A survey of ovarian maturation in a population of
Aristeus antennatus (Crustacea: Decapoda)

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ABSTRACT - Ovaries of the shrimp *A. antennatus*, as observed in other crustaceans, show several colour changes during their life-cycle that are related to growth and maturation of oocytes. In prawns, transparency of cuticles facilitates examination of gonads with the naked-eye. From colour recognition, one can assess ovarian development in a population using fresh material. Because results obtained by this method can be biased by interference with other pigments, histological examination of ovarian maturation is highly desirable. Freshly caught females from western Sicily were examined during the course of one year, either aboard a fishing-vessel or preserved material was examined under the light microscope. Comparison of the results from both "in the field" and by histological methods revealed an annual cycle of ovarian development according to a scale of colour-change. In summary, occurrence of a peak in oocyte growth was demonstrated during the summer months.

KEY WORDS : *Ovarian maturation; Crustacea Decapoda*; Aristeus antennatus; *Mediterranean Sea*

INTRODUCTION

Reproduction of decapod crustaceans is a complex phenomenon whose regulation is not yet fully understood (Meusy and Payen, 1988). During the course of gonad maturation, the ovaries of Aristeid shrimps undergo a sequence of macroscopic changes in morphology and colour that are easily detectable by the naked-eye. Changes in colour are the result of modifications in carotenoid content occurring in early oocytes which may play a role during embryogenesis (Goodwin, 1951). Each colour-change is referred to as a transition to the next phase of development of the ovaries, preceding the release of oocytes. Therefore from the sequence of colour modifications, one can determine, "in

the field", the degree of ovarian maturation of fresh material without necessarily bringing it into the laboratory for complex and tedious microscopic analyses.

This simplified procedure has customarily provided information about the stages of gonadic maturity of shrimp populations (Arculeo *et al.*, 1992; Relini-Orsi and Relini, 1979; Relini-Orsi, 1980; Relini-Orsi and Semeira, 1982; Sardà and Demestre, 1987; Badawal, 1975; Levi and Vacchi, 1988; Arrobas and Ribeiro Cascalho, 1987; Mura *et al.*, 1992). Those colours that define the macroscopic degree of gonad maturation can be arranged in a scale of four to six, going from a translucent white, typical of an immature gonad, to a deep purple hue marking a fully mature ovary ready for egg - laying. Fre-

quent inclusion of carotenoid pigments and their persistence can however overlap actual colours of the ovaries and thus mask the maturation stages thereby causing bias for results from mere visual surveys (Ceccaldi, 1968). We have faced this problem critically by comparing our macroscopic surveys of gonad colours with results of a thorough microscopic examination of ovaries. The actual reliability of the visual method has therefore been tested and new data on the biology of Tyrrhenian shrimps have been gathered.

MATERIALS AND METHODS

Collection of shrimps, measurements

Monthly samples of the red prawn, *Aristeus antennatus*, (Risso,1816), were collected from March 1988 to February 1989. Fishing surveys were carried out within a 1370 km^2 wide area off the Gulf of Castellammare, stretching from Capo Gallo to Capo San Vito (N/W Sicily) whose coordinates are: 13°15' Long. E (Greenwich) and 38°15' Lat. N to 12°45' Long. E and 38°9' Lat. N. Depths ranged from -350 m to -600 m. As many as 1436 individual females were examined. Carapace length (C.L.) ranged from 24 to 58 mm. The following parameters were taken into account:

1) wet weight of whole specimens (precision 0.01 g).
2) wet weight of gonad (precision 0.001 g).
3) presence of spermatophores.
4) diameter of ovaries and oocytes.

The female gonadosomatic index (GI) was calculated for the four seasons according to the following formula: 100 x gonad weight/body weight. The degree of maturity related to gonad colour was evaluated by using the colour scale reported by Relini Orsi and Relini (1979) and modified by Sardà and Demestre (1987).

Histological examination

We examined 214 gonads, 29 of which were of a translucent white; 27 opaque white; 39 pink; 43 lilac; 45 light purple and 31 deep purple. Ovaries removed according to method of Worsmann and Neiva (1972), were fixed immediately for 24 hours in Bouin Holland, then transferred into ethyl alcohol, stocked in buthyl alcohol for 48 hours, and finally embedded in paraffin. Slices, 5-7 µm thick, were stained with erythrosine and toluidine blue (Beccari and Mazzi, 1966).

