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Original article

Distribution, production, histology and histochemistry in *Acartia tonsa* (Copepoda: Calanoida) as means for life history determination in a temperate estuary (Mondego estuary, Portugal)

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Abstract

Acartia tonsa is a key species in the Mondego estuary and a widespread species in all temperate estuarine zooplankton communities; a fact that bestows high relevance upon the outcome of this study. One of the purposes of this study was to estimate the distribution, production and production/biomass ratio values of *A. tonsa*. Biomass/length relationship was estimated as follows: $Y = 0.15e^{3.04x}$. Length-weight relationships were used to estimate production taking into account cohort growth and mortality. The annual production was calculated to be 43.12 mg Cm^{-3} $year^{-1}$ and the production/biomass ($P/\bar{B}$) ratio was estimated to be 10.56. The other purpose of this study was to use histology, histochemistry and biometry to determine as to whether fecundity is a limiting factor in itself or are zooplankters constrained to respond to an ever changing environment. Analyses of the maturation stage of oocytes in adult ovigerous females performed in two different periods of opposed abundance (February— high abundance, representing maximum fertility—and September—low abundance, representing "sterility") demonstrated the presence of all three considered oocytical development stages: immature, vitellogenic and mature; with emphasis on the latter since it indicates a permanent capability for reproduction despite the registered population abundances. Taking into account the relevance of the species, this fact demonstrates the modulating influence of ecological parameters on general zooplankton reproductive traits.

Keywords: Zooplankton; Estuaries; *Acartia tonsa*; Production; Gonads; Histochemistry; Histology

1. Introduction

Fluctuations in numbers of zooplankton populations derive solely from the outcome of reproductive potential or are they a reflex of the modelling of this factor by means of extrinsic biological factors and physical environmental conditions? Ecological approaches indicate influences of temperature and salinity (Rodriguez et al., 1995) nutrients, growth, mortality and migration rates (Kouwenberg, 1994), behaviour *lato sensu* (Greenwood, 1981) food availability and quality (Heinle et al., 1977; Kouwenberg, 1994) and predation (Davis, 1984; Fulton, 1984). Most studies point to a complex web of interactions between factors, such as intimate correlation between the number of eggs produced/temperature/food. Here, we can discern the outline of an equilibrium that is involved between ecological factors and the intrinsic capability of organisms to reproduce. Which of them can we ultimately consider to be responsible? This study, using *Acartia tonsa* Dana (Calanoida), a key species in the Mondego estuary, tries to answer this question by means of a set of different tools, such as production study, histology, histochemistry and biometry, in order to determine whether fecundity is a limiting factor in itself. Copepoda (Crustacea) comprise the most abundant taxa of the zooplankton (Omori and Ikeda, 1984; Feinberg and Dam, 1998). There, they act as an efficient and direct path for energy transfer to higher trophic levels (Williams et al., 1994) and contribute substantially to the downward flux of organic material (Feinberg and Dam, 1998). *A. tonsa* is a widely distributed and abundant species of temperate estuarine zooplankton communities (Alcaraz, 1983; Conover, 1956; Gaudy, 1972; Kleppel, 1992). The Mondego estuary zooplankton community, at the study location, is dominated by *A. tonsa* species (Azeiteiro

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et al., 1999), a fact that bestows high relevance upon the outcome of this study, since it allows strong extrapolation due to the remarkable success of the species.

2. Materials and methods

2.1. Study site

The Mondego River estuary, located in the Portuguese west coast (40°08′ N, 8°50′ W), has an area of 3.3 km^2 and a volume of 0.0075 km^3. The hydrological basin of the Mondego, with an area of 6670 km^2, provides an average discharge of 8.5×10^9 m^3 s^{-1}. The circulation in the southern arm of the estuary (where the sampling station is located) depends on the tides and, in much smaller amounts, on the freshwater discharge from a tributary—the Pranto River, which is controlled by a sluice located 3 km from the confluence of the Mondego River (Azeiteiro, 1999). The sampling station was located in the southern arm of the estuary (Fig. 1), in the inner area of the estuary.

2.2. Determination of environmental parameters

Samples were taken monthly, from July 1999 to June 2000. All the samples were analysed in situ for salinity, temperature, dissolved oxygen and pH. The samples were also analysed in the laboratory (in triplicate) for their content of nitrate, nitrite, ammonium, phosphorus, bacterioplankton and chlorophyll *a* concentration (Strickland and Parsons, 1972; Lovley and Phillips, 1986).

2.3. Environmental data analysis

Multivariate regression analysis (LEASP) (Bacelar-Nicolau, 1998) was applied to find an explanatory model for the dynamics of the *A. tonsa* population community in terms of the environmental parameters monitored.

2.4. Phytoplankton sampling

Samples were taken monthly from July 1999 to June 2000. Sub-surface samples were collected during high spring tides, with a 25 μm mesh size net (Vieira et al., 2002).

2.5. Zooplankton sampling

Samples were taken monthly from July 1999 to June 2000. Sub-surface samples were collected during high spring tides using 63 and 125 μm mesh size nets.

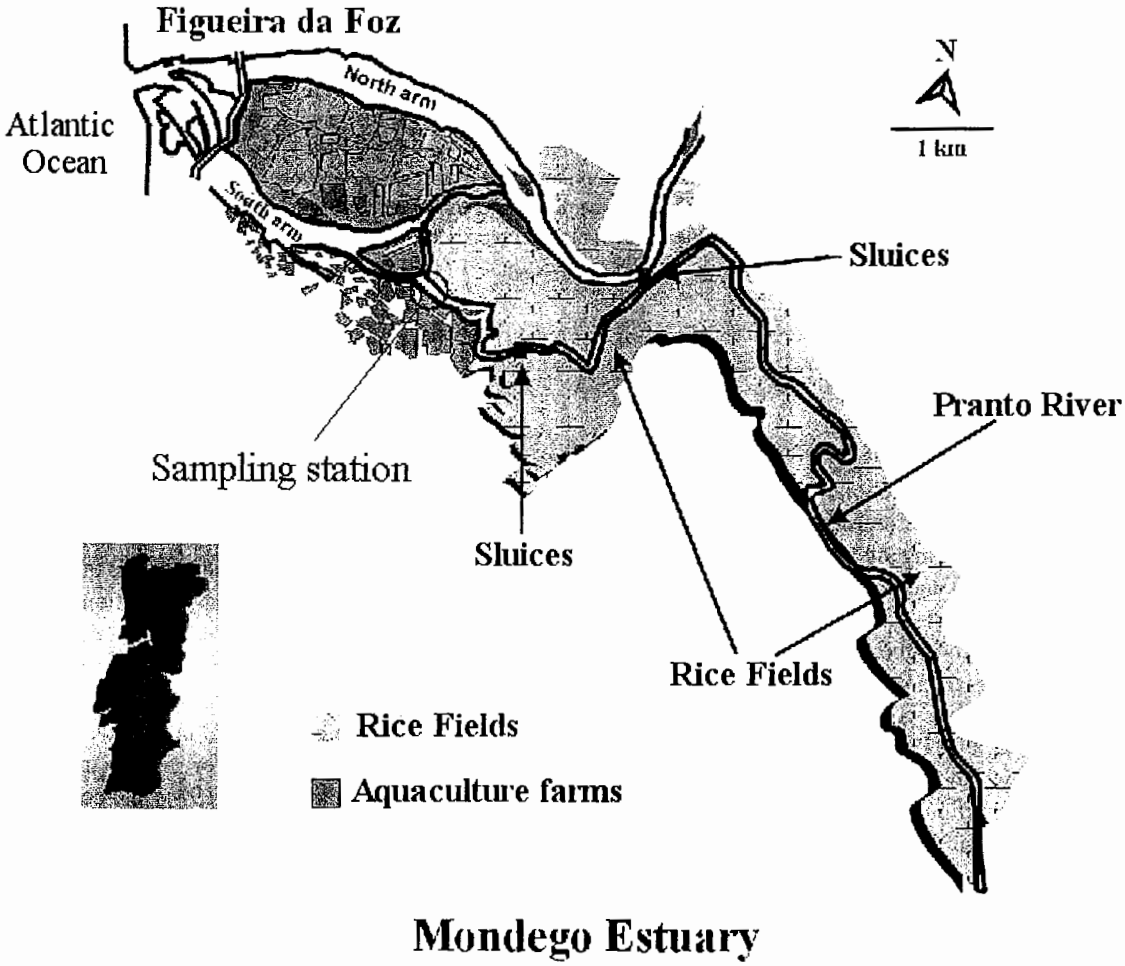

Fig. 1. Map of the Mondego estuary showing the location of the sampling station in the southern arm.

