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Research Article

Reproductive features of the deep-water rose shrimp, *Parapenaeus longirostris* (Crustacea: Penaeidae), in the Strait of Sicily

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Abstract

The deep-water rose shrimp, *Parapenaeus longirostris* (Lucas, 1846), is one of the most valuable and heavily exploited demersal species of the Mediterranean bottom trawl fisheries. The basic life traits of this shrimp, in particular its reproductive aspects, are regularly monitored during experimental trawl surveys carried out in the Mediterranean Sea. Gonadic condition and maturity status for estimating the size at onset of sexual maturity are commonly assessed in females, using macroscopic color scales, histologically validated only in a few geographical areas. In this study, histological analyses were performed on rose shrimps collected from a trawl survey carried out in the Strait of Sicily, in order to support the empirical 4-stage macroscopic scale locally employed. Ovaries from females of different sizes, ranging between 16 mm and 40 mm carapace length, were collected and used for microscopic examination of their structure, for oocytes counts and for oocyte diameter frequency distributions; oocytes diameter was measured by imaging analysis. The collected data were also used to estimate other basic vital parameters of the rose shrimp population. The histological observations show a broad correspondence between ovarian development and macroscopic features; therefore, the classifications derived by the empirical scale remain suitable for estimating the maturity parameters.

Keywords: *Parapenaeus longirostris;* Rose shrimp; Reproductive biology; Maturity scales; Mediterranean.

Introduction

The rose shrimp (a.k.a. pink shrimp in the FAO terminology) *Parapenaeus longirostris* (Lucas, 1846) is a demersal decapod crustacean showing a wide geographic distribution (Eastern Atlantic and Mediterranean) and occurring on deep sandy–muddy bottoms, preferably between 100 m and 500 m of depth (TURSI *et al.,* 1999; ABELLÓ *et al.,* 2002; SAMED, 2002). In its exploited grounds, ready-to-spawn females occur

almost exclusively on the slope (TURSI *et al.,* 1999; ABELLÓ *et al.,* 2002; SBRANA *et al.,* 2006), while in protected and unexploited areas large specimens are present on the shelf too (RINELLI *et al.,* 2005).

Despite some variability, reproduction aspects of females show a common pattern across the geographical distribution: extended spawning activity, with one-to-three peaks in spring-summer and autumn-winter (depending on location, water temperature and females' size), batch production of eggs, and absolute fecundity ranging between 20 000 and 400 000 eggs (NOUAR, 1985; DE RANIERI *et al.,* 1998; TURSI *et al.,* 1999; SOBRINO & GARCÍA, 1994; MORI *et al.,* 2000; SOBRINO & GARCÍA, 2007; GARCÍA-RODRÍGUEZ *et al.,* 2009).

The size-at-maturity (CL_m) estimates for females fall between 19.5 mm and 28.5 mm of carapace length, without apparent distinction among the Atlantic and the Mediterranean populations (FROGLIA, 1982; DE RANIERI *et al.,* 1986; RIBEIRO-CASCALHO & ARROBAS, 1987; SOBRINO & GARCÍA, 1994; SPEDICATO *et al.,* 1996; BEN MERIEM *et al.,* 2001; SOBRINO & GARCÍA, 2007; BIANCHINI & RAGONESE, 2009; GARCÍA-RODRÍGUEZ *et al.,* 2009).

Most of the reproductive data and CL_m estimates for female rose shrimps are based on the application of empirical macroscopic scales, based on ovary shape and color (from whitish-creamy to dark green-turquoise blue); it is worth noting that the carotenoid pigments (in this specific case, atoxantine; CECCALDI, 1968) are extremely sensitive to oxidative process and color changes quickly after the death of the specimen (the so-called black spot phenomenon).