RESULTS

Macroscopic colour scale

Table I summarizes observations reported hereafter. Total lack of pigments, transparency of tissues and a lowered diameter of gonads were distinctive for stage I. Individuals showing such a condition made their appearance during the entire year, absent only in June. Peaks in the number of colourless ovaries were a relevant feature of samples collected from October to April, with a mean value as high as 98% ± 2.19. Stage II was only slightly swollen with respect to earlier phases and its colour was opaque-whitish. The highest number of ovaries at stage II of maturation were recorded in September with a 9.1% mean frequency. The values observed during the rest of the year were low or even approached 0.

Individuals showing pink (stage III) and lilac-coloured (stage IV) ovaries were collected from May to September. May was the month when the peaks of both stages (35.3%) occurred together. Those at the next stage delayed their disappearance until October. After a two-month disappearance, a lower percentage of individuals at stage III, not exceeding 1.7%, was again recorded in December.

Stages V and VI, appeared in midsummer, respectively distinguished by a light-purple with a high percentage value in July, approaching 62% of the entire population. Deep-purple colour was restricted to June and August samples, with a percentage value of 12.5% registered in June. These individuals underwent a massive

Table I .
Percent frequency of colours observed in the ovaries from monthly samples

MONTH	COLOUR OF OVARIES					
	translucent white	opaque white	pink	lilac	light purple	deep purple
March	94.3	6.1	--	--	--	--
April	100.0	--	--	--	--	--
May	29.4	--	35.3	35.3	--	--
June	--	--	6.2	18.8	62.5	12.5
July	17.7	3.7	24.3	25.2	19.6	9.3
August	16.4	6.2	21.7	24.1	16.6	4.5
September	27.3	9.1	18.2	27.3	18.2	--
October	98.0	--	--	2.0	--	--
November	96.4	3.6	--	--	--	--
December	98.3	--	1.7	--	--	--
February	100.0					

increase in number at the end of June. The maximum gonadosomatic index (GI), ovarian diameter and the percentage of spermatophores coincided with stage VI (Tab.II).

Microscopic examination of the Ovaries

Stage I (Translucent white) - Ovaries showed two major cell-groups, one made up of oocytes undergoing meiotic prophase, and the other of oocytes surrounded by follicular cells as well; these showed signs of previtellogenesis (Fig. 1). A conspicuous follicular tissue was evident in samples collected in September because of the release of spawned oocytes (Fig. 2).

Stage II (opaque white) - The oocytes differed at an earlier stage, merely as slight morphological details exhibited by the follicular tissue distributed between them. Samples collected in March showed essentially a central germinative zone containing gonia, surrounded by somatic cells, and young oocytes in meiotic prophase. Near the periphery of the ovary we observed the presence of compact tissue formed by degenerating vitellogenic oocytes and a massive amount of phagocytic cells including follicular cells (Fig. 3).

Stage III (pink) - Ovaries were essentially made up of oocytes surrounded by follicular cells; no reabsorbing oocytes were found in those individuals where vitellogenesis had already occurred. A shrinking follicular tissue was reported in individuals sampled in July which were characterized by a lighter pink colour. Evidence of the release of oocytes was revealed by empty spaces observed inside the tissue. Degeneration of some vitellogenic oocytes (Fig. 4) was evident in July and indicated that these cells had failed to be spawned. Some cell fusions were evident.

Stage IV (lilac) - Vitellogenic oocytes, normally surrounded by follicular cells, characterised this

stage whose morphology was similar to the previous one. In June samples cytoplasmic fusions of oocytes were evident (Fig. 4a).

Stage V (light purple) - Full vitellogenic activity was recorded in oocytes, most of which exhibited a star-shaped structure. As pointed out by Oka and Shirahata (1965) in *Penaeus orientalis*, nucleolar modifications mark this phase of vitellogenesis (not shown).

Stage VI (deep purple) - Cortical granules were detected in the peripheral cytoplasm of vitellogenic oocytes whose follicular envelope had withdrawn (Fig. 5). All oocytes seemed to undergo this modification synchronously. Degenerative phenomena of the ovary were well evident in August, despite the persistence of the deep purple colour. They were distinguished by numerous empty spaces due to oocyte release and by a follicular shrinking tissue with follicular cells participating in yolk re-absorption (Fig. 6).