2.6. Laboratory procedures

All the samples needed for production, histology and histochemistry procedures were transported back to the laboratory in good condition, both in terms of temperature (±4 °C) and oxygen (aeration trough piston pumps).

2.7. Production study methodology

A. tonsa individuals were counted and measured by image analysis (Billones, 1999; Tackx, 1995). The conversion to biovolumes and carbon weights was carried out by the expressions and the conversion factors given by (Billones, 1999). Body volumes were calculated from length and width measurements as an ellipsoid using the following formula:

$$V_B = \frac{4}{3}\pi\frac{L}{2}\left(\frac{W}{2}\right)^2$$

where V_B = volume of the body; W = width of the body; L = length of the body; converted into carbon weight using the following conversion factors: 10^6 μm^3 body volume = 1 mg wet weight (Omori and Ikeda, 1984); Dry weight = 20% wet weight (Billones et al., 1999); Carbon weight = 45% dry weight (Heinle et al., 1977; Bernard, 1958).

The relationship between total length and ash free dry weight (AFDW) was also established and used in production estimates. The data-set of 251 individuals measured and weighed throughout the study was used to provide a single regression equation.

We estimated growth (P) production as derived by Allen (1971). The values of P for each cohort for a given time interval are expressed as:

$$P = [(N_t + N_{t+1})/2]\,(\bar{W}_{t+1} - \bar{W}_t) \text{ for } \bar{W}_{t+1} > \bar{W}_t$$

where N is the number of individuals from a cohort at each sample date, $\bar{W}$ is the mean individual biomass for each sample date, t and $t + 1$ are the consecutive sampling dates.

The total value of P for each cohort is expressed as:

$$P = \sum_{t=0}^{t=n}[(N_t + N_{t+1})/2]\,\Delta\bar{W}$$

The total value of P for the population is expressed as:

$$P = \sum_{t=0}^{t=n} P_{cn}$$

where P_{cn} is the growth production of the cohort n. $P/\bar{B}$ ratio was determined. $\bar{B}$ (mean population biomass) is expressed as:

$$\bar{B} = (1/T)\sum_{n=1}^{N}(\bar{B}_n \times t)$$

where T is the period of study, N is the number of cohorts in the period T, $\bar{B}_n$ is the mean biomass of the cohort n and t is the duration of the cohort n.

2.8. Histological technique

Matured females were selected under a binocular microscope and immediately fixed in Bouin's solution (due to the minute dimension of the organisms, selective fixation of the gonads is virtually impossible). The subsequent histological processing of the specimens was carried out using standard procedures (slightly modified due to the particularities of the biological material): paraffin embedding, sectioning (7 μm thickness), mounting and heamatoxilin-eosin staining (Drury and Wallington, 1980). The obtained slides were observed through light microscopy in order to register the arrangement of the oocytes and determine the maturity stage of the various germ cells.

2.9. Biometry

The cephalothorax of 25 females was measured using an ocular micrometer at 0.05 mm precision. All the oocytes present in the ovary of seven females were measured considering their maximum diameter (Cuzin-Roudy and Amsler, 1991). The measurement was performed using an ocular micrometer at 0.05 mm precision. The calculation of the nucleus/cytoplasm ratio (N/C ratio)—the ratio between the area of the nucleus and the area of the cytoplasm (Cuzin-Roudy and Amsler, 1991)—was always performed.

2.10. Histochemistry

In order to determine the chemical nature of the different compounds present in the oocytes, especially carbohydrate reserve material, paraffin sections were processed according to standard histochemical protocols with respect to the periodic acid Schiff (PAS) method (Kiernan, 1990). Techniques aimed at lipid identification could not be performed due to primary fixation with Bouin's solution, which allows removal of the majority of these organic compounds from tissues (by alcohol in the different dehydration and hydration series).

2.11. Statistics

All statistical analyses of collected data were performed using STATISTICA 6.0 software package. The differences between the size of adult females, mature stage of oocytes, vitellogenic stage of oocytes, immature stage of oocytes and oocyte size distributed by maturation state were investigated through a one-way analysis of variance (ANOVA). All the measurements taken in each analysed sample were considered as replicates. Prior to the analysis, data were subjected to an angular transformation which homogenised the variances in all the cases.

3. Results

3.1. Seasonal variation in environmental variables

The environmental parameters over the 11-month study (July 1999–June 2000) are shown in Table 1. Average temperature was 18.0 °C, varying between 25.0 °C in August

Table 1
Environmental results (temperature, salinity, pH, oxygen dissolved— saturation%, NO_2^-, NO_3^-, NH_3, PO_4^{3-} and chlorophyll *a* values) from monthly annual sampling cycle in the southern arm of the Mondego estuary, between July 1999 and June 2000

	July	August	September	October	November	December	January	February	March	April	May	June
Temperature (°C)	23.6	25.0	20.7	19.6	16.6	13.2	11.0	14.1	–	14.0	18.3	22.3
Salinity (‰)	18.1	26.0	31.0	22.0	19.5	29.3	23.2	29.4	–	9.5	12.0	10.1
pH	7.9	7.6	8.3	8.3	7.5	8.4	7.9	7.6	–	7.6	7.6	8.3
DO_2 (%)	72.0	85.0	48.0	60.0	48.0	79.0	91.0	72.4	–	74.0	75.2	59.0
NO_2 (mg l^{-1})	0.007	0.016	0.026	0.036	0.048	0.060	0.006	0.055	–	0.042	0.028	0.071
NO_3 (mg l^{-1})	0.031	0.033	0.035	0.076	0.106	0.135	0.193	0.163	–	0.274	0.384	0.177
NH_3 (mg l^{-1})	0.113	0.173	0.233	0.181	0.172	0.163	0.034	0.188	–	0.189	0.191	0.370
PO_4 (mg l^{-1})	0.007	0.008	0.008	0.007	0.007	0.007	0.009	0.007	–	0.008	0.008	0.005
Chlorophyll *a*	1.445	2.275	2.270	1.620	1.390	1.160	0.810	2.040	–	1.300	2.730	2.180

1999 and 11.0 °C in January 2000. The salinity varied throughout the year with a minimum of 9.5‰, in April 2000, and a maximum of 31.0‰ in September 1999. pH suffered a slight fluctuation during the studied period, varying between a maximum value of 8.4 in December 1999 and a minimum value of 7.5 in November 1999, with an average value of 7.9. Dissolved oxygen concentration presented a minimum value of 48.0% in September and November 1999, a maximum value of 91.0% in January 2000 with an annual average of 69.4%. NO_2^- presented a variation between 0.071 mg l^{-1} in June 2000 and 0.006 mg l^{-1} in January 2000. This parameter presented an average value of 0.036 mg l^{-1}. NO_3^- presented a variation between 0.384 mg l^{-1} in May 2000 and 0.031 mg l^{-1} in July 1999, with an average value of 0.146 mg l^{-1}. The values for NH_3 varied between 0.370 mg l^{-1} in June 2000 and 0.034 mg l^{-1} in January 2000, with an average value of 0.182 mg l^{-1}. PO_4^{3-} presented an average value of 0.007 mg l^{-1} and varied from a maximum of 0.009 mg l^{-1} in January 2000 and a minimum of 0.005 in June 2000. Chlorophyll *a* concentration presented a maximum of 2.730 mg l^{-1} in May 2000, a minimum of 0.810 mg l^{-1} in January 2000, and an annual average of 1.747 mg l^{-1}.

3.2. Distribution of phytoplankton

In the phytoplankton, the most abundant *taxa* were Bacillariophyceae, cyanophyta, dinophyta, euglenophyta and chlorophyta (Fig. 2). Bacillariophyceae dominated from September until December and February and May. Cyanophyta dominated in August and June. Chlorophyta exhibited significant percentages in January and March.

