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NEPHROPS NORVEGICUS: COMPARATIVE BIOLOGY AND FISHERY IN THE MEDITERRANEAN SEA. *F. SARDÀ (ed.)*

Molt pattern identification through gastrolith examination on *Nephrops norvegicus* (L.) in the Mediterranean Sea*

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SUMMARY: Samples of *Nephrops norvegicus* were collected on a monthly basis from October 1993 to September 1995 in different areas of the Mediterranean and the Atlantic, both to investigate molt periodicity and to verify the existence of a common molt pattern for this species in the Mediterranean. The presence of well-developed gastroliths in the wall of the stomach was used as an indicator of molting periods. A well defined molting periodicity was not found among juveniles, which seem to molt all year round. In each studied area molt synchronism was evidenced among both males and females after the onset of sexual maturity, even if the seasonal molting pattern differs between the two sexes. Adult females have only one molting period per year (December-March), immediately after the hatching of eggs. Adult males present a molting period in late summer-autumn (August-October). In some areas a second molting period is evident and co-occurs with that of females (late winter-early spring). This molt synchrony among adults is common to all the *Nephrops* populations of the Mediterranean Sea. The different results obtained for the Atlantic population could be due to the influence of environmental factors on molt cycle regulation.

Key words: *Nephrops norvegicus*, gastrolith, molt, Mediterranean Sea, Atlantic.

RESUMEN: IDENTIFICACIÓN DE LA MUDA A TRAVÉS DE LOS GASTROLITOS EN *NEPHROPS NORVEGICUS* EN EL MAR MEDITERRÁNEO. – Se recolectaron muestras mensuales de *Nephrops norvegicus* desde octubre de 1993 hasta septiembre de 1995 en diferentes áreas del Mediterráneo y del Atlántico. La finalidad era conocer la periodicidad de las mudas y verificar la existencia de un modelo común de muda en dicha especie en el Mediterráneo. Se tomó como claro indicador de la muda la presencia de gastrolitos bien desarrollados. En los individuos juveniles no se detectó ningún período de muda definido; que parecen mudar durante todo el año. Se ha evidenciado sincronismo entre las mudas de machos y hembras en cada área estudiada y también en los patrones de mudas estacionales en ambos sexos. Las hembras maduras sólo tienen un período de muda al año (diciembre-marzo), inmediatamente después de la eclosión de los huevos. Los machos adultos presentan un período de muda al final del verano (entre agosto-octubre). En algunas áreas se observa un segundo período y coincide con el de las hembras (final de invierno-principio de primavera). Esta sincronía entre las mudas de los adultos es común en todas las poblaciones de *Nephrops* en el Mediterráneo. Los diferentes resultados obtenidos en el Atlántico podrían ser debidos a la influencia de los factores ambientales en la regulación del ciclo de muda.

Palabras clave: Nephrops norvegicus, gastrolitos, muda, Mediterráneo, Atlántico.

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INTRODUCTION

In Crustaceans, growth is a discontinuous process with a succession of molts separated by intermolt periods. During each molt the old exoskeleton is shed and the animal grows very quickly, before the new exoskeleton hardens. Two components of growth have been recognized (Hartnoll, 1982): the increase in size at each molt (growth factor) and the time between two successive molts (intermolt period). To identify molting periods and periodicity is of great importance in growth studies; in some decapods (i.e. lobsters) this is facilitated by the presence of gastroliths. In species with a very hard exoskeleton and to which calcium is not readily available in the environment, calcium is stored in these transitory structures. Gastroliths are situated in the wall of the cardiac chamber of the stomach, between the epithelium and the cuticularized layer. Here, during pre-molt phases, calcium, mobilized from the old exoskeleton to the blood by the molt hormone, is stored as calcium carbonate crystals (calcite) (Sardà, 1981; Skinner, 1985). After ecdysis, gastroliths are quickly dissolved and the calcium is used to harden the new exoskeleton.

The duration of the molt cycle (from proecdysis to anecdysis) differs among species and is highly influenced by environmental factors (in particular, temperature and light) which affect molt hormone secretion (Skinner, 1985). As a consequence, the period in which gastroliths are present is variable. To define the duration of molt phases, identified in accordance with the Drach (1939) scale, the structural changes in pleopods of lobsters were studied by Aiken (1973) on *Homarus americanus*, and by Sardà (1983) on *Nephrops norvegicus*.

