

The oogenic cycle of *Nereis diversicolor* (O. F. Müller, 1776) (Annelida: Polychaeta) in shallow water environments in southwestern Portugal

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ABSTRACT

This study presents data on the oogenesis and sex ratio of *Nereis diversicolor* (O. F. Müller, 1776) from April 1993 to May 1994 on the southwestern coast of Portugal: Odeceixe (ODX), Aljezur (ALZ) and Carrapateira (CAR). The results obtained showed that *N. diversicolor* presented, during the entire period of study, adults with gametes as well as a constant presence of juveniles. The average (\pm SD) oocyte diameter were, respectively, $136 \pm 39 \mu\text{m}$ ODX, $140 \pm 37 \mu\text{m}$ for ALZ, and $130 \pm 49 \mu\text{m}$ for CAR. The sex ratio was always favourable to females, being 1: 2,9 for ODX, 1: 3,1 for ALZ and 1: 4,1 for CAR. In the warmer waters of CAR, accelerated maturation, reduced size, and a strong female-biased sex ratio were observed.

Keywords: *Nereis diversicolor*; reproduction, oogenic cycle, recruitment, sex ratio.

RESUMEN

El ciclo oogénico de *Nereis diversicolor* (O. F. Müller, 1776) (Annelida: Polychaeta) en ambientes someros del suroeste de Portugal

En este estudio se presentan datos sobre oogénesis y proporción de sexos de *Nereis diversicolor* (O. F. Müller, 1776) durante los meses de abril 1993 a mayo 1994 en la costa suroeste de Portugal: Odeceixe (ODX), Aljezur (ALZ) y Carrapateira (CAR). Los resultados obtenidos demuestran que *N. diversicolor* presenta adultos con gametos a lo largo del periodo de estudio, y también una constante presencia de juveniles. Las medias (\pm el error estándar) del diámetro de los oocitos fueron, respectivamente: $136 \pm 39 \mu\text{m}$ para ODX; $140 \pm 37 \mu\text{m}$ para ALZ; y $130 \pm 49 \mu\text{m}$ para CAR. La proporción de sexos siempre fue favorable para las hembras, siendo de 1: 2,9 para ODX, 1: 3,1 para ALZ y 1: 4,1 para CAR. En las aguas más cálidas de CAR se observó maduración más rápida, tamaño reducido de los individuos y proporción de sexos fuertemente desviada hacia las hembras.

Palabras clave: *Nereis diversicolor*, reproducción, oogénesis, reclutamiento, proporción de sexos.

INTRODUCTION

The study of aspects of reproductive biology, and more specifically of gametogenesis in populations of *Nereis diversicolor* (O. F. Müller, 1776), started with

the detailed work of Dales (1950) in the Thames estuary. More recent papers, e.g. Chambers and Milne (1975), Heip and Herman (1979), Mettam (1979), Olive and Garwood (1981), Möller (1985), Ozoh and Jones (1990), Francés Zubillaga and Saiz Salinas

(1997) and Scaps (2002) focused on aspects of biology, ecology and potential use of these Polychaeta.

The life cycle of *N. diversicolor* is particularly interesting, considering that during gametogenesis the energy invested in gamete biomass can be as high as 70 % of total energy, and the oocytes develop in the coelomic fluid and not in ovaries (Olive, 1983; Grémare and Olive, 1986). As a result of this considerable investment in gametogenesis, a reduction of somatic body mass occurs, and growth ceases (Mettam, 1979; Olive, 1983; Fischer, 1984). This is not surprising, since this species reproduces only once, dying a few days after releasing the gametes (Dales, 1950; Olive and Garwood, 1981; Olive, Garwood and Bentley, 1981; Mettam, Santhanam and Havard, 1982; Fischer, 1984; Humphreys, 1985).

In different species, the oogenic patterns present considerable variation, so the choice of the maturity index should be made carefully. Determination of the reproductive period should include both a detailed observation of the availability of mature males and the presence or absence of females with mature oocytes. Samples with the purpose of determining the presence of newly-settled larvae in the habitat, are also strongly recommended.

The method most frequently used in oocyte growth analysis is the average diameter of a sample of oocytes contained in the coelomic cavity. Another indicator is size distribution of oocytes in a sample of individuals from a certain population. As spawning approaches, the variation in oocyte sizes diminishes (Olive and Garwood, 1981; Olive, 1983; Fischer, 1984).

