

## Structure and absolute growth of a population of *Hippolyte inermis* Leach 1815 (Decapoda: Caridea) from *Zostera marina* (L.) meadows (Malaga, southern Spain)

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**SUMMARY:** The *Hippolyte inermis* Leach 1815 population from *Zostera marina* beds in southern Spain showed two recruitment periods that occurred simultaneously for both sexes (from September to December and from April to June), in a size range between 1.67 and 1.90 mm carapace length, due to gonadal activity and eggs hatching in summer and winter. The estimated Von Bertalanffy parameters were used to determine absolute growth and showed that males live for around 8 months and females for around 12 months; consequently, four cohorts for males and 7 to 8 for females can coexist throughout the cycle. The sex ratio favours females throughout the entire life cycle. Data published on the reproductive biology of *H. inermis* support the idea that this is a protandric hermaphrodite species, though recent studies have revealed that there is no histological proof of hermaphroditic sexuality in adult specimens of this species. The results obtained here indicate that the Cañuelo Beach *Hippolyte inermis* population has a gonochoric structure. If *H. inermis* were to have hermaphroditic sexuality, the sex reversal of adult males would occur in a single moult in the size range between 2.42 and 3.22 mm. These new, secondary females would be incorporated into the primary female cohort at practically the same size, although they would be 0.12 to 5.20 months younger. Our results, compared with those from other population studies, suggest that this species has a highly plastic population structure, which seems to be determined by external factors and which varies between the protandric and gonochoric condition, depending on the conditions of the habitat.

**Keywords:** *Hippolyte inermis*, Caridea, growth, population dynamic, reproductive biology.

**RESUMEN:** ESTRUCTURA Y CRECIMIENTO ABSOLUTO DE UNA POBLACIÓN DE *HIPPOLYTE INERMIS* LEACH 1815 (DECAPODA: CARIDEA) DE LAS PRADERAS DE *ZOSTERA MARINA* (L.) (MÁLAGA, SUR DE ESPAÑA). – El estudio de una población de *Hippolyte inermis* Leach 1815 de fondos de *Zostera marina* del Sur de España muestra dos periodos de reclutamiento simultáneo para ambos sexos, de septiembre a diciembre y de abril a junio, en un rango de talla de 1.67-1.90 mm como consecuencia de la maduración gonadal y la eclosión de la puesta en verano e invierno. El estudio de los parámetros de Von Bertalanffy muestra que el modelo de crecimiento absoluto es de tipo indeterminado y que los machos viven alrededor de 8 meses, mientras que las hembras son más longevas (12 meses); consecuentemente durante el período de estudio coexisten 4 cohortes para los machos y 7-8 para las hembras. El estudio de crecimiento poblacional revela que éste viene determinado por el de las hembras, ya que el *sex-ratio* siempre es a favor de estas últimas. Datos publicados sobre *Hippolyte inermis* apoyan que se trata de una especie hermafrodita proterándrica, aunque estudios histológicos en adultos no apoyan esta hipótesis. En el caso de la población estudiada, el conjunto de resultados obtenidos justifican sobradamente que se trata de una población gonocórica. Si existiera cambio de sexo en los machos adultos este se produciría en una sola muda, en el rango de talla de 2.42-3.22 mm y estas nuevas hembras se incorporarían a las cohortes de hembras primarias de la talla más o menos similar pero entre 0.12-5.20 meses más jóvenes. Los resultados obtenidos, en comparación con otras poblaciones de otras áreas geográficas, muestran que posiblemente esta especie tenga una estructura y dinámica poblacional muy versátil, pudiéndose manifestar como una especie proterándrica o gonocórica dependiendo de factores externos propios de cada hábitat.

**Palabras clave:** *Hippolyte inermis*, Caridea, crecimiento, dinámica de la población, biología reproductiva.

## INTRODUCTION

There are many studies available in the literature that focus on different aspects of caridean biology, such as larval development, reproductive cycles and population structure and dynamics (Allen, 1959; Butler, 1964; Berreur-Bonnenfant and Charniaux-Cotton, 1965; Yaldwyn, 1966; Fréchette, *et al.*, 1970; Noel, 1976; Nakashima, 1987; Gherardi and Calloni, 1993; *Guerao et al.*, 1994; Company and Sardá, 2000; Colloca, 2002; Maiorano, *et al.*, 2002; Cartaxana, 2003; Kim and Hong, 2004; Kim, 2005; Chilari *et al.*, 2005; among others). The number of studies that focus on the reproduction and sexual behaviour of these shrimps is progressively increasing.