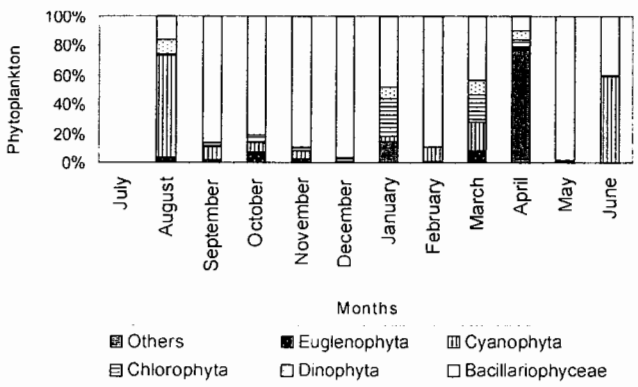

Fig. 2. Percentage of different phytoplankton groups contributing to the total phytoplankton percentages.

3.3. 63 μm taxocenosis—copepoda community

The characterisation of the copepoda community (nauplii, copepodits and adults) and the relative distribution of the *A. tonsa* species in the community can be seen in Fig. 3A-C. *A. tonsa* registered a peak in October (29 070 ind. m^{-3}) and significant densities in August (3793 ind. m^{-3}), November (2121 ind. m^{-3}), December (2724 ind. m^{-3}) and April (1966 ind. m^{-3}) (Fig. 3C).

3.4. 125 μm taxocenosis—copepoda community

In the 125 μm taxocenosis (Fig. 3D-F), *A. tonsa* exhibited peaks in November (6520 ind. m^{-3}) and February (3500 ind. m^{-3}), and significant densities in October (1650 ind. m^{-3}), December (2960 ind. m^{-3}) and April (2520 ind. m^{-3}) (Fig. 3F).

3.5. Multiple regression analysis

Multiple regression analysis (r = 0.964; r^2 = 0.929) (Table 2) indicated that the abundance of *A. tonsa* increases with an increase in dissolved oxygen and temperature.

3.6. Length-weight relationships

The following biomass/length relationship was estimated for the specimens of *A. tonsa* (Fig. 4): $Y = 0.15e^{3.04x}$, with an R^2 of 0.62. The population structure throughout the year is shown in Fig. 5. Size-frequency polymodal distributions were analysed for *A. tonsa* recognisable cohorts (Fig. 6).

3.7. Production

Length-weight relationships were used to estimate production taking into account cohort growth and mortality. The annual production was calculated to be 43.12 mg C $m^{-3}year^{-1}$ and the production/biomass ($P/\bar{B}$) ratio was estimated to be 10.56.

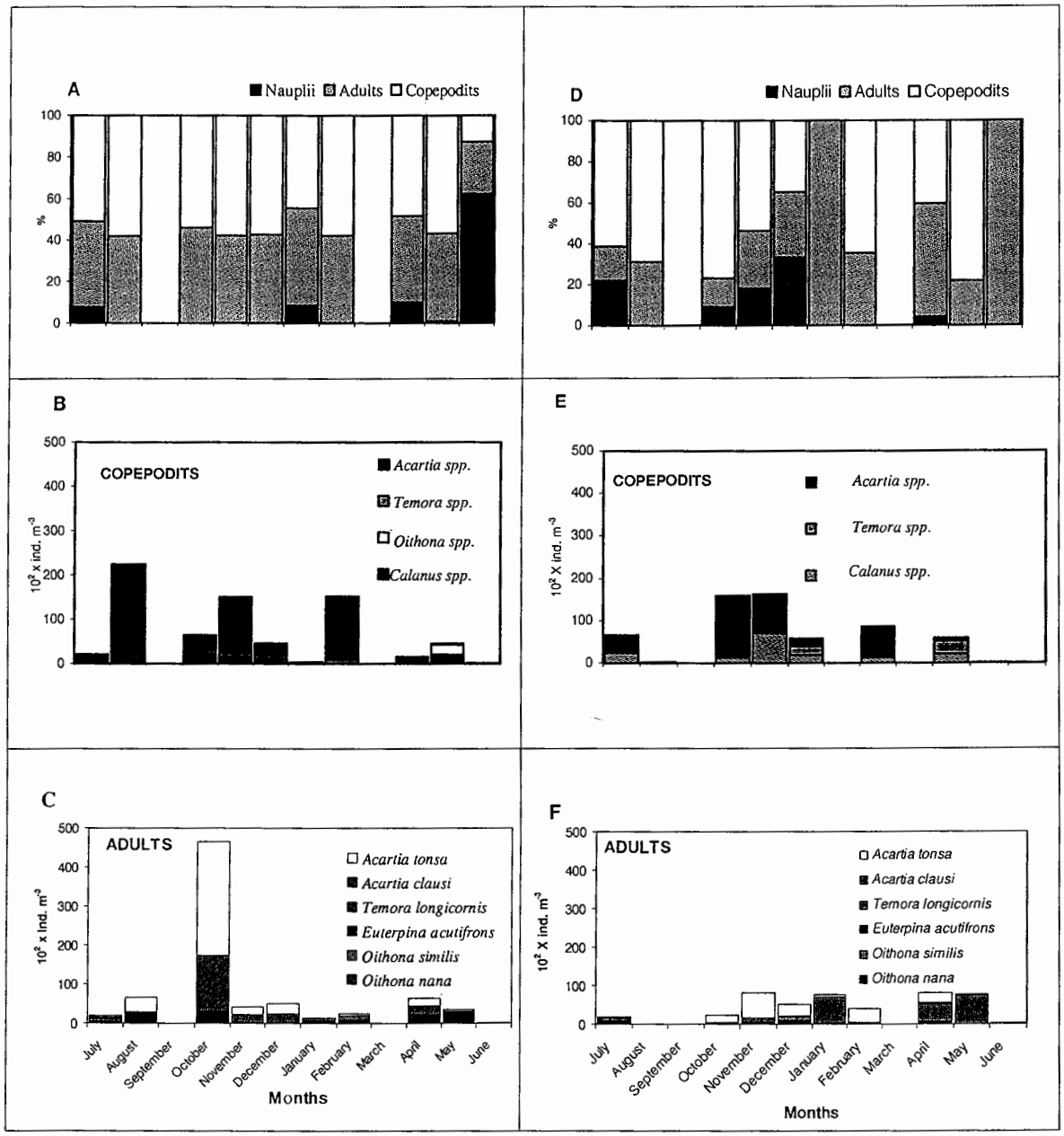

Fig. 3. Percentages and seasonal variation of copepoda community in 63 μm (A, B and C) and 125 μm (D, E and F) taxocenosis. (A and D) Percentage of the three stages of copepoda development groups; (B and E) seasonal variation of copepodits; (C and F) seasonal variation of the six most abundant adult copepoda species.

Table 2
Variables in the equation of multiple regression for the variation of the abundance of *A. tonsa*, over the sampling period; $r = 0.964$; $r^2 = 0.929$

Variables	Regression coefficient	Standard deviation of the regression coefficient
Chlorophyll *a*	–0.290	0.215
Dissolved oxygen	0.148	0.129
pH	–1.05	0.092
Salinity	–0.122	0.130
Temperature	0.271	0.165

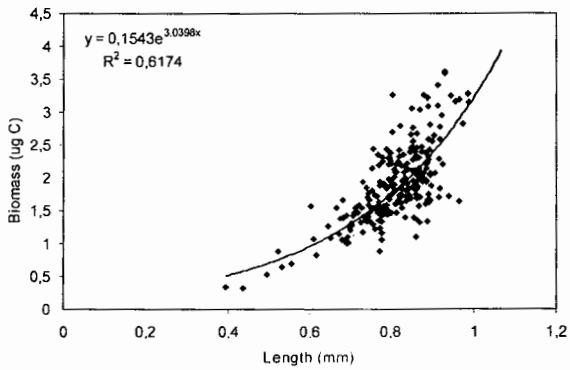

Fig. 4. Regression model for biomass-length relationships of *A. tonsa* in the Mondego estuary. The correlation coefficient (R^2) is indicated.