Macroscopic scales for *P. longirostris* have been validated by histological analysis only in a few stocks, both in the Atlantic Ocean (CROSNIER *et al.,* 1970; SOBRINO & GARCÍA, 1994; SOBRINO & GARCÍA, 2007) and the Mediterranean Sea. For the Mediterranean, histological evidence was provided for the Algerian waters (NOUAR, 1985), the northern (DE RANIERI *et al.,* 1986; 1998) and southern (ARCULEO *et al.,* 1992) Tyrrhenian Sea, and the Israeli waters (TOM *et al.,* 1987).

An empirical, not-validated 4-stage scale (LEVI, 1991; LEVI *et al.,* 1995) has been used for many years to study the reproductive features of the rose shrimps of the Strait of Sicily, identifying an extended spawning activity from the maturity data , with a peak occurring from August to March.

The aim of the present study is to support with histological images the empirical macroscopic scale used for the deep-water rose shrimp *P. longirostris* (Lucas, 1846) of the Strait of Sicily, where the species represents one of the most abundant and valuable catches (LEVI *et al.,* 1995; CHAOUACHI & BEN HASSINE, 1998; RAGONESE & BIANCHINI, 2006).

Material and Methods

Rose shrimps were gathered during an experimental bottom trawl survey (Grund program) carried out in the northern part of the Strait of Sicily (GSA15 and GSA16, according to GFCM) in late autumn 2003 (midday, October 09). The general aspects concerning the protocols (statistical design, gear, haul operations and locations, etc.) of the Grund program are found in LEVI (1991).

The specimens were sexed and measured with a caliper(carapace length, CL; 1 mm): moreover, on the basis of the color and shape of the gonad, each specimen was classified according to the macroscopic empirical color scale in Table 1. For further histological analyses, 120 females (16-40 mm)

Table 1
Macroscopic empirical scale for females of *Parapenaeus longirostris*.

stage	maturity definition	color of the ovary	general appearance	comparisons and remarks
1	immature	transparent / whitish	ovary translucid and string-shaped, almost not visible in transparency (if exposed after dissection of tegument, small, whitish or translucid with anterior lobes poorly developed and thin)	1st type in Crosnier *et al.,* 1970 1st type in Nouar, 1985 1st type in De Ranieri *et al.,* 1986 5th stage by De Ranieri *et al.* (1986), as postspawning 1 & 2e stages in the Medits (2007) scale
2	developing	beige-cream (orange when stored in formalin)	visible ovary with the anterior extensions and the lateral lobes distinguished but not much developed; the abdominal extensions are thin and not much visible but colored	2nd type in De Ranieri *et al.,* 1986 2a & 2b stages in the Medits (2007) scale
3	advanced development	pale-green or green-gray (orange-yellow when stored in formalin)	ovary well visible even without dissection; it covers a good extension of cephalotorax segments, and the cephalic and lateral lobes are turgid	2nd type in Crosnier *et al.,* 1970 2nd type in Nouar, 1985 3rd type in De Ranieri *et al.,* 1986 2c stage in the Medits (2007) scale
4	near-ripe and ripe	olive to dark-green (light orange when stored in formalin)	the swollen ovary occupies most of the cephalotorax, hiding the lower organs; the anterior and lateral lobes are well developed, and the abdominal extensions are much evident	3rd-4th type in Nouar, 1985 3rd type in Crosnier *et al.,* 1970 4th type in De Ranieri *et al.,* 1986 2d stage in the Medits (2007) scale

were dissected and the ovary extracted directly, on board.

In previous histological studies (SOBRINO, 1998; SOBRINO & GARCÍA, 2007) no morphological differences were observed in different sectors of the ovary, and therefore only one fragment (1st abdominal segment) of the ovary was taken and stored, first in Bouin solution and then in alcohol 70%. Once in the laboratory, the ovary tissue was dehydrated, embedded in Paraplast and finally included in acetylic resin boxes. The inclusions were serially sectioned at 6 μm, and each section stained; thereafter the sections were permanently mounted in Bio Mount. Various staining techniques were employed: hematoxylin-eosin G, toluidine blue; Gomori's trichromic; P.A.S.