The Hippolytidae family it is not well known. Reports have been published on several genera (see Baeza and Bauer 2004; Baldwin and Bauer, 2003; Bauer, 1998, 2000, 2005, 2006; Bauer and Newman 2004, among others). However, in many cases, the research carried out does not include the population dynamics or absolute growth. This is surprising because these aspects provide an overall vision of the life histories of caridean species and are thus necessary for understanding the peculiarities of caridean reproduction.

Previously published data about the reproductive biology of *Hippolyte inermis* Leach, 1815 support the idea that this species is a protandric hermaphrodite. Zupo (1994; 2000) reached this conclusion after studying a population of *H. inermis* in seagrass meadows of *Posidonia oceanica* (Linnaeus) Delile. This author found two reproductive periods that were synchronic with the seasonal growth of this seagrass. Both females ( $\beta$ ) and males were recorded in the first recruitment period of *H. inermis* juveniles to the seagrass beds, but during the second period, only juvenile males were found. From these results Zupo suggested that all the males in the population undergo sex reversal, which is determined by environmental factors (Zupo, 2000; Nappo *et al.*, 2006), and transform into large females ( $\alpha$ ) in the following year, just after the spring season.

However, a recent study from our laboratory (Cobos *et al.*, 2005) that included a careful examination of the morphology of the reproductive system of this species did not find any proof of hermaphroditic sexuality in the studied population of *Hippolyte inermis*.

The main aim of the present work is to complete the published studies on the biology of *Hippolyte*

*inermis*, focussing on population structure, dynamics and absolute growth of *H. inermis* from *Zostera marina* (Linnaeus) meadows on the southeast Spanish coast.

## MATERIAL AND METHODS

### Sampling

In 2000 and 2001, two replicates of monthly samples were collected from *Zostera marina* (Linnaeus) meadows between 11 and 14 m depth, at El Cañuelo Bay (36°44'40.51''N, 03°47'56.26''W - 36°44'23.20''N, 03°47'22.71''W), Mediterranean Sea, Malaga, Spain (Fig. 1). An Agassiz trawl (76.6 x 30 cm, with a net of 3 x 3 mm from knot to knot) was used for 10 min (total sampled area: 235 m<sup>2</sup>). In the laboratory, the leaves of *Z. marina* and the sediment were washed with marine water, and the *H. inermis* specimens separated from other fauna (Zariquiey Álvarez, 1968; Udekem d'Acoz, 1996) and fixed in different fixative solutions depending on the histological technique used (see below for details).

### Sex identification and morphometry

The sex of the sampled individuals was determined by observing the morphology of the first pleopod and the presence or absence of the male appendage on the second pleopod.

A stereoscopic microscope and a light camera were used to measure the length of the carapace (CL: maximum length from the posterior margin of the ocular orbit to the posterior margin of the carapace). A total of 176 males and 986 females was measured.

### Population structure, absolute growth and breeding periods

In order to analyze the length-frequency histograms, the measurements were grouped into size classes calculated in accordance with Christiansen (1983), i.e. 27 size classes ranging from 1.62 to 6.8 mm carapace length. Von Bertalanffy (1934, 1938) parameters were determined by the Bhattacharya (1967) and Ford-Walford (1946) methods, which were run using FISAT software (as proposed by Gayanilo *et al.*, 1989, 1994). The  $\phi$  index (Munro and Pauly, 1983, modified by Sparre, 1987) was

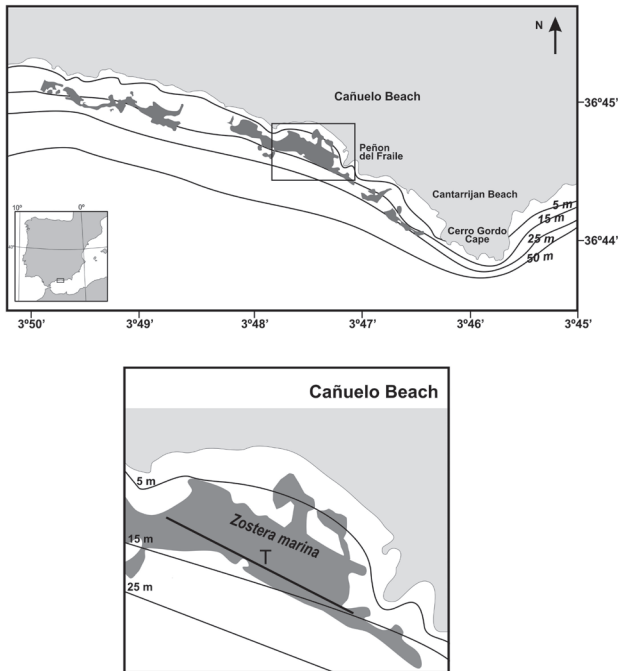


FIG. 1. – Study area. “El Cañuelo” Beach on the Malaga coast (Mediterranean Sea, Spain). T: trawl transect. N: North.

applied to analyze the differences between these parameters and the absolute growth model obtained for both sexes.