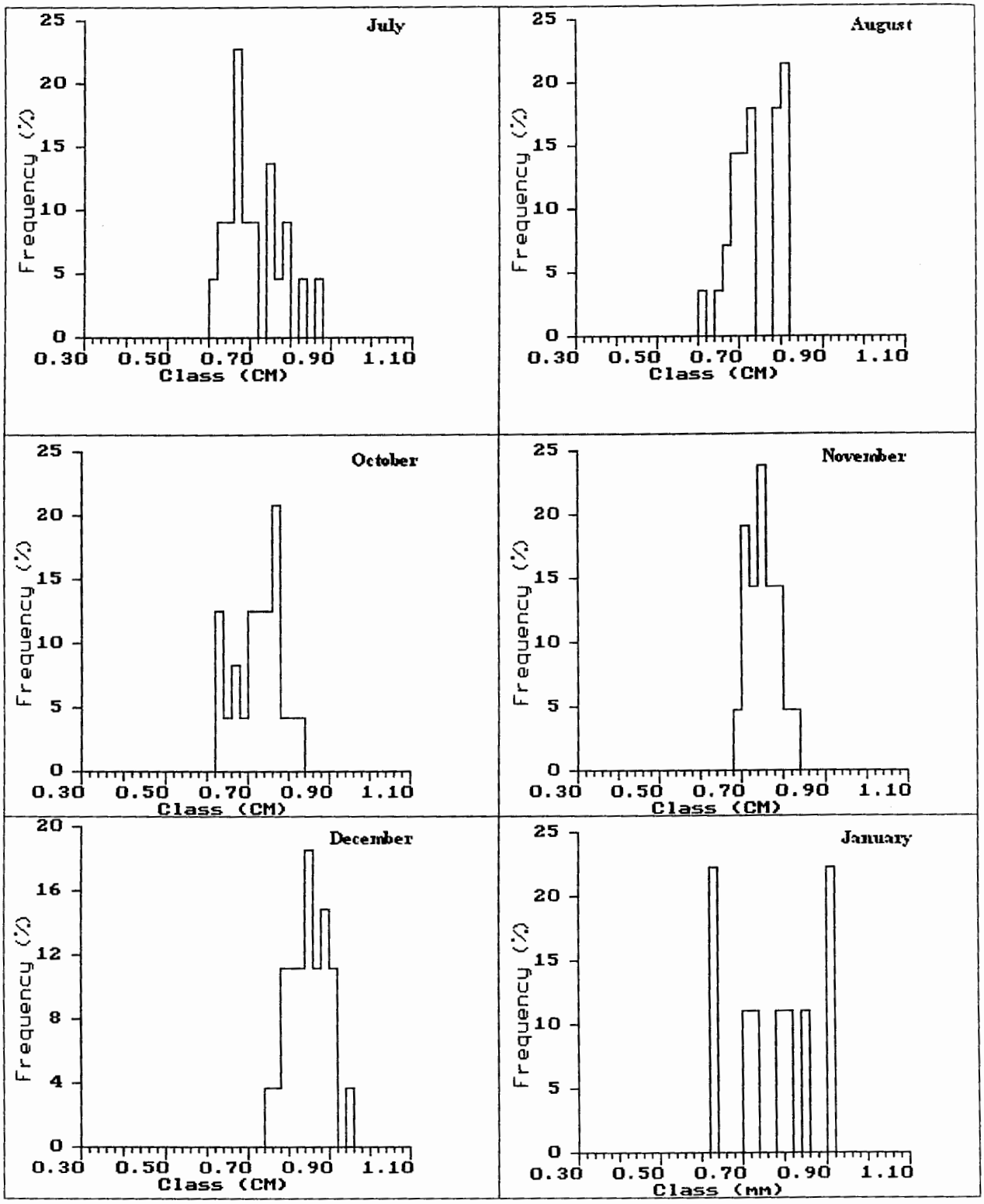

Fig. 5. Size-frequency polymodal distribution of *A. tonsa*, in the southern arm of Mondego estuary. The sampling dates are indicated.

3.8. Biometrics

3.8.1. Female size

The size of the cephalothoracic portion of 25 mature ovigerous females in each selected sample was measured using an ocular micrometer at 0.05 mm precision. The organisms from September demonstrated smaller minimum, maximum and average sizes (5.17, 6.69 and 5.94 mm, respectively) when compared with those from February (6.08, 7.14 and 6.55 mm, respectively) (Table 3).

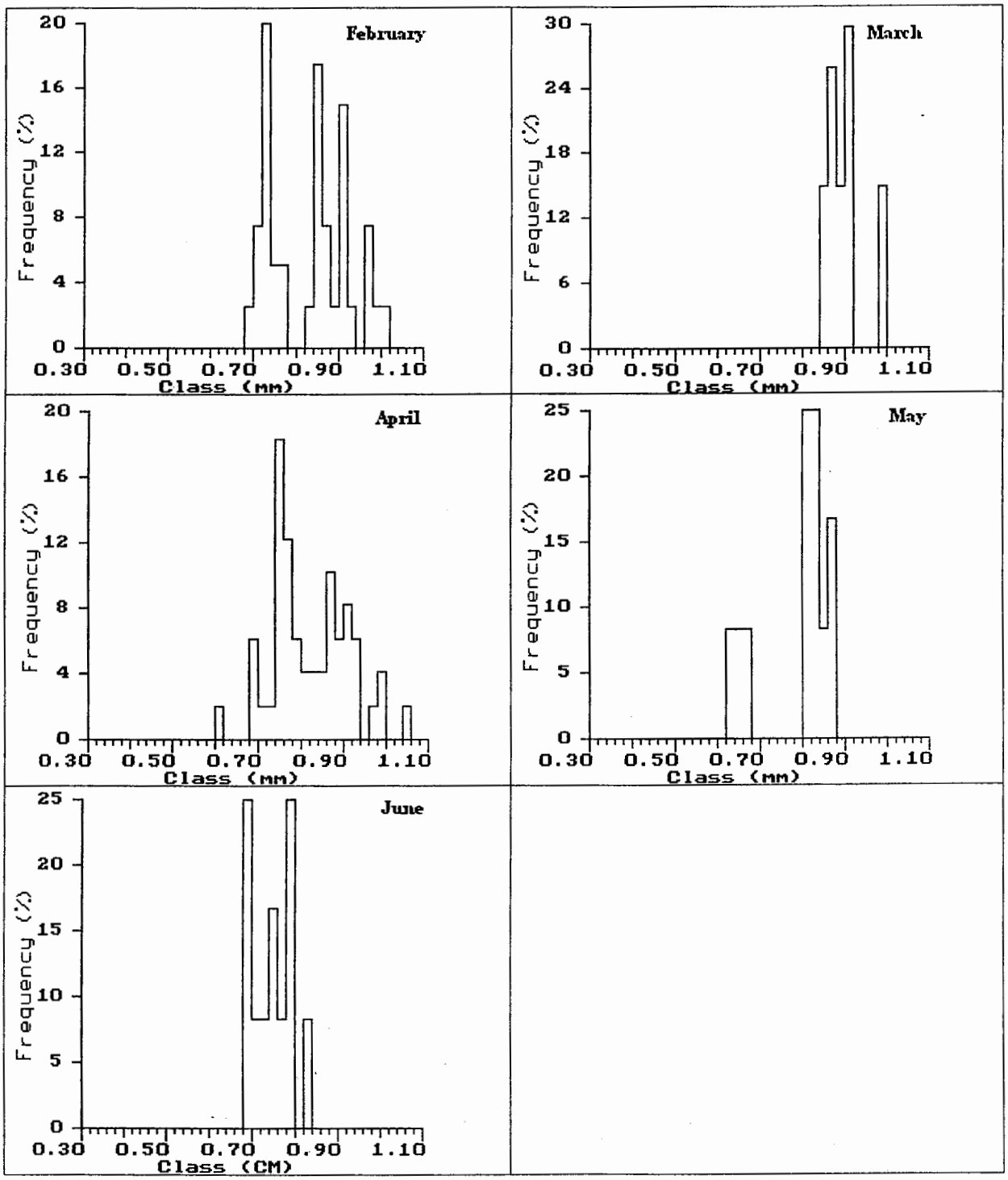

Fig. 5 (suite)

3.8.2. Cell size

The size of the oocytes varied from 5 μm (immature stage in February) to 130 μm (mature stage in September). The obtained average for each development stage in the study periods for immature cells was 20.62 and 12.47 μm (September and February, respectively), for vitellogenic cells, it was 67.76 and 50.76 μm, and for mature cells, it was 112.02 and 92.81 μm (Table 4). A one-way ANOVA showed highly significant values ($P < 0.001$) for the distribution of oocytical size by each considered maturation state, for both months of the sampling (Table 5). Similar results ($P < 0.001$) were obtained for the analysis of the three stages of maturation (immature, vitellogenic and mature) and its variation between samples (Table 5).