Experiments on the variation in calcium concentration in different body structures indicate that in *N. norvegicus* the maximum increment in gastrolith volume takes place during last phases of premolt (*D2* to *D4*). Gastroliths reach full development about 15 days before molt, and are reabsorbed very quickly (less than 24 hours) after ecdysis (Sardà and Cros, 1984).

Therefore, the presence in this species of well-developed gastroliths indicates that the animal is ready to molt within a few days. The use of gastroliths as indicators of molting periods can also support results obtained with other techniques such as percentage of 'soft' specimens in size frequency distributions (Conan, 1975; Sardà, 1991), or studies in aquaria (Thomas, 1965; Conan, 1978; Sardà, 1985).

The aim of this paper, based on samples from different areas of the Mediterranean and the Atlantic coast, was to improve knowledge on molting periodicity and to compare the molting pattern among different *Nephrops* populations.

MATERIAL AND METHODS

Samples of *Nephrops norvegicus* were collected monthly from October 1993 to October 1995 in some areas of the Mediterranean Sea and in the Eastern Atlantic. The areas sampled were the Aegean Sea (Euboikos Gulf), the Adriatic Sea (off Ancona), the Tyrrhenian Sea (off Elba island), the Ligurian Sea (off Genoa), the Catalan Sea (off Barcelona), the Alboran Sea (off Malaga) and the eastern Atlantic (off Faro, southern coast of Portugal).

The samples were collected by commercial bottom trawlers in all the areas, except in the Adriatic where a research vessel was used. The gears used had meshes of 40 mm (stretched) in the codend except for Greece (32 mm) and Portugal (55 mm).

The sampling techniques were different among the areas. In Portugal, Euboikos Gulf and Adriatic Sea, *Nephrops* catches were systematically sampled with the aim to obtain at least 10 specimens per sex and length class (each length class had a range of 5 mm *CL*). In the other areas, the whole catch of *N. norvegicus* (or a random sample if the catch was too large) was examined. Unfortunately in some areas and in some seasons it was impossible to have regular samples for rough sea, trawler unavailability, etc. The number of specimens examined (by area and sex) is reported in Table 1.

All the specimens in the sample were sexed and measured (*CL*) with a dial calliper to the mm below. Then the carapace was opened at stomach level to check for gastroliths; only gastroliths whose crystals were noticeable by touch were considered as 'present'.

From growth studies on the species (Conan, 1985; Sardà, 1985; Froglia and Gramitto, 1988) it is known that molt frequency is higher in juveniles than in adults, and differs between adult males and females, because females do not undergo molting during the period they are carrying external eggs. For these reasons, in data processing each sex was treated separately and for each sex three groups were formed based on information on size at sexual maturity (Orsi Relini *et al*., 1998). The same class limits were chosen for all the areas, except for the Alboran Sea. The first

TABLE 1. – Number of specimens of *Nephrops norvegicus* per sex and area collected monthly for gastrolith examination. M, males; F, females