The breeding period and fertility studies were established with monthly counts of the ovigerous females and the eggs they carried (fecundity studies). An image analysis method based on a Visilog 6.0 computer system (Benhamou *et al.*, 2004) was used to estimate the number of eggs and their average diameter (the entire brood of each female was measured). The embryo stages were determined by the percentage of vitellogenine, and the development stage of the polar disc, the appendages and the eyes, so that six stages were defined: S1, the complete egg is a homogeneous vitellogenine mass; S2, the tissue cap stage (clear area at the animal pole) of the developing embryo; S3, the cephalothorax and abdominal regions are well defined; S4, the embryo occupies more than two thirds of the total egg volume and eye development starts; S5, the pereopods and telson are wrapped by the cephalothorax of the embryo; S6, eye development is complete and the vitellogenine mass has almost disappeared. Specimens were considered

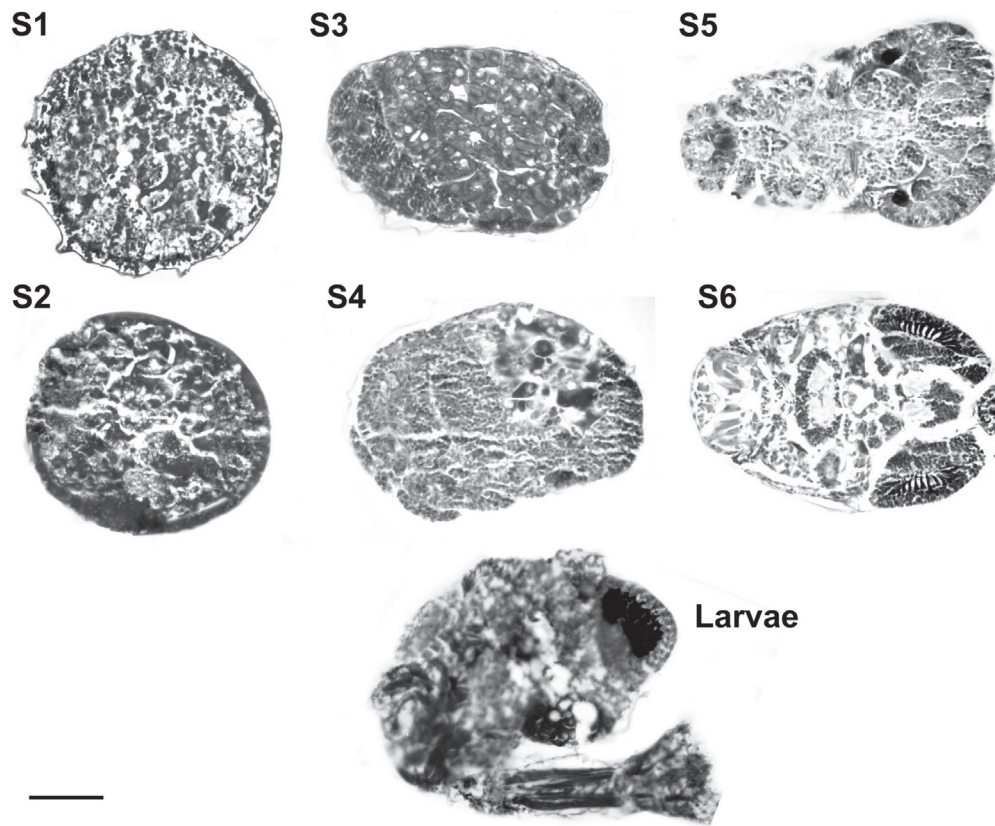


FIG. 2. – Sections of eggs with embryos in different development stages. S1: stage 1 (transversal section), S2: stage 2 (frontal section), S3: stage 3 (sagittal section), S4: stage 4 (sagittal section), S5: stage 5 (frontal section), S6: stage 6 (frontal section), Larvae: First larvae just hatched from the egg. Bar: 1 mm.

to be larvae at the first appearance of zoeal larval features (Glas *et al.*, 1997) (Fig. 2).

## Histology

Specimens were fixed in a methanol–acetone–distilled water solution (2:2:1) for 8 to 12 hours or in 4% formaldehyde. Samples were then dehydrated in an ethanol series followed by butanol, and embedded in paraffin. Serial sections of the ovaries and eggs were obtained using a Lieitz microtome; the sections were collected on poly-L-lysine-coated slides. After drying, sections were dewaxed in xylene, hydrated in an ethanol series, washed in distilled water, and stained with Mallory's trichrome (Pauley, 1967), VOF (light green + orange G + acid fuchsin) (Gutiérrez, 1967), and P.A.S. (periodic acid–Schiff) (Kiernan, 1990).