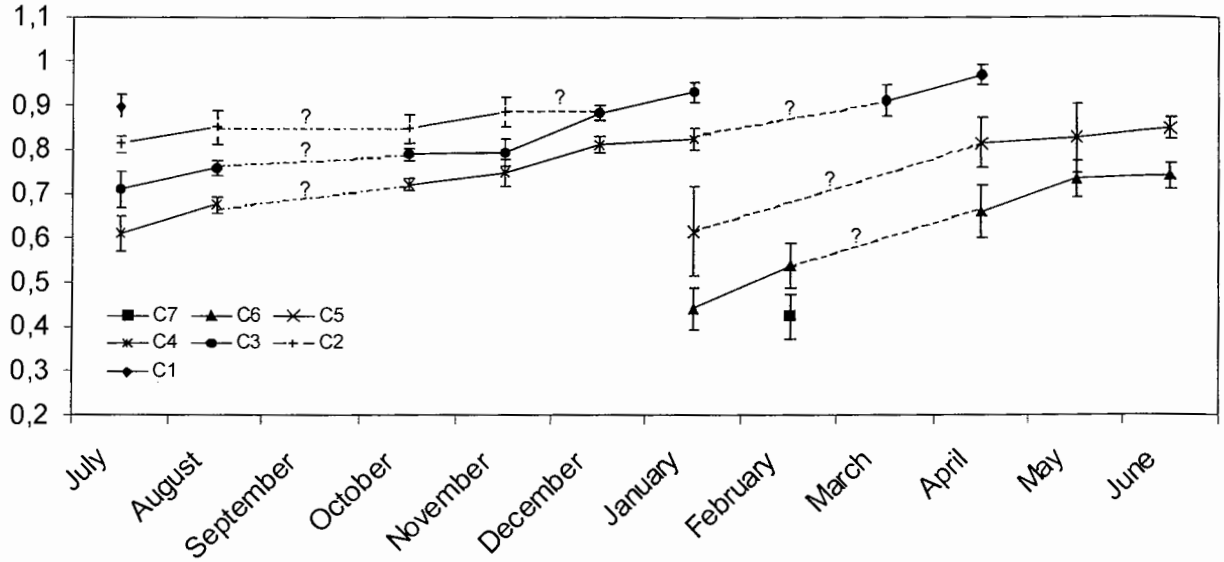

Fig. 6. Estimated growth of *A. tonsa* cohorts, average total length ± standard deviation during the study period.

3.8.3. Staging of the oocytes

A total of 45 gonadic masses from females of *A. tonsa* containing oocytes in different developmental stages were analysed. Microscopic observations of histological sections, both stained with haematoxylin-eosin and treated by the PAS technique (this analysis was complemented by descriptions for other crustaceans) (Johnston and Nothcote, 1989; Ladurantaye et al., 1980; Ianora and Butino, 1990), allowed the identification and subsequent separation of the cells into three generic developmental stages: stage I—immature: the cells are in the Oogonial state or are young oocytes. These cells generally lie in the ventral zone of the ovary, are spherical in shape and measure 17 μm (average of averages from the 2 months; Table 4). The cytoplasm is extremely narrow, the nucleus is centrally located, with an N/C ratio of 73.46% (average of averages; Table 6) and basophilic (haematoxylin binding). Negative response to the PAS technique (Figs. 7–10); stage II—vitellogenic: the cells are maturing oocytes that initiated and continued the process of accumulating yolk. The range from an early stage—previtellogenesis—characterised by the accumulation of glycoproteins and a later stage—vitellogenesis— characterised by the accumulation of lipidic yolk. They generally, lie in the intermediate/dorsal zone of the ovary, are cubic or prismatic, measuring, on average, 59.26 μm (average of averages from the 2 months; Table 4). The nucleus is centrally located, with an N/C ratio of 46.98% (average of averages; Table 6) and the cytoplasm is mainly basophilic. There is a great variability in the response to the PAS technique, mirroring the existence, according to each case, from sparse droplets of glycoproteins to the occupation of high percentages of the cytoplasm (Figs. 7–11); stage III—mature: the cells are fully mature oocytes that have completed yolk synthesis/accumulation (Ianora and Butino, 1990) (or close). They are generally located in the dorsal-lateral wall of the ovary, present a spherical appearance, measuring 102.41 μm (average of averages from the 2 months—Table 4). The nucleus is located in the central position in the cell, with a C/N ratio of 20.70% (average of averages from 2 months—Table 6) surrounded by a thin layer of cytoplasm (running all around the inner side of the cellular membrane, very basophilic and with a strong positive reaction to the PAS technique), since this is almost totally filled by yolk, it causes the cell to swell very prominently. The yolk inclusions register discrete positive reaction to the PAS technique due to the accumulation of abundant lipid droplets (which constitute the majority of the reserve materials) (Figs.

Table 3
Size of the cephalothoracic portion of adult ovigerous females of *A. tonsa* in each sample (values in mm)

	September	February
Minimum	5.17	6.08
Maximum	6.69	7.14
Average	5.94	6.55
Standard deviation	0.35	0.31

Table 4
Minimum, maximum and average size of immature (Im), vittelogenic (Vit) and mature (Mat) oocytes in *A. tonsa* and respective standard deviation (values in μm)

	September			February		
	Im	Vit	Mat	Im	Vit	Mat
Minimum	10	50	97.5	5	35	75
Average	20.62	67.76	112.02	12.47	50.76	92.81
Maximum	32.5	87.5	130	25	72.5	107.5
Standard deviation	5.51	9.98	10.14	4.65	8.35	10.38

Table 5
One-way ANOVA tests applied to the size of adult ovigerous females (I), oocyte size in the months of September (II) and February (III) and to three stages of oocytical maturation (immature (IV); vittelogenic(V); mature (VI)) of *A. tonsa*. df = degrees of freedom; MS = mean square; *Fs* = test value; *P* = probability value

Source of variation	df	MS	*Fs*	*P*
I-sample	I	0.0004	38.93	$P < 0.001$
II-sample	2	0.008	764.28	$P < 0.001$
III-sample	2	0.011	752.33	$P < 0.001$
IV-sample	1	0.015	114.17	$P < 0.001$
V-sample	I	0.004	78.92	$P < 0.001$
VI-sample	1	0.002	36.86	$P < 0.001$

(I) One-way ANOVA of the size of adult ovigerous females of *A. tonsa* during the period of the study. The null hypothesis is that the size of the organisms does not differ between samples.

(II) One-way ANOVA of the oocyte size of *A. tonsa* distributed by maturation state, for the month of September. The null hypothesis is that the oocyte's size does not differ between maturity status.

(III) One-way ANOVA of the oocyte size of *A. tonsa* distributed by maturation state, for the month of March. The null hypothesis is that the oocyte's size does not differ between maturity status.

(IV) One-way ANOVA of the immature stage of oocyte of *A. tonsa* during the period of the study. The null hypothesis is that all the immature cells do not register any variation between samples.

(V) One-way ANOVA of the vittelogenic stage of oocyte of *A. tonsa* during the period of the study. The null hypothesis is that all the vittelogenic cells do not register any variation between samples.

(VI) One-way ANOVA of the mature stage of oocyte of *A. tonsa* during the period of the study. The null hypothesis is that all the mature cells do not register any variation between samples.