In Portugal, several studies have been made on the biology and ecology of *N. diversicolor*: in the Ria de Aveiro (Abrantes, Pinto and Moreira, 1999, Mondego estuary (Gonçalves and Ribeiro, 1997), St. André lagoon (Cruz, 1989), Mira estuary (Lopes da Silva, 1993), and the southwest coast (Fidalgo e Costa and Cancela da Fonseca, 1998; Fidalgo e Costa, Sardá and Cancela da Fonseca 1998). Specifically concerning oocyte development, Gonçalves and Ribeiro (1987) and Abrantes, Pinto and Moreira (1999) have determined the oogenic cycle for this species, but only for the northern part of the country.

In the present work, we studied the oogenic cycle and sex ratio of *N. diversicolor* in three small estuaries on the southwest coast of Portugal. These shallow sites were selected to provide a wide range

of regional variability and to determine whether local factors within these sites are more important to set the conditions for the oogenic cycle of this species than regional factors. The development of oocytes in each individual was correlated with the measure of L_3 (length of prostomium, plus peristomium and first chaetiger segment) and the reproductive period was also determined through the analysis of larval recruitment peaks.

MATERIALS AND METHODS

Study area

The studied sites are within the Parque Natural do Sudoeste Alentejano e Costa Vicentina (Sudoeste Alentejano and Costa Vicentina Nature Park). The sampling stations were established at the terminal part of the Odeceixe (ODX), Aljezur (ALZ) and Carrapateira (CAR) estuaries which form temporary lagoons (figure 1). These water basins extend through about 250, 200 and 110 km² respectively, and partially drain the western part of southwest Portugal. These brackish environments, despite

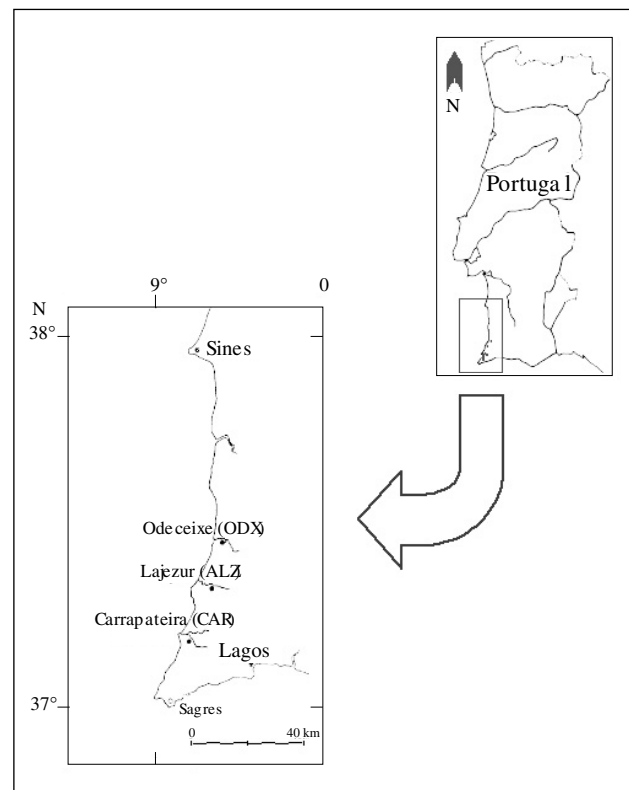


Figure 1. Sampling locations on the southwest coast of Portugal

their high geomorphological diversity, are, from an ecological point of view, lagoon-estuarine systems (Day, Hall, Kemp and Yáñez-Arancibia, 1987).

Environmental conditions

Salinity (at the surface and at the bottom layer) and temperature (at the bottom layer) were measured in the vicinity of sampling sites using sensors (YSI Model 33, Engineered Systems and Designs-Model 600, Philips W9424). Monthly data on precipitation and atmospheric temperature were obtained from the National Meteorological Institute and the General Administration of Natural Resources.