Mean diameters of oocytes and ovaries are shown in figs 7 and 8.

Gonadosomatic Index

The lowest calculated mean value was 0.57% in winter and the highest was 7.74% in summer (Table II). This finding suggests that GI can also be useful in determining the occurrence of this macroscopic stage of maturity.

DISCUSSION

Six macroscopic stages of gonad maturation have been identified, to a great extent coincident with the sequence described by Sardà in Spanish populations (1987), but only partly consistent with observations made by Relini in the Ligurian Sea (1979). Stage VI, corresponding to egg-laying, occurred in a three-month interval from June to August, in agreement with the observations of Mura *et al.* (1992) in Sardinia. This 90-day period was, however, much shorter than the 6-month interval reported by Relini (1979) and 5-monthly by Sardà (1987) in more Western and Northern waters.

The regular seasonal rise of other parameters (GI values, the diameters of oocytes and ovaries) from spring to summer when they attain the peaks, further supported our observations. The microscopic screening also confirmed some phenomena that had not been detected by examination at macroscopic levels.

Table II
Seasonal frequencies of the following parameters: female Gonado-somatic index (GI) 100 x gonad weight/body weight; percent spermatophore - bearing females (% Sperm); Mean diameter of oocytes (Diamet. oocytes) in μm; Mean diameter of ovary in mm. (Diam. ovary); Total no. of individuals (Tot. n. indiv.).

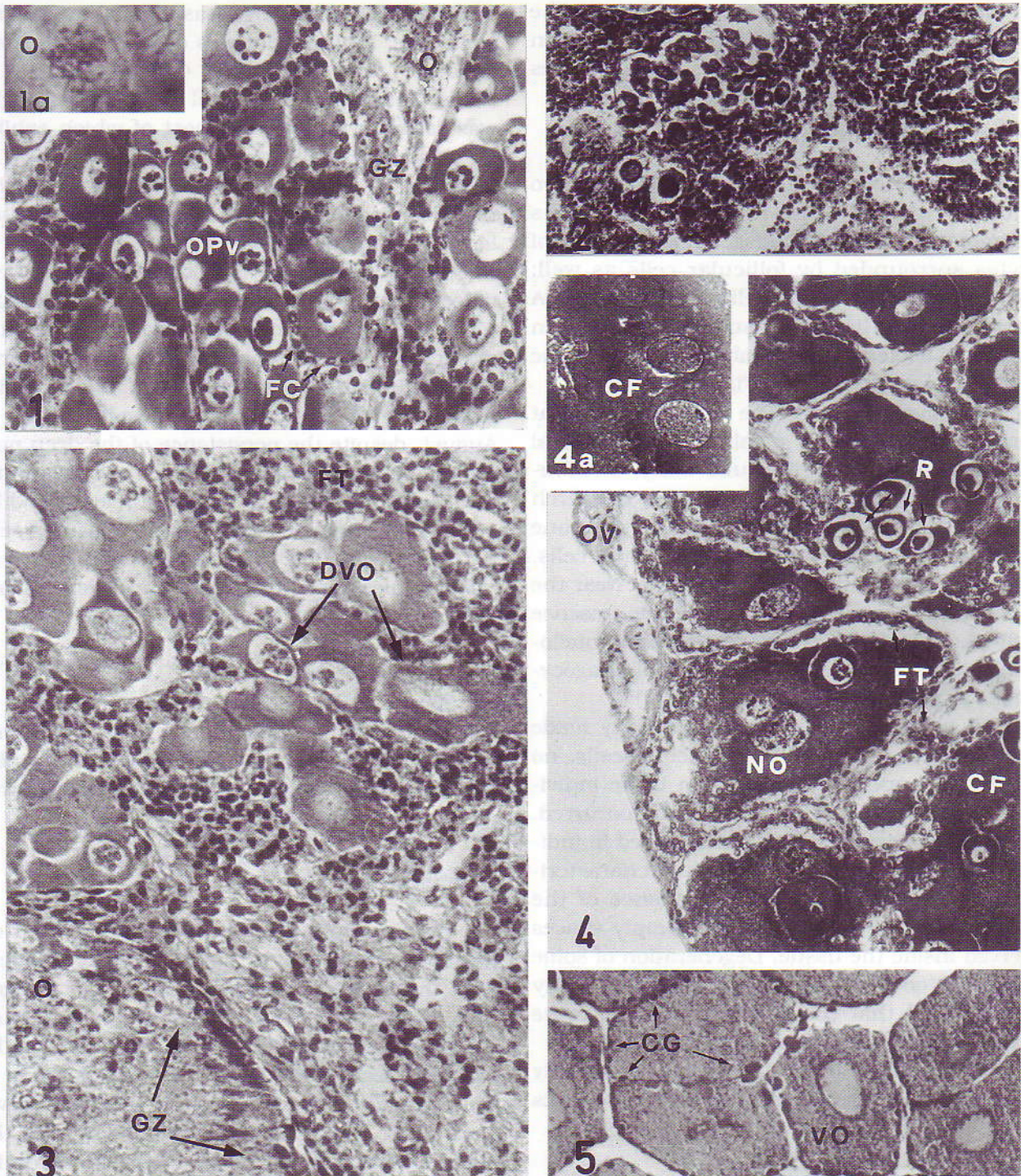| SEASON	Total n. indiv.	G.I.	% Sperm	Diam. oocytes	Diam. ovary
Spring	361	0.80±0.18	34.1	103.1±22.4	1.12±0.39
Summer	286	7.74±1.93	98.4	197.8±27.9	2.10±0.75
Autumn	418	2.21±0.33	39.2	148 ±12.3	1.18±0.33
Winter	236	0.57±0.09	5.3	49.5±1.2	0.75±0.11