AREA	Euboikos		Adriatic		Tyrrhenian		Ligurian		Catalan		Alboran		Atlantic	
DATE	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Oct 93	279	155	87	62	155	156	—-	—-	106	91	156	250	—-	—-
Nov 93	474	341	80	62	150	150	129	100	880	655	168	113	67	49
Dec 93	228	214	79	50	148	219	—-	—-	353	263	225	183	38	24
Jan 94	245	188	107	83	150	185	—-	—-	209	151	116	56	66	44
Feb 94	203	199	127	105	148	163	514	435	496	554	68	247	72	39
Mar 94	206	196	117	79	150	160	365	353	194	281	150	154	75	56
Apr 94	208	219	131	95	257	333	—-	—-	293	399	105	171	61	48
May 94	236	216	111	119	202	199	97	87	222	203	195	162	59	48
Jun 94	209	193	138	111	200	200	337	477	363	406	165	217	60	64
Jul 94	201	213	131	112	195	200	—-	—-	299	284	198	203	66	71
Aug 94	203	203	130	121	198	215	—-	—-	307	237	150	135	53	51
Sep 94	209	211	129	131	161	248	193	173	441	367	—-	—-	73	58
Oct 94	180	175	119	102	200	200	125	103	197	219	81	98	—-	—-
Nov 94	202	149	166	115	200	200	490	370	249	174	106	98	96	83
Dec 94	183	125	—-	—-	200	200	—-	—-	236	239	150	113	55	33
Jan 95	179	112	—-	—-	200	200	435	345	375	395	163	168	38	38
Feb 95	188	163	157	90	200	205	208	107	279	252	201	191	—-	—-
Mar 95	146	163	—-	—-	150	150	—-	—-	361	491	156	111	51	37
Apr 95	226	299	207	120	200	199	171	180	290	393	167	160	59	59
May 95	150	140	175	150	200	200	159	166	335	373	78	125	64	81
Jun 95	123	137	145	128	200	200	477	435	471	587	120	149	75	81
Jul 95	115	111	148	113	200	200	1158	1176	453	371	—-	—-	69	59
Aug 95	122	126	257	214	200	200	560	490	295	216	179	203	—-	—-
Sep 95	239	216	155	103	200	200	479	534	382	310	135	164	—-	—-
Oct 95	—-	—-	—-	—-	199	200	373	340	464	349	136	105	—-	—-

group (*CL*<25 mm) included young specimens, while the third one (*CL*≥35 mm) included the largest animals, all adults. The intermediate group (25≤*CL*<35 mm) could include both juveniles and adults, so that it had been excluded from further analysis. The *Nephrops* population sampled in the Alboran Sea seemed to be composed of larger specimens. The smallest berried female had 30 mm *CL* and the 100% of maturity was over 40 mm *CL*. Only for this area the upper limit of the first group and the lower limit of the third one were therefore moved to 30 and 40 mm *CL* respectively.

The rather small size of the samples examined for the Atlantic population and the unusually low gastrolith frequency make the identification of molting periods difficult. In an attempt to increase sample size, 'soft' specimens were included in gastrolith frequency calculations. 'Soft' specimens are animals in the first post-ecdysis stage, whose exoskeleton is not yet completely hardened (parchment-like).

For each sex and month, the frequency of occurrence of gastroliths (*F%*) was calculated as the percentage of specimens with gastroliths on the total of specimens in the size range considered. For each percentage, the 95% confidence interval was also calculated. To avoid large confidence intervals due to paucity of data, only length classes with 10 or more specimens were considered. Negative values of the lower confidence limit were considered unrealistic and discarded.

RESULTS

Gulf of Euboikos

In juveniles, specially in females (Fig. 1F), a molting period in winter-early spring was well evidenced in both the sampling years. Other molting peaks were present in summer and early autumn, but their existence can only be supposed, because the confidence intervals are quite large. Adult males (Fig. 2A) showed a preferential molting period at the end of summer with gastrolith frequency higher than 20%. Adult females (Fig. 2G) clearly underwent only one molt per year, in winter (January-March). This molting period is not well evidenced in the second sampling year, but supported by data of the first year.