7–10 and 12). It is important to note that the main objective presiding the elaboration of this staging scale was not descriptive but, instead, aimed at creating a tool in which to anchor comparisons between oocytes from different sampling periods and maturity stages.

4. Discussion

The distribution patterns of the environmental parameters observed are mostly in agreement with previous studies (Azeiteiro, 1999; Vieira et al., 2002). During the study period, *A. tonsa* was positively correlated with temperature according to the species preferences (Alcaraz, 1983).

The estuarine and marine phytoplanktonic communities are frequently dominated by dinoflagellate and diatom species (Lehman, 2000; Philippart et al., 2000; Vieira et al., 2002). Diatoms and dinoflagellates were the most abundant phytoplankton species found in this study. Chlorophyta, euglenophyta and cyanobacteria were also found. This flora conditions the primary consumers, not only for the form of the cells and the associations that they establish with each other, but also because of the elements and molecules transmitted by the different species (Vieira et al., 2002) (the cyanotoxins); although it has not conditioned *A. tonsa* distribution or reproduction, as reported by other authors (Uye, 1996).

Estuarine copepods typically reach very high densities and tend to dominate the neritic and estuarine fauna, such as the one present in the Mondego estuary. Despite copepod domination, only a limited number of species were commonly present in the zooplankton assemblages (Azeiteiro et al., 1999) making them important pelagic producers. The production values estimated in this study are in accordance with the values reported by other authors (Table 7). The $P/\bar{B}$ value obtained revealed an expected modal turnover rate (Valiela, 1995; Banse and Mosher, 1980). This $P/\bar{B}$ value indicates that although the individual biomass of small sized species may be small, the higher specific production makes them important producers. These results indicate that *A. tonsa* play a significant role in transferring energy to higher trophic levels. Although production by nauplii is not included in the present study, this does not typically exceed 25% of copepod total production (Mullin, 1988; Liang and Uye, 1996a; Liang et al., 1996).

Most copepods reproduce throughout the year. The cohorts represent the maximum of possible generations and the duration of the cohorts represents the longevity of the generations (Binet, 1977). Copepoda life cycle in temperate regions have an average of 25–45 d. In temperate regions, the adult longevity rarely exceeds 2 months (Gaudy, 1972). This study found seven annual generations. Similar results were reported for other systems (Table 8). The most notable difference is the longer longevity found in the Mondego estuary. Annual generations depend on latitude and trophic availability. Both temperature and food availability are known to play a significant role in the copepod production activity (Klein

Table 6
N/C ratio in percentage for the three considered maturity stages in the 2 months of study

	Immature		Vitellogenic		Mature	
	September	February	September	February	September	February
Average	73.91	73.02	41.64	52.31	22.56	19.00
Total average	73.46		46.98		20.78	

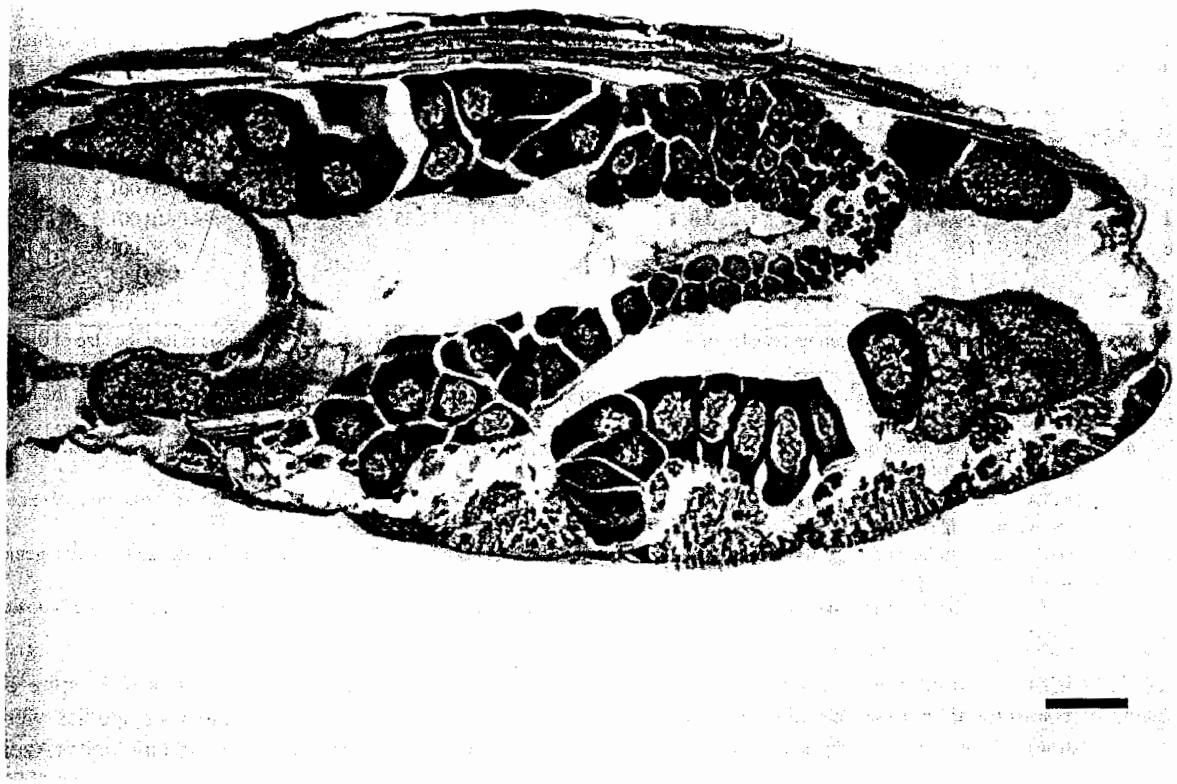

Fig. 7. February 2000. *A. tonsa*. Longitudinal section. Note the coexistence of mature (m), vitellogenic (v) and immature oocytes (i). HE colouring, 235×, and tool bar: 50 mm.

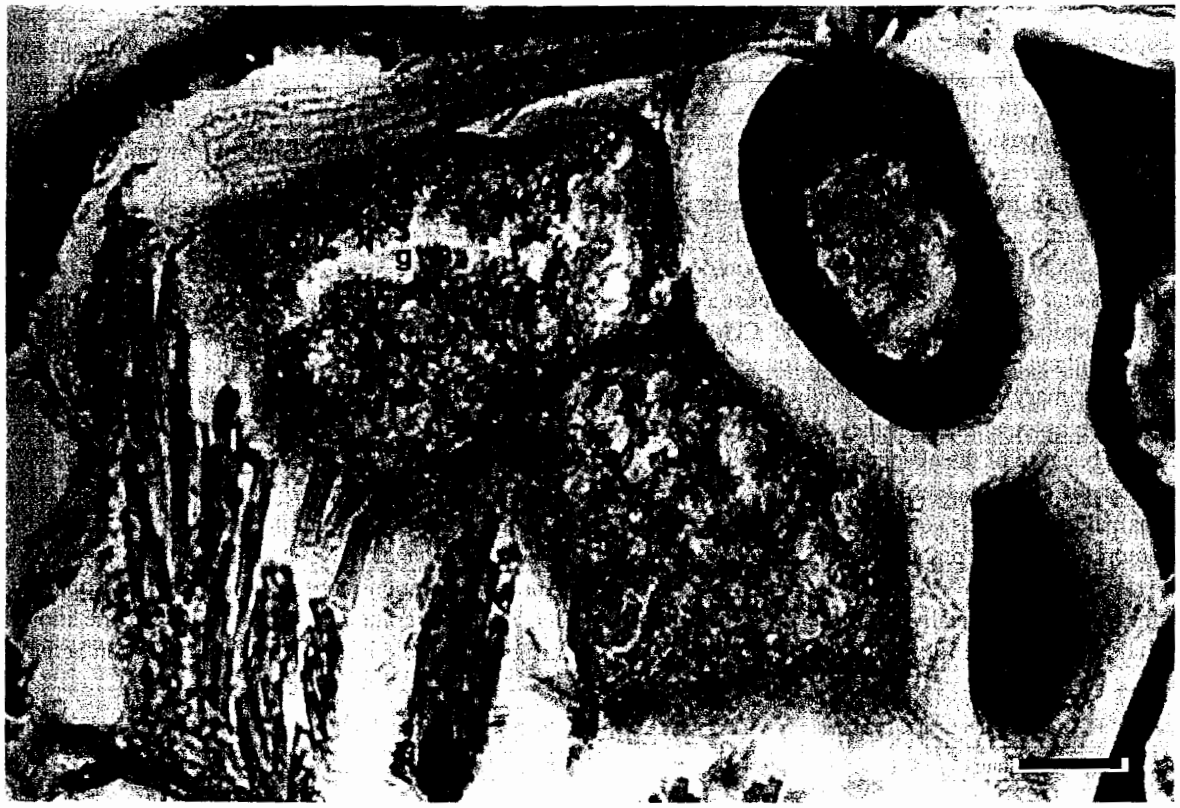

Fig. 8. February 2000. *A. tonsa*. Longitudinal section. Mature oocyte (granulous aspect). Note the existence of gaps (g) in the cytoplasm due to lipid removal. HE colouring, mag: 923×, tool bar: 50 mm.