Sampling techniques

All samples were collected between April 1993 and May 1994 at three sites: at low tide on the sublittoral fringe of ODX and ALZ, and in the upper sublittoral area of CAR. This last site is a small coastal lagoon formed by the partial closing of the river mouth by a sand barrier which remains closed most of the year, and opening only during winter storm events. At each site five samples of the first 30 cm of sediment were collected with a hand-corer sampling tube (area = 0.02 m², totalling an area of 0.1 m²). The samples were sieved through a 0.5 mm square mesh (Olive and Garwood, 1981; Humphreys, 1985). Moreover, for larvae and juveniles, three samples were taken (total area = 19.8 cm²) from the sediment to a depth of 10 cm, preserved and sieved through a 0.2 mm square mesh.

Size determination

All specimens were measured, based on the value of L₃ (length of prostomium, plus peristomium and first chaetiger) according to Gillet (1993) using a binocular microscope equipped with a camera lucida and a digitising table (Houston Instruments HIPad) connected to a computer. This measure showed the best correlation with biomass ($r^2 = 0.914$; $N = 259$; < 0.001), being a valuable conversion factor (see Fidalgo e Costa, Sardá and Cancela da Fonseca, 1998) which avoids problems caused by autotomy, which may occur very frequently in polychaetes.

Sex ratio and oocyte measurements

The analysis of the coelomic content required the use of iris scissors, mainly for the smaller individuals. Animals without sexual products were not considered in the analysis. Males were identified through the visualization of sperm plates or free sperm. For each female a maximum of 36 oocytes (for specimens in late stages of maturation) and a minimum of 10 oocytes (for small specimens), were measured. This procedure follows the one used by other authors in similar studies (Mettam, 1979; Olive and Garwood, 1981; Mettam, Santhanam and Havard, 1982; Möller, 1985; Hefferman and Keegan, 1988; Francés Zubillaga and Saiz Salinas, 1997, Abrantes, Pinto and Moreira, 1999). Some individuals were not intact, presenting a reduced number of gametes in the coelomic cavity (less than 10). In these cases, only the sex was determined.

RESULTS

Environmental conditions

The water temperature during the sampling period at all sites followed closely the atmospheric temperature values (figure 2), oscillating between 12-21°C (ODX), 12.7-24.7 °C (ALZ) and 13.3-26.3 °C (CAR). The averages at the bottom were, respectively, 15.6, 17.5, and 19.5 °C.

Precipitation was the same at all sites, the highest values occurring during October and November. Changes in salinity at several parts per thousand were recorded in these habitats (figure 2), and could be explained by tidal influences, distance from the sea, and the different water entries. Stratification was observed, specially in ODX and ALZ, throughout the sampling period, clearly reflecting the fact that in CAR, the junction with the sea remains closed for most of the year. The obtained annual average values at the bottom were 31.4 (ODX), 29 (ALZ) and 15 (CAR).

Oocyte cycle

Nereis diversicolor showed continuous reproduction, presenting females with oocytes and frequent affluence of new recruits in the three systems studied, during almost the entire sampling period. Its