## Statistical analysis

SPSS 14.0 v. software was used for statistical analysis. To define the relationship between ovigerous females, the number of eggs per female and the carapace length, the Pearson coefficient ( $r$ ) was computed with a significance level of  $P = 0.0001$ ; a Kolmogorov-Smirnoff analysis was used for normal variables. A regression analysis was used to find the best model (logistic or exponential curves) to explain the distribution of the results.

## RESULTS

### Abundance and sex ratio

The evolution of the monthly relative abundance in each cycle is shown in Figure 3A. In the summer period the general abundance decreases, mainly due to the absence of males. Since the number of males remained very low throughout the study period (Fig. 3B), the sex ratio (M/F) clearly favoured females ( $0.19 \pm 0.17$ ).

### Breeding periods, fertility and fecundity

Females bred throughout the entire year (Fig. 3C). There was an abundance peak of ovigerous females in spring and summer and a minimum in autumn and winter. During the spring breeding period the ovigerous females, which tend to have vitellogenetic ovaries, carry eggs at different developmental stages

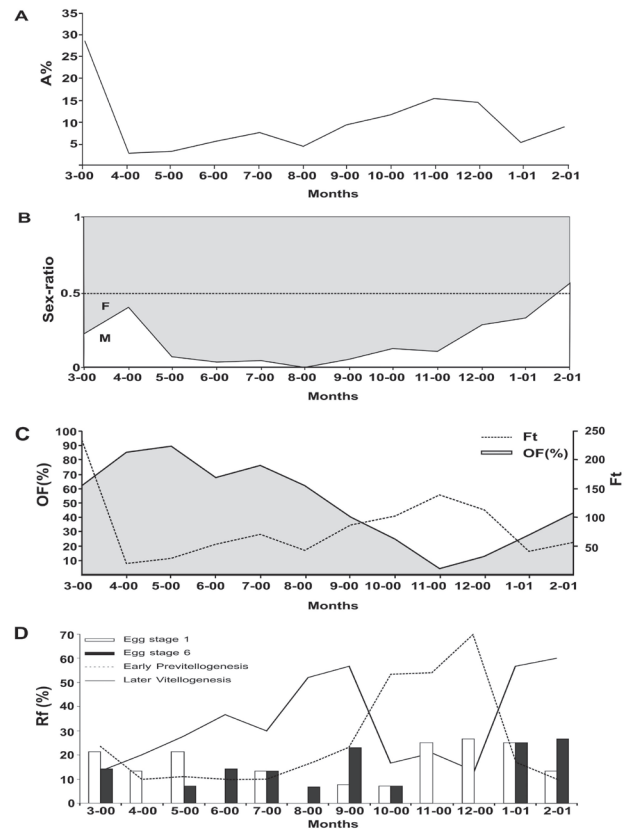


FIG. 3. – A. Monthly relative abundance (A%) throughout the cycle. B. Changes in sex-ratio. C. Monthly evolution of the frequency of ovigerous females (OF (%)) and total females (Ft). D. Monthly evolution of gonad maturation and egg stages 1 (white) and 6 (black) of embryo development. Rf (%): Relative frequency

(Fig. 3D). In summer, ovaries are in the late vitellogenesis stage and carry eggs in the last development stage (S6). These eggs mainly hatch during the summer and the juveniles are recruited to the population in September (Fig. 3D, 4). In autumn, recruitment is continuous until November-December when a new period with low abundance of ovigerous females begins. However, there is an increment in the total number of females as a consequence of new, small, previtellogenetic females (which do not carry eggs) in the population (Fig. 3C, D) and small, older females that carry eggs at earlier development stages. In winter, most of the eggs in Stage 6 begin to hatch, which leads to another recruitment event in March (Fig. 4).

The biometrical analysis of the ovigerous females and size-class distribution shows that a carapace length of 3.98 mm is the optimal reproductive size (Fig. 5A). Moreover, at this size 50% of females are ovigerous (Fig. 5B), although ovigerous females were also found in the first and following size classes (minimum size = 1.62 mm).