Oocyte development phase and (maximum) diameter were checked and measured both directly (under a Leica DMR optic microscope) and indirectly (using the imaging software Image-Pro^r Plus 4.0); microscopic maturity types were assigned taking into consideration the cytological changes of the oocytes during the development of the ovary.

Results

The length-frequency distribution of the rose shrimp female catch is presented in Figure 1; two peaks are evident, with modes of 14 mm and 22-24 mm CL respectively.

The percentage of the various maturation stages in the sample, according to the macroscopic scale, was 66.8% of immature (stage 1), 22.8% maturing (stage 2), 7.5% advanced (stage 3), and 2.9% of ripe (stage 4) female shrimps.

The wide size range of the rose shrimp specimens examined and the period of sampling made possible the observation of every macroscopic variation in color and shape of the ovaries (Table 1); in fact the ovary, symmetrically extending along the longitudinal axis of the shrimp, changes from an inconspicuous and uncolored appearance to dark-green, greatly swollen, well distinct lobes throughout one complete cycle of oocyte maturation.

The histological analysis allowed the characterization of the ovarian structure and the detection of broadly distinct phases of oocyte development. The ovary parenchyma of the rose shrimp, in particular, showed separated lobes with a mosaic presence of oocyte populations at different development phases (i.e., asynchronous ovary organization). Only when hydration occurs, does a clear separation appear between advanced (vitellogenesis) and hydrated oocytes, underlying both the macro- and the microscopic maturation type of the ovary.

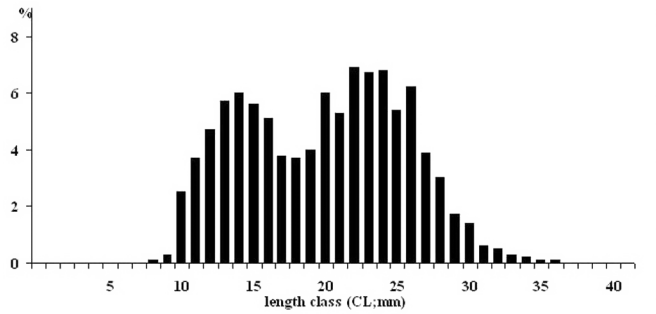

Fig. 1: Length-frequency distribution of *Parapenaeus longirostris* females captured in autumn 2003 in the Strait of Sicily.

Table 2
Histological types of ovary condition for females of *Parapenaeus longirostris*.

ovary type	phase definition	oocyte development (and indicative %)	advanced oocytes diameters (µm)	comparisons and remarks
I	proliferation	oogonial cells (65%) primary oocytes (35%)	20-80	1st phase in De Ranieri *et al.,* 1986 1st phase in Demestre and Fortuño, 1992
II	meiosis	oogonial cells (10%) oocytes with peripheral nucleola (15%) oocytes with dense bodies (45%) primary oocytes (30%)	60-120	2nd phase in De Ranieri *et al.,* 1986 2nd phase in Demestre and Fortuño, 1992
III	early vitellogenesis	cells of the previous kinds (45%) maturing oocytes (45%) primary vitellogenic oocytes (5%) precocious secondary vitellogenesis oocytes (5%)	140-220	3rd phase in De Ranieri *et al.,* 1986 3rd-4th phases in Demestre and Fortuño, 1992
IV	secondary vitellogenesis	immature and maturing cells (20%) secondary vitellogenesis oocytes (80%) presence of ready-to-spawn oocytes (up to 25%) sometimes presence of atresic oocytes	180-300	4th phase in De Ranieri *et al.,* 1986 5th-6th phases in Demestre and Fortuño, 1992

In correspondence to the 4 stages of the empirical scale (Table 1), it is possible to recognize distinct microscopic types: I, proliferation; II, meiosis; III, early vitellogenesis; IV, secondary vitellogenesis (Table 2 and Fig. 2). A further type, characterized by resorptive oocytes (oosorption phenomenon), whose presence is a sign of spent, post-spawning ovaries (DE RANIERI *et al.,* 1986), was not considered, because only a few, sparse atresic cells (Fig. 3d) were detected in 'normally-developing' ovaries.