Fig. 1. Histological aspect of an ovary at stage I. Two major categories of germ-cells can be distinguished: oocytes in meiotic prophase (**O**) near the germinative zone (**GZ**) and oocytes in previtellogenesis (**OPv**) surrounded by follicle cells (**FC**) (arrows). (x 320).

Fig. 1a. Oocytes (**O**) in meiotic prophase (probably at pachytene stage). (x 640)

Fig. 2. Ovaries at stage I. Conspicuous follicular tissue observed in September samples. (x 320)

Fig. 3. Periphery of an ovary at stage II. Note the important follicular tissue (**FT**) surrounding few degenerating vitellogenic oocytes (**DVO**) (arrows). **GZ**, germinative zone; **O**, oocytes in meiotic prophase. (x 320).

Fig. 4. Periphery of an ovary at stage III showing the degeneration of non-spawned oocytes (**NO**) and remnant of oocytes (**R**). **FT**, shrunken follicular tissue; **Ov**, ovarian wall. (x 320).

Fig. 4a. Cytoplasmic fusion (**CF**) between oocytes in a stage IV ovary. (x 640)

Fig. 5. Ovary at stage VI. Presence of cortical granules (**CG**)(arrows) lining the cytoplasm of vitellogenic oocytes (**VO**) whose follicular tissue has retracted. (x 320).

Fig. 6. Degeneration phenomena of the ovary at stage VI. Empty space (**ES**) due to oocyte release; follicular shrinking tissue (**FT**); follicular cells participating to yolk reabsorption (**FC**); Nucleoli (**NU**). (x 320).
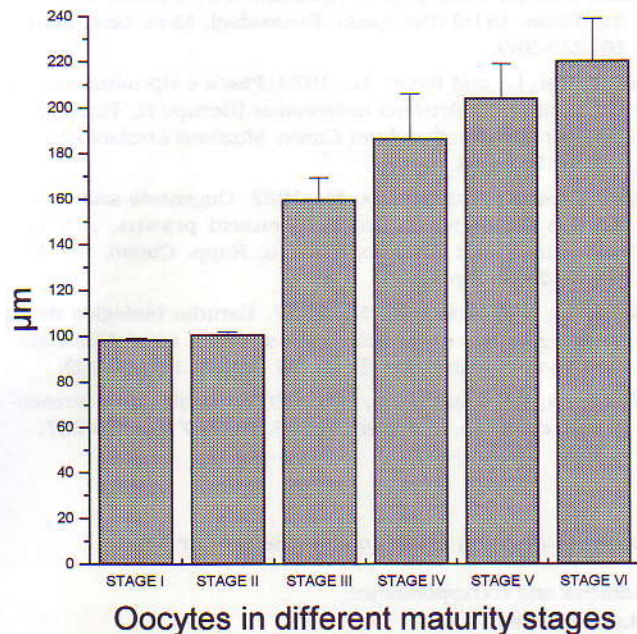