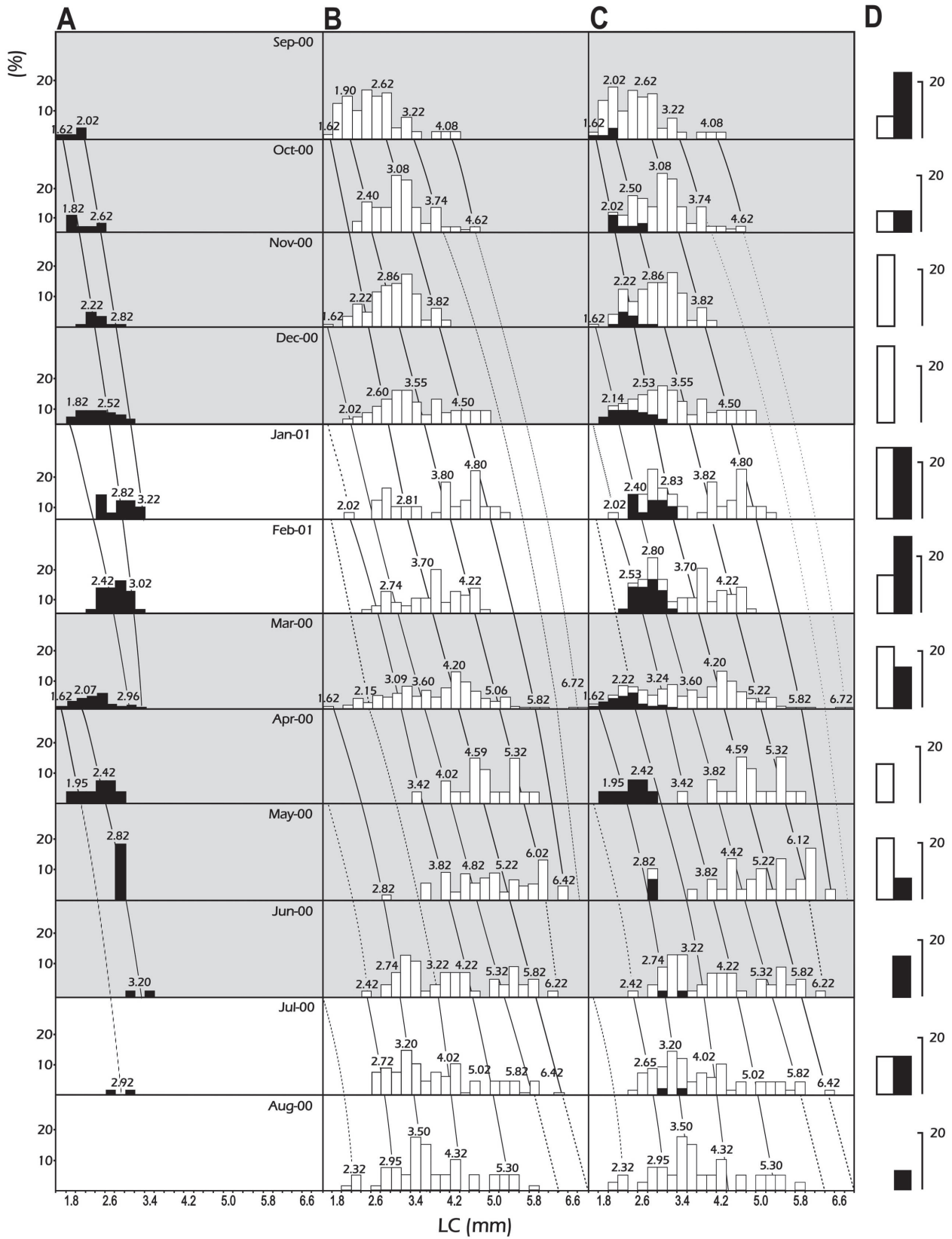


FIG. 4. – Frequency histograms of size classes. Size evolutions are indicated with diagonal lines between the mean size values of the cohorts. A: males; B: females; C: whole population. D: Monthly evolution of embryo development stages. 1 (white): earliest stage. 6 (black): last stage.