The characteristics of these 4 ovarian types in terms of cell histology, cell differentiation and cell frequency can be described as:

- Type I, proliferation (Fig. 2a). The ovary primordium, i.e. the 'germinative centrum' formed by the primordial gonia and oogonia, appearing as basophile cells with a prominent nucleus in division, is located in the peripheral area, similarly to what was observed in *Aristeus antennatus* (ORSI-RELINI & SEMERIA, 1983).

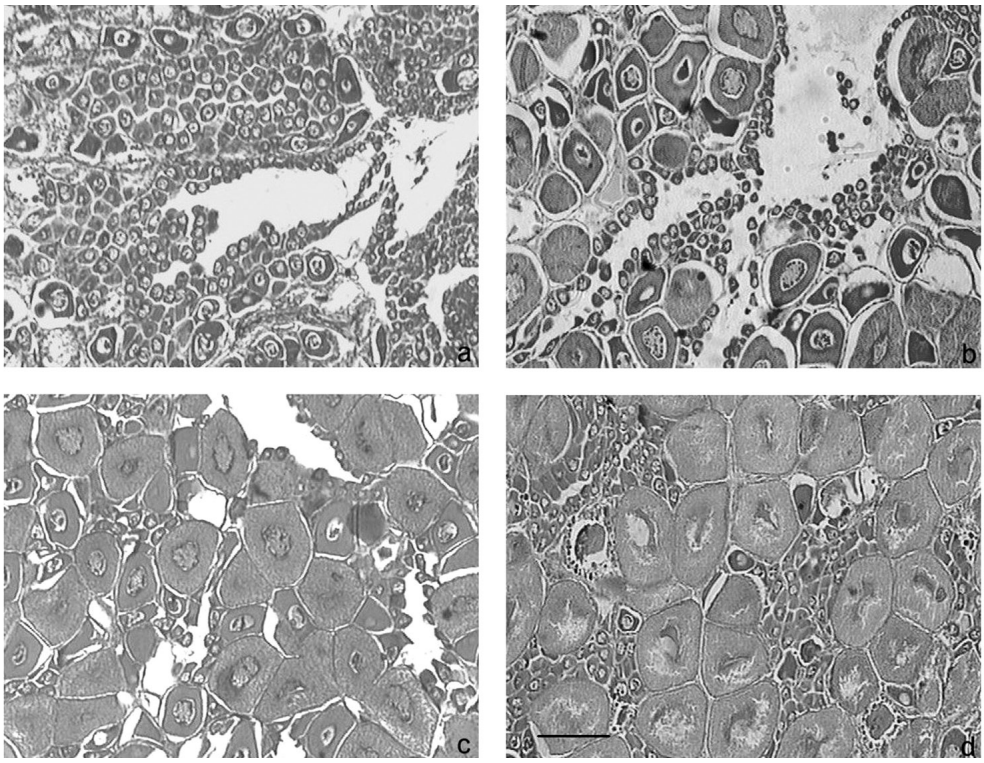

Fig. 2: : Histological sections of the ovaries of *Parapenaeus longirostris,* in different development phases (hematoxylin-eosin stain, 10x; the black line is 0.2 mm long), made from specimens all of carapace length CL= 32 mm. **a)** Proliferative phase: primordial gonia and oogonial cells both grouped in the germinative zone, and early primary oocytes migrating out from it. **b)** Meiotic phase: developing oocytes exhibiting chromatin, nucleolus and dense bodies; note also the surrounding follicolar cells. **c)** Early vitellogenesis phase: oocytes with accumulated yolk globules (endogenous vitellin platelets) in the ooplasma and a still prominent nucleus with several peripheral nucleola. **d)** Secondary vitellogenesis phase: oocytes create a mosaic structure; the germinal zone and 'islands' of oocytes in lower developmental phases remain present; abundant exogenous platelets are visible.