Adriatic Sea

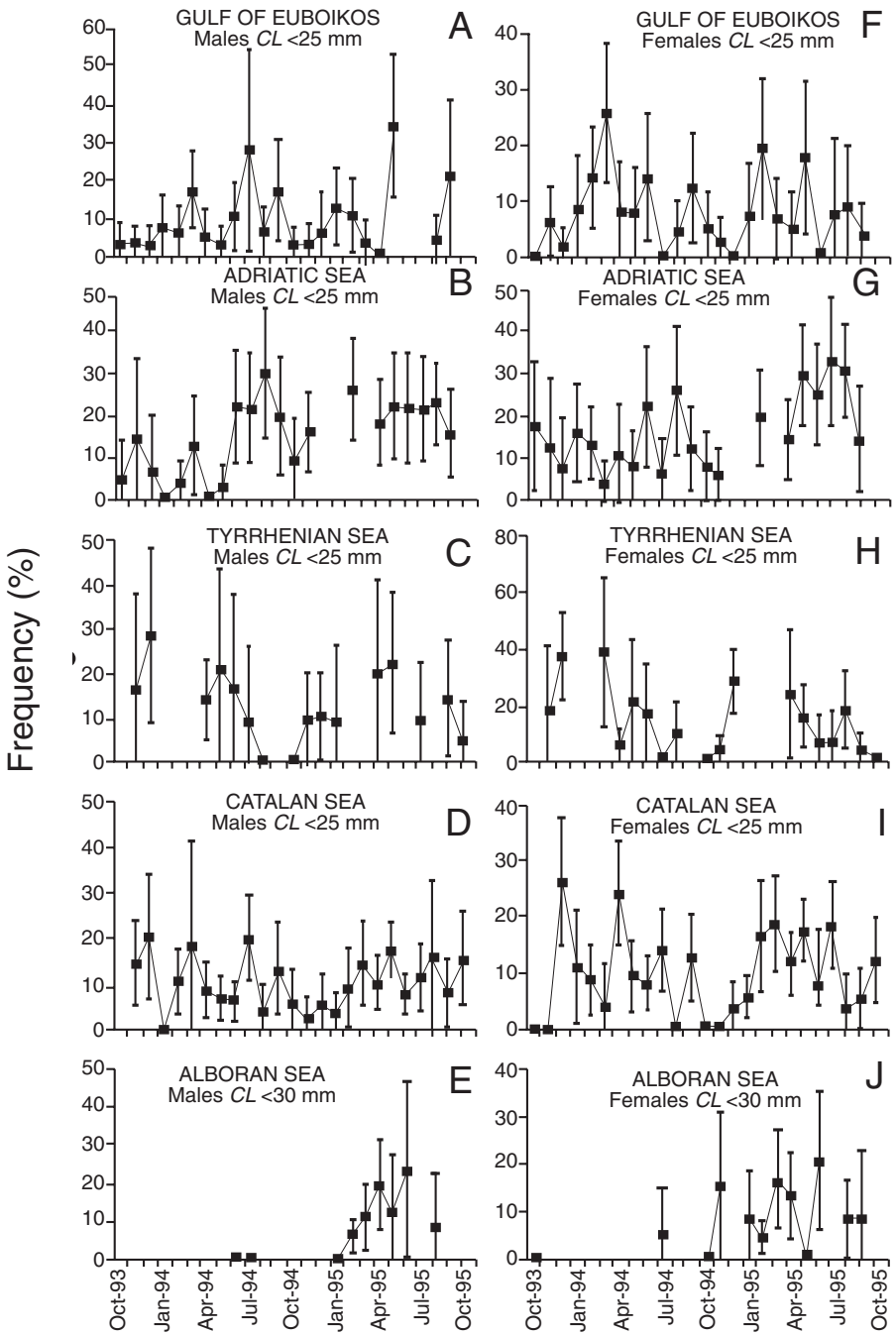

FIG. 1. – Frequency of occurrence of gastroliths (in percentage) in young males (A-E) and females (F-J) in the studied areas. Vertical bars indicate the 95% confidence interval

Trends were very muddled in both sexes for juveniles (Fig. 1B, G), with very wide fluctuations in gastrolith frequency between months, evidenced by large confidence intervals. Only in the first sampling year, there was a molting peak in young males between June and September. The data for adult males showed a main molt peak between June and September in both years (Fig. 2B). In adult females, the frequency of gastroliths was always very low (Fig. 2H), so that little can be said on their molt cycle, other than adult females do not molt between August and January, when they carry external eggs (Froglia and Gramitto, 1981).