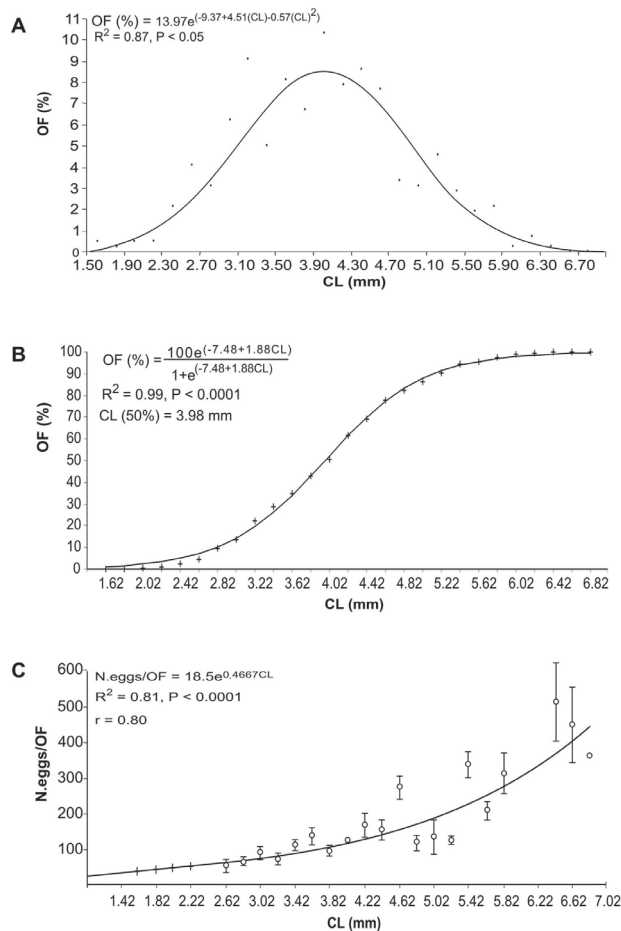


FIG. 5. – A. Relationship between relative frequency of ovigerous females (OF (%)) and size (CL (mm)). B. Relationship between accumulated frequency of ovigerous females (OF (%)) and size (CL (mm)). C. Relationship between the mean number of eggs (Neggs/OF) by ovigerous female and the size (CL (mm)) (exponential regression).

The relation between the mean number of eggs (Neggs/OF) per ovigerous female and body size follows a potential function (Fig. 5C). The number of eggs per brood ranged from 56 to 513. The maximum and minimum averages for the egg diameter were  $0.37 \pm 0.04 \text{ mm}$  and  $0.29 \pm 0.02 \text{ mm}$  respectively.

### Structure of the population and absolute growth

In the population studied (Fig. 4), both sexes were well represented in the first size classes, but there were only females in the largest size classes. The maximum carapace length observed for males was 3.42 mm and 6.72 mm for females. There was a large overlap between mean sizes of the two sexes in the small size classes (Fig. 4C). Although the minimum sizes found for both sexes were similar (1.62 mm), females were slightly larger than males (up to 1.90 mm CL).

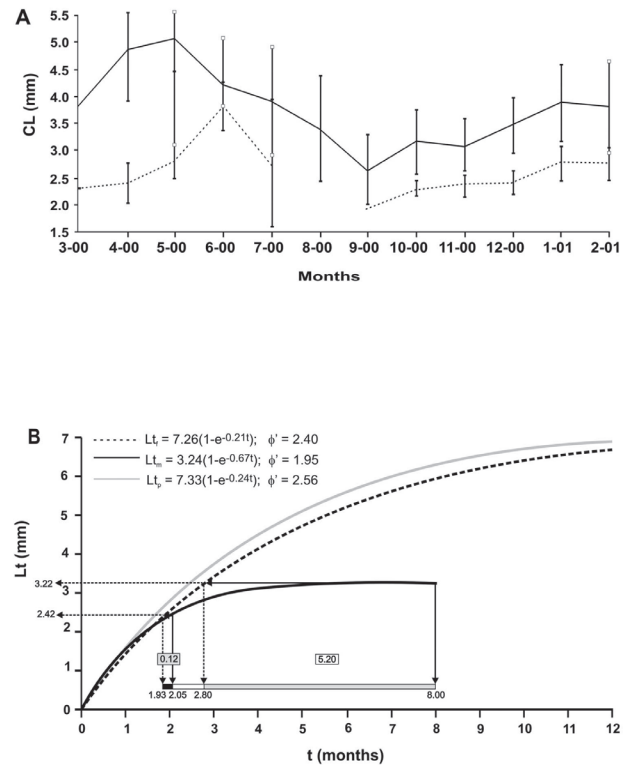


FIG. 6. – A. Monthly evolution of the mean size (CL: carapace length) and its standard deviation by sexes. Females: continuous line, males: discontinuous line. B: Absolute growth models of *H. inermis*. Females: discontinuous line (L<sub>t</sub>: total length for females), males: continuous black line (L<sub>tm</sub>: total length for males), and the whole population: continuous grey line (L<sub>tp</sub>: total length for the whole population). Continuous line with arrows: sizes and ages of males. Discontinuous line with arrows: sizes and ages of females. Bar: Possible sex reversal age ranges; black: minimum, grey: maximum. Lt: total length.

Modal regression analysis identified 2 to 4 cohorts (modal classes) for males and 5 to 9 cohorts simultaneously in one month (Fig. 4A, B) for females, which had similar mean sizes for both sexes. Recruitment was more or less continuous during two four-month periods, from March to May-June and from September to December (the most important) (Fig. 4, 6A). There were two peaks, one in March and one in September, which originate from the eggs hatching in January-February and July-August (Fig. 3D, 4D).