This ovary is characterized by two kinds of cells: a majority of oogonial cells, with the rest primary oocytes, showing an irregular shape and a large nucleus containing patches of dense chromatin. These small cells (20-80 μm in diameter) are grouped around the germinative centrum, and are always detectable at the subsequent maturation phases, although pushed to the periphery.

- Type II, meiosis (Fig. 2b). Four cell categories characterize this ovary: a few oogonial oocytes; some oocytes with peripheral nucleola; abundant oocytes with nucleola appearing as dense bodies (Fig. 3a), spread over a wide size range (60-120 μm); many primary oocytes, forming groups close to the germinative cells. The oocyte cytoplasm is more basophile than in the oogonial cells.
- Type III, early vitellogenesis (Fig. 2c). Early vitellogenesis is characterized by a quick growth of the oocytes, which undergo shape changes as their size increases (from irregular, almost amoeboid form to more regular contours).

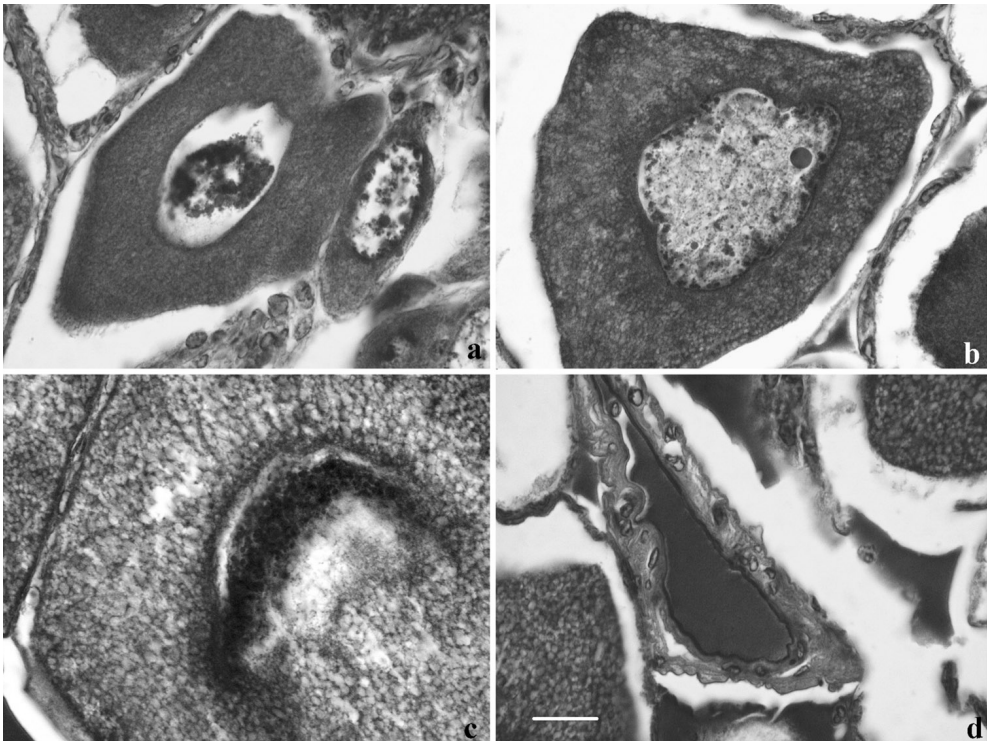

Fig. 3: Details of some cell features in the ovaries of *Parapenaeus longirostris,* in different development phases (Gomori's trichromic stain, 100x; the white line is 20 μm long). **a)** Oocyte undergoing meiosis, showing the so-called dense bodies in its nucleus; follicolar cells border and 'nourish' the oocyte. **b)** Early-vitellogenesis oocyte, whose nucleola have migrated to the nucleus periphery; the cytoplasm appears granular by the abundance of endogenous vitellin platelets. **c)** Ready-to-spawn oocyte, with compressed, peripheric nucleus; the bordering follicolar cells are almost crushed between the mature oocytes. **d)** Resorptive oocyte, of irregular shape, undergoing phagocytosis.

