa*), the majority of which were of high commercial value.

Catch species composition changed greatly with sampling period (Fig. 2). Thus, *Mullus surmuletus* and *Scorpaena scrofa* dominated the catches in November 1996 whereas *Spicara maena* and *Boops boops* dominated the catches during the same month after the deployment of the units (Fig. 2). Overall, *Sardinella aurita* dominated the catch of all mesh sizes combined in terms of both number and weight (Fig. 3), a fact attributed to its occasional high abundance in April 1997 (Fig. 2).

TABLE 1

Species caught with experimental fishing in the study area, all seasons and mesh sizes combined. * denotes species that were only caught after the deployment of the artificial reefs.

Apogon imberbis (Linnaeus, 1758)
Boops boops (Linnaeus, 1758)
Chromis chromis (Linnaeus, 1758)
Coris julis (Linnaeus, 1758)
*Dentex dentex** (Linnaeus, 1758)
Dicentrarchus labrax (Linnaeus, 1758)
Diplodus annularis (Linnaeus, 1758)
Diplodus vulgaris (Geoffroy St. Hilaire, 1817)
Epinephelus alexandrinus (Valenciennes, 1828)
Gobius niger jozo Linnaeus, 1758
Labrus bimaculatus Linnaeus, 1758
*Labrus merula** Linnaeus, 1758
Mullus surmuletus Linnaeus, 1758
Oblada melanura Linnaeus, 1758
Octopus vulgaris Cuvier,
Pagellus bogaraveo Brönnich, 1768
Pagellus erythrinus (Linnaeus, 1758)
*Pagrus pagrus** (Linnaeus, 1758)
Palinurus elephas (Fabricius, 1787)
Phycis phycis (Linnaeus, 1766)
Raja radula Delaroche, 1809
Sardinella aurita Valenciennes, 1847
Scorpaena notata Rafinesque, 1810
Scorpaena porcus Linnaeus, 1758
Scorpaena scrofa Linnaeus, 1758
Sepia officinalis Linnaeus, 1758
Serranus cabrilla (Linnaeus, 1758)
Serranus scriba (Linnaeus, 1758)
Spicara maena (Linnaeus, 1758)
Spicara smaris (Linnaeus, 1758)
*Sphyraena sphyraena** (Linnaeus, 1758)
*Spondyliosoma cantharus** (Linnaeus, 1758)
Symphodus melanocercus (Risso, 1810)
Symphodus melops (Linnaeus, 1758)
Symphodus rostratus (Bloch, 1791)
Symphodus tinca (Linnaeus, 1758)
Trachurus mediterraneus (Steindachner, 1868)
Trachurus picturatus (Bowdich, 1825)
Trachurus trachurus (Linnaeus, 1758)
Trisopterus minututs capelanus (Linnaeus, 1758)
Trigloporus lastoviza (Bonnaterre, 1788)
*Umbrina cirrosa** (Linnaeus, 1758)
Uranoscopus scaber Linnaeus, 1758
Zeus faber Linnaeus, 1758

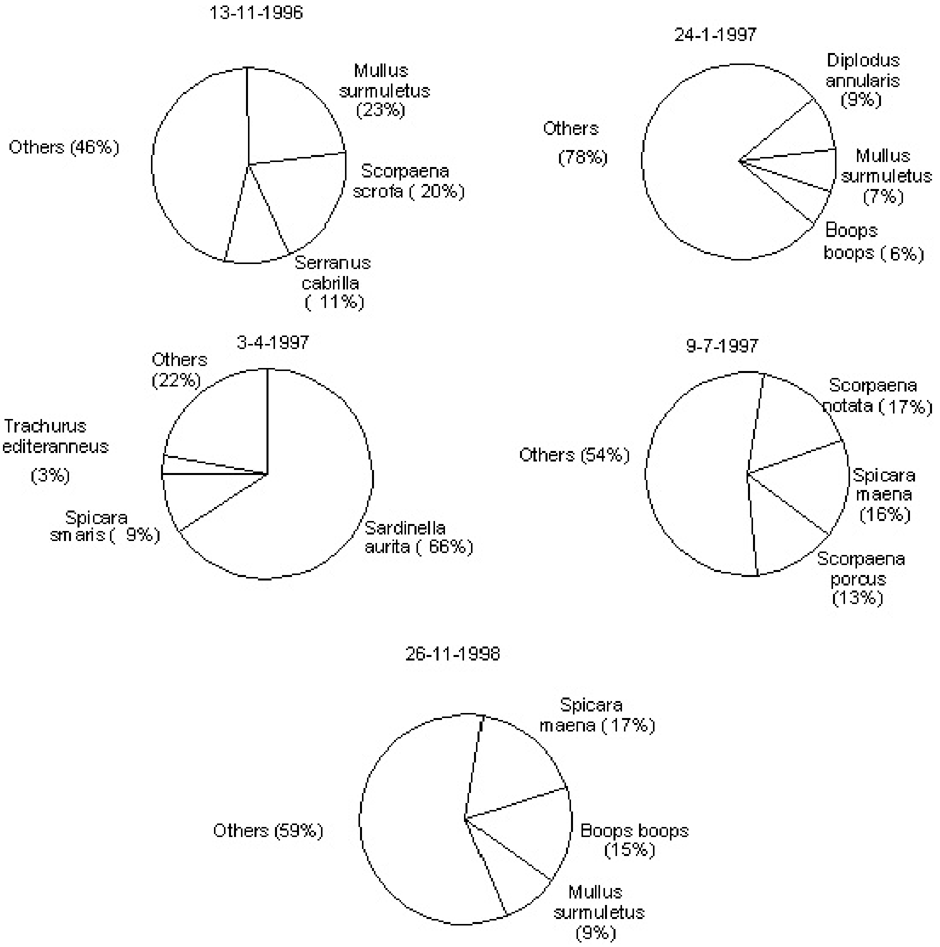


Fig. 2. – Seasonal species composition by weight of the total catch, all mesh sizes combined.