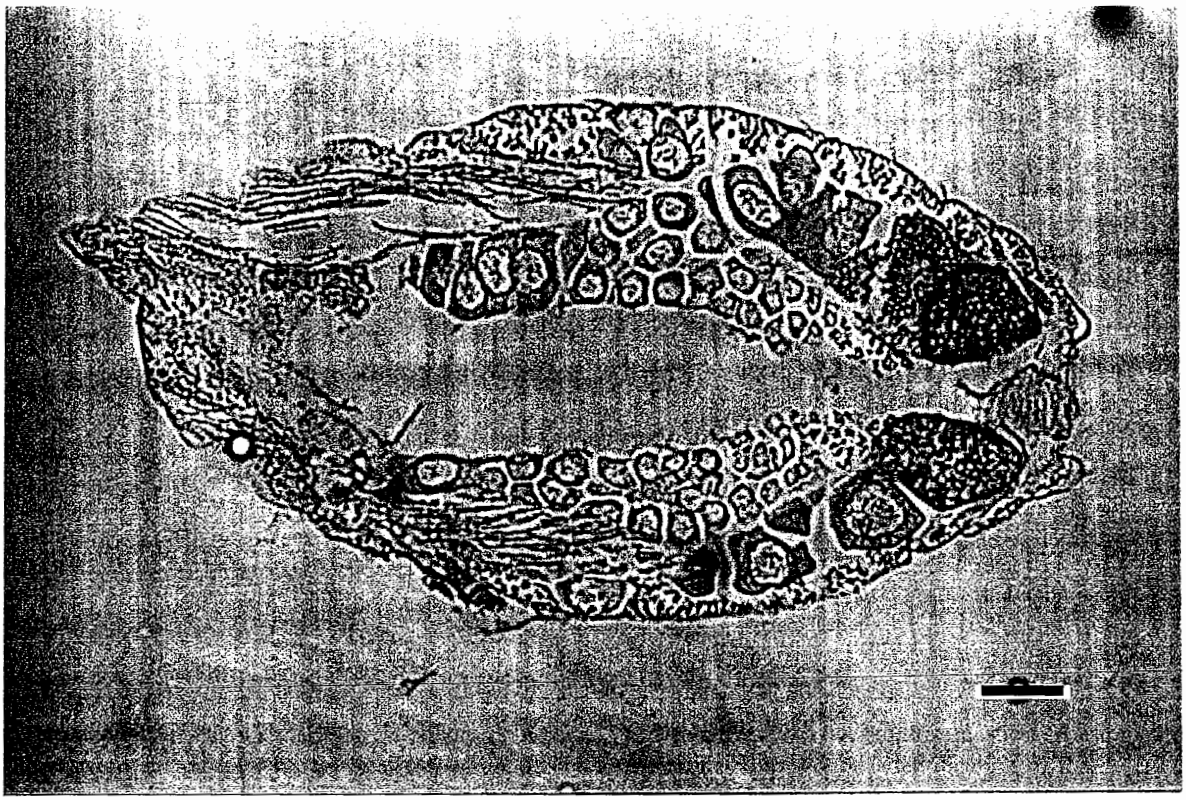

Fig. 9. February 2000. *A. tonsa*. Longitudinal section. Note the coexistence of mature (m), vitellogenic (v) and immature oocytes (i). PAS technique, mag: 235×, tool bar: 50 mm.

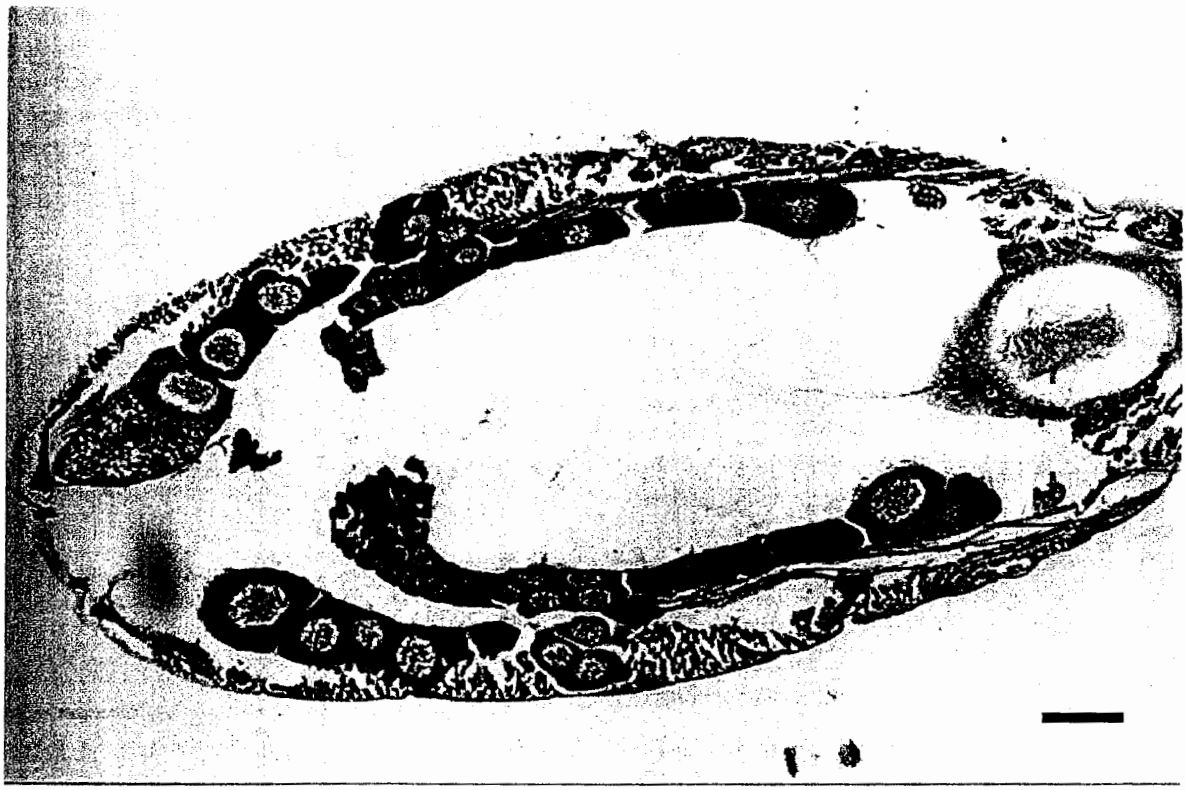

Fig. 10. September 1999. *A. tonsa*. Longitudinal section. Note the coexistence of mature (m), vitellogenic (v) and immature oocytes (i). HE colouring, 235×, tool bar: 50 mm.

Fig. 11. September 1999. *A. tonsa*. Longitudinal section. Vitellogenic oocytes. Note the deposition of reserve materials (d). HE colouring, n: nuclei. mag: 923×, tool bar: 50 mm.