### Gonochoric growth model

The Von Bertalanffy parameters ( $L_\infty$ , K) were determined for males ( $L_\infty = 3.24 \text{ mm}$ ,  $K = 0.67$ ), females ( $L_\infty = 7.26 \text{ mm}$ ,  $K = 0.21$ ) and for the whole population ( $L_\infty = 7.33 \text{ mm}$ ,  $K = 0.24$ ) (Fig. 6B). The  $\phi'$  index showed significant differences between sexes, but not between females and the whole population ( $\phi' = 1.95$ , males;  $\phi' = 2.40$ , females;  $\phi' = 2.56$ , population) (Fig. 6B).

### Protandric growth model

In this case the Von Bertalanffy parameters were assumed to be those calculated for the whole population, taking into account that the same specimen reverses from male to female. According to this growth model, the sex reversal of males that become secondary females should occur in the size range between 2.42 mm and 3.22 mm. The secondary females would be incorporated into the cohorts of primary females with the same mean size range (Fig. 5C), although the secondary females would be between 0.12 and 5.20 months younger than the primary females (Fig. 5C, 6B).

### DISCUSSION

The *Hippolyte inermis* population from *Zostera* beds studied here shows a population structure that is very similar to the Italian population from *Posidonia* beds studied by Zupo (1994). In both populations, abundance increases in summer and reaches maximum values in autumn as a consequence of recruitment; however, we observed another abundance peak in spring.

Females dominate the population composition throughout the entire life cycle, so that the sex-ratio favours females, similarly to other decapod species (Manjón-Cabeza and García Raso 1994, 1996, 1998). The Spanish population recruits a month later than the Italian population. In both cases, the recruitment lasts four months due to the gradual incorporation of males and female juveniles. There are some differences between the Spanish and the Italian populations probably due to the fact that they live in different seagrasses. In the Spanish population, males are not present in August, and the medium size of female cohorts continues to increase until they reach a larger size in the following months. The Italian population (Zupo, 1994), however, has a single male recruitment in the same month and females are absent. The females in this period come from the sex reversal of the male cohorts of previous months.

The growth parameters (Von Bertalanffy,  $L_{\infty}$ , K) show significant differences between sexes (see  $\phi'$ ), but the model for the whole population is very similar to that of females. We believe this is mainly due to the abundance of females (see sex ratio), the  $L_{\infty}$  value (population maximum size = female maxi-

um size) and the similarity between mean values for males and females that belong to the same cohort. Females live around 12 months, whereas males live for no more than 8 months or they become females at a size between 2.42 mm and 3.22 mm (the last size is very similar to the sex reversal size given by Zupo;  $LT = 17 \text{ mm} \sim CL = 3.20 \text{ mm}$ ). This sex reversal (which was not detected in the population studied here) most probably occurs in a single moult, probably without an increase in size, as the shrimps must put all their energy into the sex reversal process. These secondary females are incorporated into the primary female cohorts that include specimens with a similar size, but different ages. Therefore, it is difficult to fit a growth model to a protandric hermaphrodite species with primary females, at least in natural populations.

Three important conclusions can be drawn from the above analysis and the results in the literature: (1) there is no evident sex reversal in the gonads (Reverberi, 1950; Cobos *et al.*, 2005); (2), there is no evidence of any change in the relative growth of the male appendix (Cobos *et al.*, 2005); and (3) studies of different populations obtained different results for the life cycle of *H. inermis* (Zupo, 1994; the present study).

There are two possible explanations for the complex reproductive biology of *H. inermis*: (1) it is a gonochoric species that shows large sexual dimorphism and males do not change, dying as males; or (2) it is protandric species with its sex regulated by environmental factors such as temperature (Regnault, 1969a), nutrition (Regnault, 1969b; Zupo, 1994, 2000; Zupo and Buttino, 2001; Nappo *et al.*, 2006), parasitism (Veillet *et al.*, 1963) or genetic factors (Reverberi, 1950). In this case, sex reversal could occur during larval development and/or the adult life cycle.

Most previous studies have defended the protandric hypothesis because small females (primary females) were not found in populations and therefore only males were thought to be involved in the recruitment cohorts. However, in the present study and in the Italian population studied by Zupo (1994), there are primary females and males in the same size class. Nevertheless, in the present work no male recruitment was found, while the Italian population seems to involve only males in one of the two recruitments (Zupo, 1994). This new evidence strongly suggests that a revision of the sex determination and life cycle of this species is required.