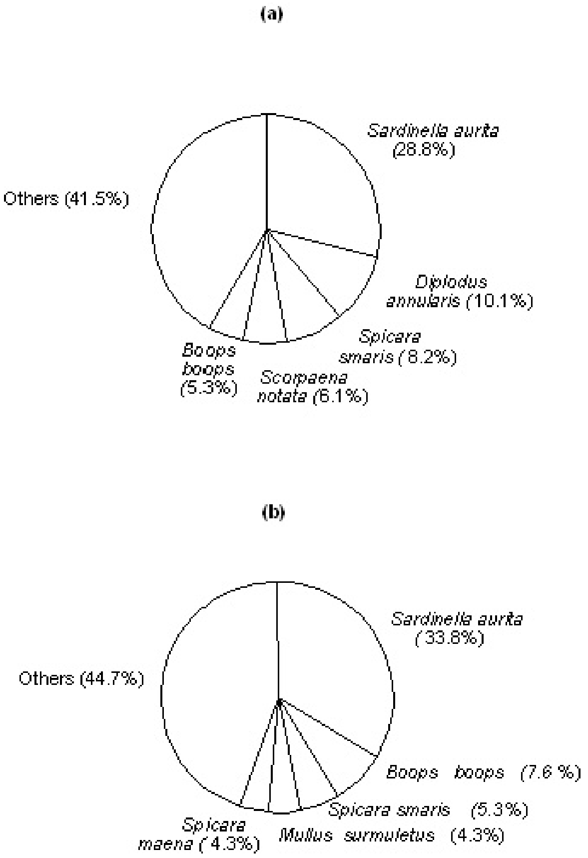
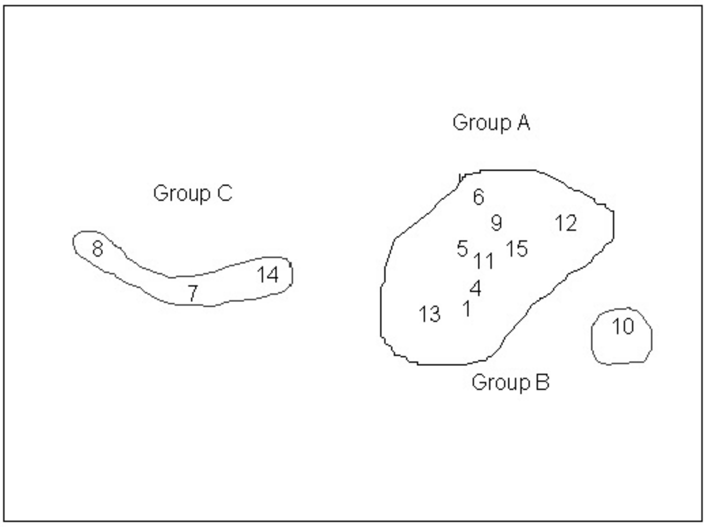


Fig. 3. – Species composition of the total catch by (a) number and (b) weight, mesh sizes and seasons combined.

The results of multidimensional scaling (Fig. 4) agreed with those of cluster analysis (not shown here) and revealed three main groups of gear/season combinations: (a) Group A, including various gear/season combinations, (b) Group B, including the sample taken with the 18 mm gill net in July 1997 and (c) Group C, which included the samples collected with the 22 mm mesh in April 1997 and November 1998 and that caught with the 18 mm mesh in April 1997. It must be pointed out that the samples taken with the three different mesh sizes after the deployment of the units (Fig. 4: combinations 13, 14 and 15) did not form a distinct group by themselves. Yet, two out of three (i.e. combinations 13 and 15) were separated from the remaining combinations of the subgroups in which they belong (Fig. 4).

To sum up, our results indicated that there is a tendency: (a) of sessile organisms to inhabit the reef units and (b) for fish to find refuge in the reef units with higher number of species generally recorded after the deployment of the reef units. It must be stressed, however, that it takes many years for the results of the establishment of artificial reefs to become apparent (Bombace et al., 1993). In addition, we also believe that the results would have been rather different if commercial fishing was prohibited in the wider area of reef deployment, a fact that was not true in the study area. Seasonal monitoring of the artificial reef units is also in progress for 2000.



	Mesh size (mm, bar length)		
Date	18	22	28
13/11/1996	1	2	3
24/1/1997	4	5	6
3/4/1997	7	8	9
8/7/1997	10	11	12
26/11/1998	13	14	15

Fig. 4. – Results of multidimensional scaling analysis applied to the similarity matrix between numbers of individuals per species/mesh-size/season combinations. The combinations 1 to 15 are shown in the legend.

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