The maturing oocytes show a variable number of highly basophile nucleola, which migrate towards the border of the cell, while the nucleus remains central; the oocytes show endogenous vitellin platelets in their ooplasma, which become more granular (Fig. 3b). As vitellogenesis progresses, the oocytes (140-220 μm) move toward the periphery of the ovary.

- Type IV, secondary vitellogenesis (Fig. 2d). This phase is characterized by vitellin platelets of exogenous origin (entered through micropinocytosis), i.e. acidophile lipo-proteic vesicles (yolk globules), which render the cells opaque. The nucleus changes its previous spherical aspect and localization, migrating to the peripheral zone (Fig. 3c). In this phase, many oocytes are at the ready-to-spawn stage, and the immature/maturing fractions are reduced to less than 25%. The oocytes' size ranges between 180 μm and 300 μm (mode around 220-240 μm).

Discussion

The analysis of the mature condition of the ovaries, as an index of the spawning activity and a tool to estimate the size at onset of sexual maturity, is a common procedure in the stock assessment of commercial Penaeoid shrimp, which do not incubate eggs and shed their fertilized gametes directly into the water. The maturity condition of shrimps is assigned macroscopically; looking at the fresh color and morphological appearance of the ovary, there is a general consensus in using no more than seven stages, including the 'spent' condition (DEMESTRE & FORTUÑO, 1992 for the blue-and-red shrimp *Aristeus antennatus*).

In Penaeoid shrimps, ripe oocytes could form special tubular ('rouleau') structures, e.g. *Aristaeomorpha foliacea* (KAO *et al.,* 1999), or stay as single units, as in P. longirostris (SOBRINO & GARCÍA, 2007); no matter what the intimate architecture of the ovary, it seems that the macroscopic chromatic changes truly reflect the microscopic development of the reproductive tissues. Nevertheless, different studies used a different number of stages to classify the maturity of rose shrimp females: macroscopically, 2 stages were employed in the Medits program (ABELLÓ *et al.,* 2002), which are now split in 6 sub-stages (MEDITS, 2007); 3 stages by CROSNIER *et al.* (1970) and by NOUAR (1985); 4 stages by DE RANIERI *et al.* (1998), by SOBRINO (1998) and in the present study; 5 stages by DE RANIERI *et al.* (1986). A similar situation is true also for the microscopic types, when available: 3 types were observed by CROSNIER *et al.* (1970); 4 types by NOUAR (1985) and 5 types by DE RANIERI *et al.* (1986).

The macroscopic development in the color and shape of the ovaries of the shrimp from the northern part of the Strait of Sicily is in agreement with the general pattern described for other Mediterranean (HELDT, 1938; NOUAR, 1985; DE RANIERI *et al.,* 1986; TOM *et al.* 1987; ARCULEO *et al.,* 1992; DE RANIERI *et al.,* 1998) and Atlantic (CROSNIER *et al.,* 1970; SOBRINO & GARCÍA, 1994; SOBRINO & GARCÍA, 2007; GARCÍA-RODRÍGUEZ *et al.,* 2009) stocks. LEVI (1991) and LEVI *et al.* (1995) observed mature females and juveniles throughout the year: the beginning of the peak reproductive phase in spring (whitish ovaries), with development in summer (gonads cream-orange) and autumn (gonads light-green), and full maturity (dark-green ovaries) reached between autumn and winter (but by late summer on the Tunisian side of the Strait of Sicily; BEN MERIEM *et al.,* 2001); in addition, the histological analyses

of the present study indicate that the rose shrimp of the Strait of Sicily is an asynchronous batch spawner, in which all types of oocytes are present in the mature ovary at the same time.