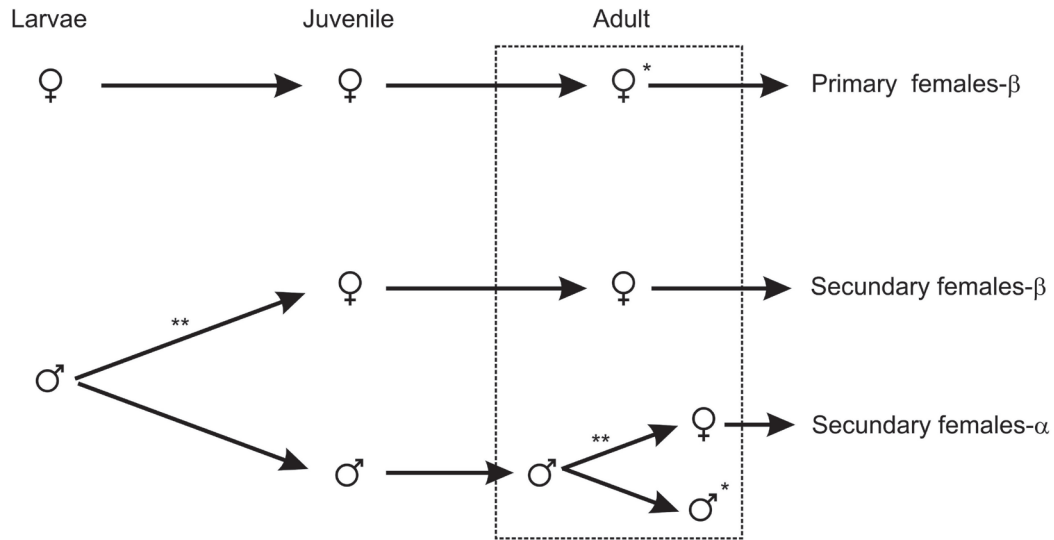


FIG. 7. – Sexual development of *H. inermis*. Summary of life cycle strategies extracted from literature and the present work. \*: Gonochoric condition. \*\* sex determination or sex reversal  $\alpha$ ,  $\beta$ : following Zupo. Primary: without sex reversal. Secondary: with sex reversal.

Zupo (1994) suggests that the presence of small females ( $\beta$  females) in the Italian population is a hypothetical sex reversal in later postlarval stages due to the ingestion of the diatoms *Cocconeis neothumensis* (Zupo, 2000) or *Cocconeis scutellum*, (Nappo *et al.*, 2006), which are very abundant in *P. oceanica* meadows during the reproduction period of *H. inermis* (Zupo and Mazzocchi, 1998). This could explain the high presence of postlarval female stages in culture media rich in *C. neothumensis*, but cannot explain the 5.06% of females already present in control cultures with no *C. neothumensis*. To uphold Zupo and Mazzocchi's hypothesis (1998), this difference in the feeding behaviour of *H. inermis* should be constant throughout the species' entire life cycle under natural conditions.

Our samples come from *Zostera marina* meadows and we have no data on the presence of this diatom in this kind of substrate. However, *Posidonia oceanica* and *Zostera marina* show differences in the surface area of their leaves in their annual cycle (Rueda, 2007; Guidetti *et al.*, 2002), which influences epiphyte settling. Thus, we cannot know whether this is the cause of the different population dynamics of *H. inermis* presented in this study; however, it should be emphasized that the number of females in the small size classes during the recruitment period was always much higher than the number of males (for both recruitment periods). This could be explained by a possible increase in the concentration of these diatoms. However, the easiest explanation could be that the *H. inermis* population we studied recruits females and males, as occurs in the laboratory under

normal conditions (Zupo, 2000), which would suggest that this species has gonochoric characteristics rather than hermaphroditic ones (Cobos *et al.*, 2005). A recent study (Terossi, *et al.*, 2008) shows the same gonochoric conditions in *Hippolyte obliquimanus* Dana, 1852.

A few caridean species have been described as protandric hermaphrodites with primary females, mainly *Processa edulis* (Risso, 1816) (Nöel, 1976; Bauer, 2004), but even in these cases, differences in the relative growth of the male appendix and some intersex specimens have been found. This does not seem to occur in our *H. inermis* population (it did not occur in the French population analyzed by Veillet *et al.*, 1963 either) and only a few intersex specimens have been cited by Reverberi (1950), all of which were parasitized by Bopyrids. The data presented in this work suggest that *H. inermis* could have a highly plastic population structure, with a unique kind of partial protandry that involves primary females and males (following Bauer, 2004), with sex determination occurring in the last larvae stages (Zupo, 2000; Zupo and Messina, 2007). In these life cycle strategies (Fig. 7) the male larvae can continue on to become juvenile males or reverse to become females (secondary or  $\beta$  female according to Zupo, 1994). These adult males can reverse to become females (secondary or  $\alpha$  according to Zupo, 1994) or continue to be males until they die (according to our data). In addition, female larvae do not change and remain female until death (primary, or  $\beta$  according to Zupo, 1994). This high plasticity seems to be determined by external factors, which could vary



depending on environmental conditions and habitat; thus, justifying the results found in other studies of different populations in which both protandric and gonochoric conditions have been described.

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