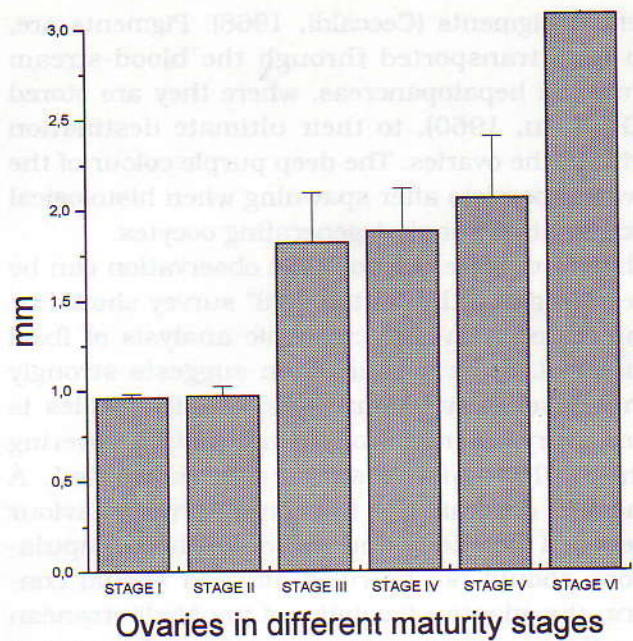Fig. 8. Mean diameter (mm) of ovaries in different maturity stages.

Fig. 7. Mean diameter (μm) of oocytes in different maturity stages.

Stages I and II were similar, differing only in their stromal component. Both stages were represented in autumn and winter by numerous post reproductive and quiescent ovaries. An early (previtellogenic) phase with a progressive increase in the number of previtellogenic oocytes occurred in spring. Some degenerative phenomena were recorded at stage II in which phagocytic cells, including follicle cells, were massively involved. This disruption is comparable to the one that occurs following experimental conditions (Payen, 1975; Payen and Costlow, 1977). These were characterized by formation of follicle cells in clusters, the nuclei of which were found to be enlarged and engaged in cell division.

Vitellogenic oocytes surrounded by follicle cells characterized stages III and IV. A number of degenerating vitellogenic oocytes, together with some cytoplasmic fusions, were also evident in the June samples of stage IV. Reproduction occurred at stages V and VI and was marked by the appearance of star-shaped vitellogenic oocytes filled with cortical granules characteristic of the egg-laying phase. Degenerating oocytes, retaining their typical deep-purple background, characterized most individuals entering stage VI at the end of August. Fading off of pink and orange colours from early oocytes to more advanced stages was assumed to be due to disappearance of proteins associated with caro-

tenoid pigments (Ceccaldi, 1968). Pigments are, in fact, transported through the blood-stream from the hepatopancreas, where they are stored (Goodwin, 1960), to their ultimate destination with in the ovaries. The deep purple colour of the ovaries persists after spawning when histological examination shows degenerating oocytes.

Therefore, mere macroscopic observation can be deceiving and the "in the field" survey should be integrated with a microscopic analysis of fixed material. Such a conclusion suggests strongly that laboratory examination of subsamples is necessary when thorough information covering the biology of the species is requested. A marked dissimilarity in reproductive behaviour between northern and more southern populations should not be ruled out and should confirm the adaptive flexibility of the Mediterranean benthos, reflecting the astonishing diversity of the Mediterranean systems (Margalef, 1985).

After examining of Sardinian populations, Mura and Cau (1989) emphasized a strict correlation between morphological changes in the course of individual growth and maturation of the male gonad to the seasonal flux of solar energy, obviously coinciding with summer. These data are quite consistent with our results.

As a conclusion, our survey has pointed out a marked homogeneity in reproductive behaviour of shrimp populations in the southern Tyrrhenian Sea, data quite in agreement with the homogeneity of other Mediterranean waters at depths over -200 m (Ekman, 1967). Differences in population behaviour from northern subbasins may be referred to as an expression of a biogeographical gradient, or, possibly, as an adaptation to the bathyal system.

Acknowledgements

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