Tyrrhenian Sea

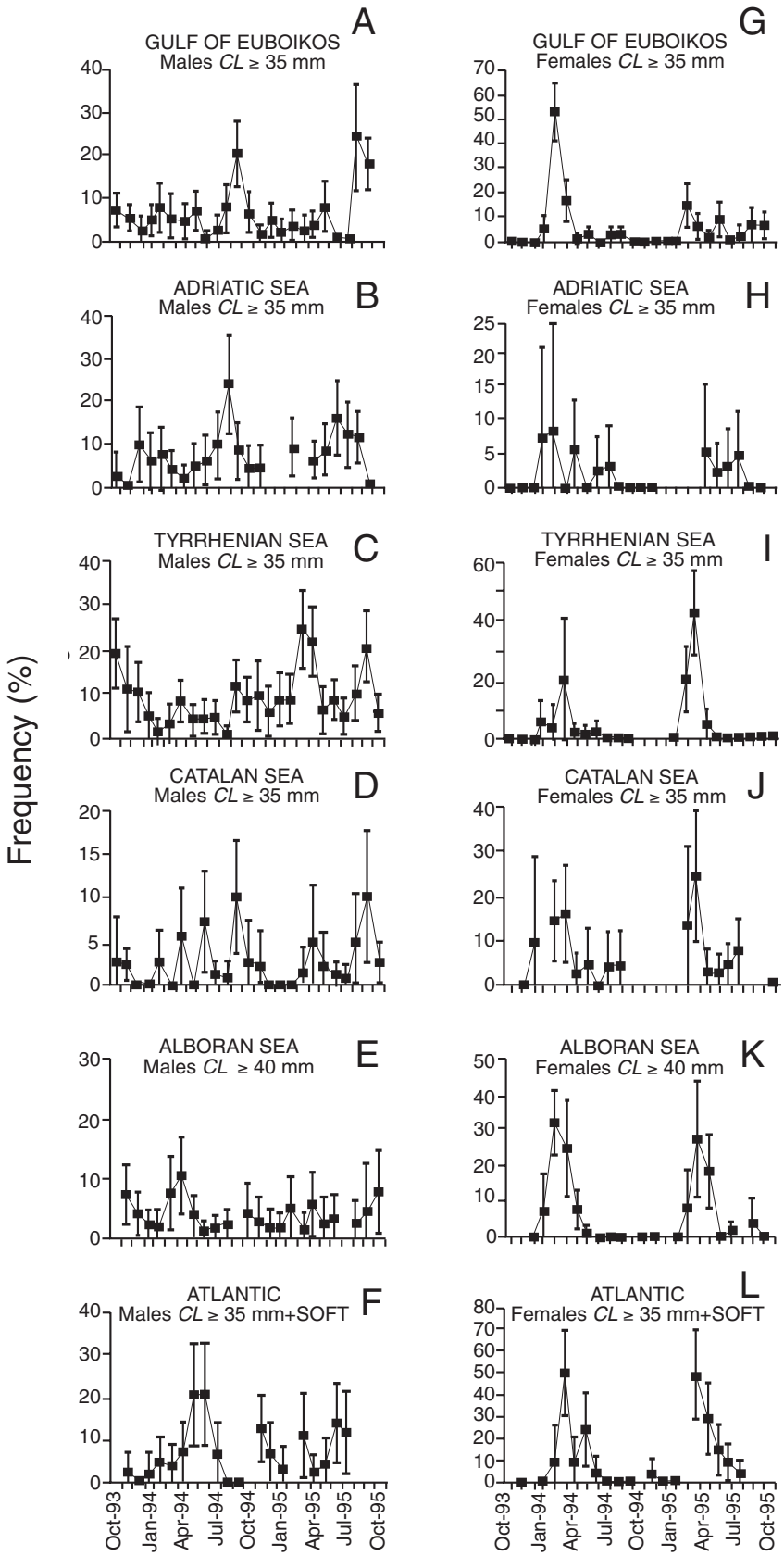

FIG. 2. – Frequency of occurrence of gastroliths (in percentage) in adult males (A-F) and females (G-L) in the studied areas. Vertical bars indicate the 95% confidence interval

Nothing can be said about juveniles. The paucity of specimens sampled (Fig. 1C, H) was unsuitable for an analysis (less than 10 specimens) or resulted in very wide confidence intervals of the estimated gastrolith frequencies. Adult males showed two molting periods: spring (March-April) and late summer (August-October) (Fig. 2C). These peaks were more evident in 1995 than in 1994, when the frequency of gastroliths was quite low (generally less than 10%) in spite of the high number of specimens examined. Adult females confirmed the existence of only one molt period per year (winter-early spring) (Fig. 2I).