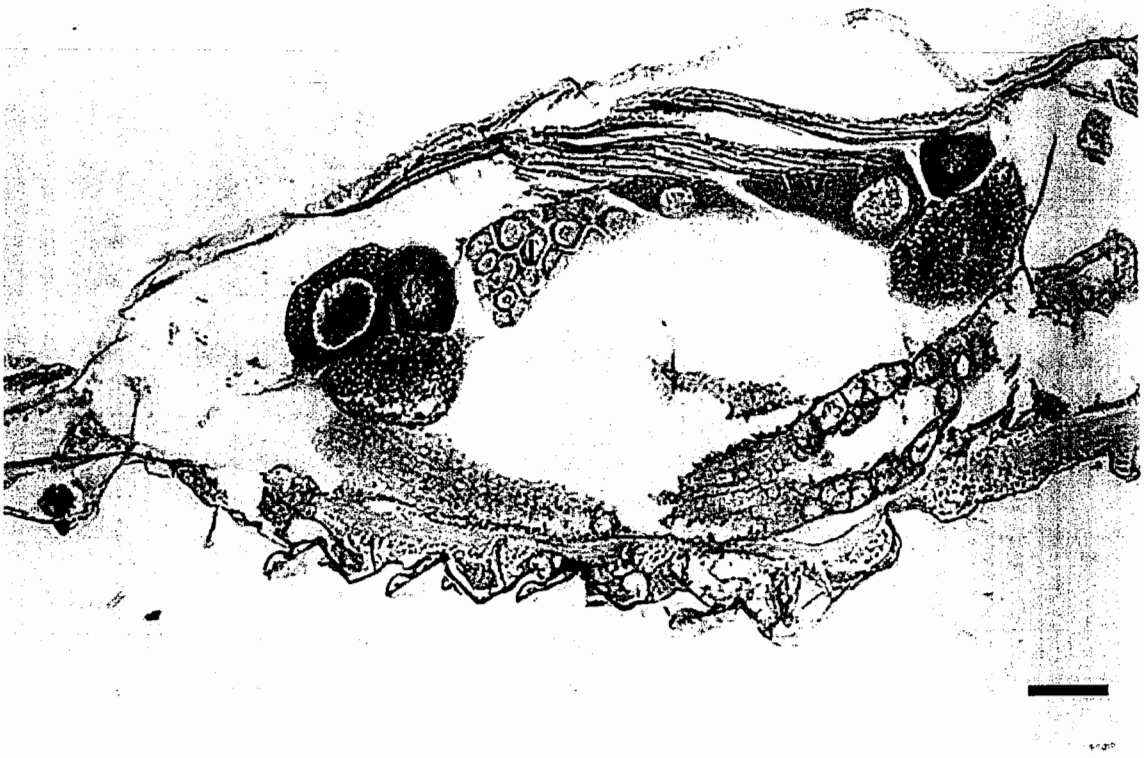

Fig. 12. September 1999. *A. tonsa*. Longitudinal section. Note the coexistence of mature (m), vitellogenic (v) and immature oocytes (i). PAS technique, 235×, tool bar: 50 mm.

Table 7
Copepoda production and production/biomass ratio value data

Species	Reference	Study site	Methods	*P* (annual)	*P/B*
Acartia spp.	Hirst et al. (1999)	Coastal station, Solent UK	Weight	17.62 mg C m^{-3} $year^{-1}$	
Acartia bifilosa	Irigoien and Castel (1995)	The Gironde (SW France)	Size		28
Acartia omorii	Liang and Uye (1996a)	Inland sea of Japan	Egg production	5.62 g C m^{-3} $year^{-1}$	
Acartia spp.	Escaravage and Soetaert (1995)	Westerschelde estuary, Nether	Growth rate methods	5 g C m^{-3} $year^{-1}$	
A. tonsa	Kleppel (1992)	Southern California	Egg production rates	Varies along the year	
Copepoda	Hirst et al. (1999)	Coastal station, Solent UK	Weight	32.2 mg C m^{-3} $year^{-3}$	
Centropages abdominalis	Liang et al. (1996)	Inland sea of Japan	Egg production	2.66 g C m^{-3} $year^{-1}$	
Centropages hamatus	Hirst et al. (1999)	Coastal station, Solent UK	Weight	8.16 mg C m^{-3} $year^{-1}$	
Copepoda	Banse and Mosher (1980)	Landry 1976, 1978		60.3 kcal m^{-2} $year^{-1}$	58/32
Copepoda	Liang and Uye (1996b)	Fukuyama Harbor	Growth rate estimation	18.4 g C m^{-2} $year^{-1}$	
Eurytemora spp.	Escaravage and Soetaert (1995)	Westerschelde estuary, Nether	Growth rate methods	6 g C m^{-2} $year^{-1}$	
Oithona davisae	Uye and Sano (1998)	Fukuyama Harbor	Weight	650 mg m^{-3} $year^{-1}$	
Paracalanus parvus and *Pseudocalanus elongatus*	Hirst et al. (1999)	Coastal station, Solent UK	Weight	1.67 mg C m^{-3} $year^{-2}$	
Paracalanus spp.	Liang and Uye (1996b)	Inland sea of Japan	Egg production	5.36 g C m^{-3} $year^{-1}$	
Copepoda	Liang and Uye (1996a)	Inland sea of Japan	Egg production	51 mg C m^{-3} $year^{-1}$	
Pseudodiaptomus marinus	Liang and Uye (1997)	Inland sea of Japan	Egg production	0.38 g C m^{-2} $year^{-2}$	
Temora longicornis	Hirst et al. (1999)	Coastal station, Solent UK	Weight	4.77 mg C m^{-3} $year^{-1}$	

Breteler et al., 1988; Kleppel, 1992). In the Mondego estuary, temperatures were never below 10 °C (the multiple regression analysis showed that *A. tonsa* abundances were significant and positively correlated with temperature) and chlorophyll *a* concentration was always high. These two factors should explain the *A. tonsa* biological cycles features in the Mondego estuary. As mentioned by Gaudy (1972), the biological cycle and number of generations of *A. tonsa* vary with latitude as a function of temperature and food availability.

One of the aims of this paper was to try and discern the relevance of ecological parameters in the outcome of reproductive potential from zooplanktonic organisms or, in other words, what is the balance between biotic and abiotic parameters and the intrinsic reproductive potential of the elements of the zooplankton? Histological observation of the positioning and distribution of cells and different cellular structures, qualitative evaluation of chemical contents, through histochemistry, pointing existence of differences between development stages in terms of the accumulation of reserve substances (nominately glycoproteins and, by indirect extrapolation, lipids), measurement of cellular size and determination of the C/N ratio, were used in order to obtain a clear and convincing answer to this question. These methods and techniques allowed the identification of three very distinct stages of maturation of oocytes and hence the elaboration of a functional scale, numerically based and statistically validated, allowing comparisons between samples. When compared, it was verified that all the three maturation stages were present in both the sampling epochs. Special attention must be paid to stage III oocytes (mature). Their presence in the gonadic masses of the females means that these females are fully capable of reproduction and only fecundation has to occur, since these cells possess all that is necessary for the juvenile to be viable. The simultaneous existence of fully functional reproductive cells in registered high density epochs (February—which would correspond to a hypothetical greater fecundity) and in minimum density periods (September—which would correspond to "sterility"—given their certified disappearance in well studied estuaries) of the reproductive cycle of the organisms, indicates that the main modulating influence (and limiting factor!) over these cycles comes out of ecological biotic and abiotic parameters which notoriously superimpose over the continuous reproductive capabilities of zooplanktonic organisms, creating the well described fluctuating patterns.

As a corollary one could conclude that seasonal variability in zooplankton densities reflects advantageous or disadvantageous assemblages of external factors acting over a latent reproductive potential.

Table 8
Number of generation values

Number of generations	Study site	Reference
2	Occidental Atlantic	Deevey (1971)
3, 4	Adriatic Sea	Vucetic (1957)
4	Roscoff	Razouls (1965)
4	Long Island Sound	Conover (1956)
5	Plymouth	Digby (1950)
6, 7	Sebastopol	Greze and Baldina (1964)
8	Black Sea	Porumb (1968)
9	Karadag	Tchaianova (1950)
Continuous	Mediterranean	Bernard (1958)

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