A broad agreement also exists regarding the phase and size of the oocytes (Table 2), when taking into consideration the effect of the different (histological or binocular) techniques employed: mature or ready-to-spawn oocyte diameter ranges between a minimum of 230 μm (HELDT, 1938) and a maximum of 250 μm to 390 μm, the latter threshold independently of the Atlantic (CROSNIER *et al.,* 1970; SOBRINO & GARCÍA, 2007) or Mediterranean (TOM *et al.,* 1988; ARCULEO *et al.,* 1992) localization examined. Moreover, the (maximum) diameter of the mature oocytes appears to be correlated (r^2= 0.92) with the shrimp size (Fig. 4).

The present results did not allow the establishing of a distinct 'spent' phase, nor observing the cytological structures used as 'definitive' criterion to judge the ready-to-spawn condition of the mature oocytes.

In fact, no female was found with spent ovaries, a condition recorded by CROSNIER *et al.* (1970), ABDEL RAZEK *et al.* (2006) and SOBRINO & GARCÍA (2007); however, the lack or very rare occurrence of spent females has already been indicated for other Penaeoid shrimps (e.g., in *A. foliacea;* KAO *et al.,* 1999). Various hypotheses can be advanced to justify this phenomenon, among which are the wrong sampling period, sudden death after spawning or the inaccessibility of spent females (KAO *et al.,* 1999); still, at least for *P. longirostris,* with a generally-accepted life span of 2-3 years (TURSI *et al.,* 1999), the condition of batch spawner might be a sound explanation. Batch spawning is supposed to be an adaptive strategy to produce numerous large eggs within the constraints of limited food or body-cavity space (MURUA & SABORIDO-REY, 2003); in fact , it is remarkable that the gonadosomatic index of rose shrimps does not exceed 6-7% (SOBRINO & GARCÍA, 2007).

The absence, or at least scanty occurrence, of spent females might be in some degree related to the rarity of histological detection of peripheral rod-like corpuscles (a.k.a. as cortical granules) in mature oocytes, granules which have been considered the only valid criterion for distinguishing the nearly-ripe from the fully-ripe, ready-to-spawn phase in shrimps (CLARK *et al.,* 1980). In *P. longirostris,* these 'star-shaped' oocytes

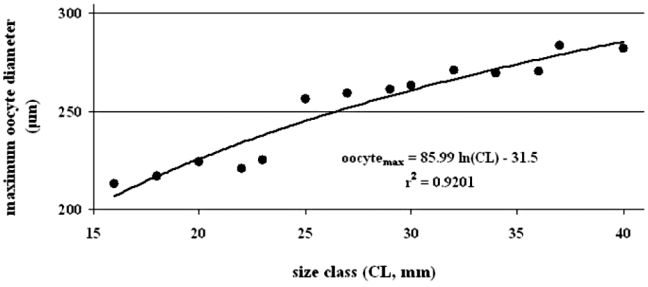

Fig. 4: Relationship between maximum diameter of the mature oocytes and size class in *Parapenaeus longirostris.*

have been detected in two geographical areas (Israeli waters; TOM *et al.,* 1987; and the southern Tyrrhenian Sea; ARCULEO *et al.,* 1992), and as a single occurrence by SOBRINO & GARCÍA (2007); none was observed in the present study nor in other situations. The question is not marginal since the continuous spawning generally reported in literature for *P. longirostris* cannot be maintained when using the presence of the corpuscles as the sole valid criterion for distinguishing ready-to-spawn females: spawning events could happen at periods of the year not sampled by the trawl surveys or in areas not accessible to bottom trawling. Moreover, the almost continuous recruitment reported for the rose shrimp stocks (LEVI, 1991; SAMED, 2002) cannot be proof of an extended and not discrete spawning, since populations might be formed by smaller, discrete units (stock-lets), each with a discrete, specific and variable, spawning activity.

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