Catalan Sea

In juveniles of both sexes (Fig.1D, I) gastrolith frequency fluctuated widely from month to month over the two years, without any evidence of molt periodicity. In adult males (Fig. 2D) the gastrolith frequency was always very low (less than 8%) and the confidence limits very large. It can be argued that molt occurred the year round with a possible maximum peak in September (10% in both years). Adult females (Fig. 2J) were the less abundant, but the percentage of specimens with gastroliths reached peak values in February and March (14-15% in 1994, 13-24% in 1995). Data collected in the first sampling year can be sustained by those of the second one. This means that, as in the other areas examined, adult females molt once per year, in late winter. The lack of data between September 1994 and January 1995 is due to paucity of adult females in the samples. It may be the result of the lower vulnerability of berried females.

Alboran Sea

Young specimens were almost completely absent in the first sampling year, and the gastrolith frequencies obtained in the second year are meaningless, due to the paucity of specimens in the samples (Fig. 1E, J). In adult males only a small peak was detectable in spring 1994 (Fig. 2E). In adult females (Fig. 2K), the trend is the classical one, with a well defined molting period, from January to April (F_{max} > 25%, in March).

Atlantic

Samples obtained off Faro, were characterized by the almost complete absence of young specimens (always less than 10 specimens in each length class). Available data for adults (Fig. 2F, L) suggest a molting period extending from March to May for females and a slightly shorter one for males (from May to June).

DISCUSSION AND CONCLUSIONS

To define molting periods for juveniles is quite difficult for the wide fluctuations in gastrolith frequency (Fig.1). Also from data presented in this paper, sex does not seem to influence molt periodicity in young specimens, confirming a common growth pattern for males and females in the juvenile phase (Hillis, 1979; Sardà, 1985; Froglia and Gramitto, 1988). Results obtained in areas where the number of specimens caught is sufficiently high (Catalan Sea and Adriatic Sea), suggest that molt synchrony does not exist among juveniles which can molt all year round (see Sardà, 1991 for the Catalan Sea). In other areas (Tyrrhenian and Alboran Sea, Atlantic) juveniles are scarce or almost absent from samples. Probably the main cause of this low catchability is the behaviour of young specimens, which spend most of their time within burrows, where they also feed. They either build their own burrows (Crnkovic, 1968) and find food by digging tunnels in the sediment (Chapman, 1980), or they can live in small tunnels linked to the burrows of adults (Chapman, 1980), feeding on remains of food caught by adults inhabiting the same burrow system (Baden *et al.*, 1990).

Within each area, slight shifts in molt peaks of adult *Nephrops* are evident between the two years studied (Fig. 2). This may be due to the influence of environmental factors on molt cycle regulation (Conan, 1985; Skinner, 1985). Even so, the synchrony in seasonal molt pattern appears quite evident, particularly in adult females. Differences in molting periodicity between males and females after the onset of sexual maturity (Charuau and Conan, 1977; Froglia and Gramitto, 1988; Sardà, 1995) is also evidenced in gastrolith frequencies (Fig. 2).

Adult females molt before mating, immediately after the hatching of eggs (Conan, 1985; Sardà, 1991). In the Mediterranean they undergo molt once per year between December and March, their molting period off Faro (Atlantic) seems to shift towards spring (March-May).

The molting period of adult males is situated in late summer-autumn. In some areas (Tyrrhenian

and Alboran Sea) there is also evidence for a second molting period, co-occurring with that of females in late winter-early spring. Probably this period is present also in other Mediterranean areas, but it is not so evident as the first one. In the Atlantic area, only a single period in late spring can be noted.

Data from the Ligurian Sea were too scattered to allow any analysis on molting periodicity. Even so, the available values for gastrolith frequency suggest a molting period in late spring both for males and females, as in the Atlantic area.

The existence of a common seasonal molting pattern among *Nephrops* populations in the Mediterranean Sea suggested by Sardà (1995) was only partially confirmed by present work for adult specimens. There is no such evidence among juveniles.

The low frequencies of gastrolith occurrence observed in most of the samples may be due to extended molting periods or to inappropriate sampling periodicity, as well-developed gastroliths are present for about 15 days before molt (Sardà and Cros, 1984). Probably, better results could be obtained with a more intense sampling frequency.

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