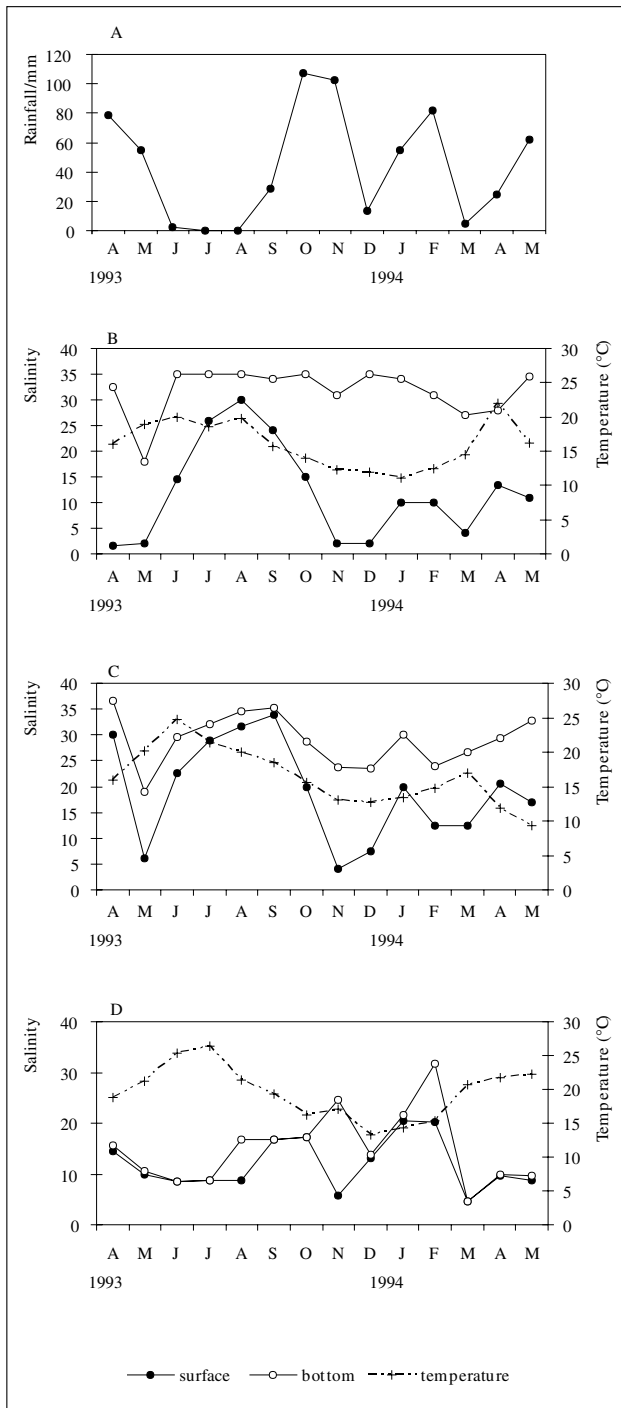


Figure 2. (A): rainfall fluctuation on the southwestern coast of Portugal; (B): monthly values of salinity (surface and bottom) and temperature in ODX; (C): monthly values of salinity (surface and bottom) and temperature in ALZ; (D): monthly values of salinity (surface and bottom) and temperature at CAR

population has a large proportion of juveniles, and the breeders is dominated by small group of old individuals (figure 3). From a total of 950 presumed

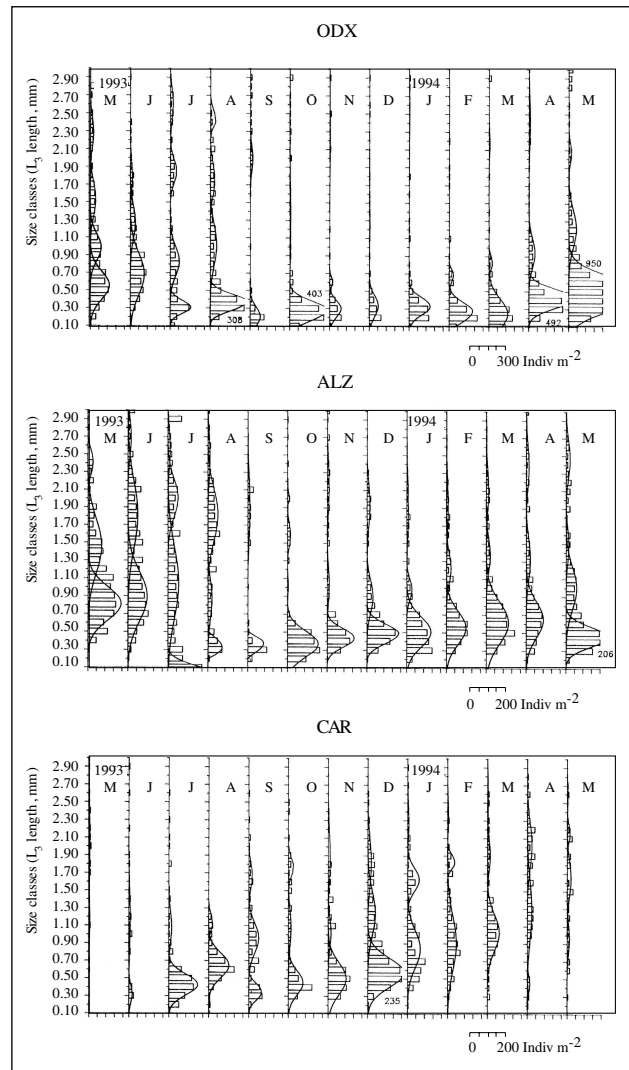


Figure 3. Size frequency histograms (indv m⁻²) of *N. diversicolor* population throughout the sampling period at Odeceixe (ODX), Aljezur (ALZ), and Carrapateira (CAR). Curved lines are normalized expected frequencies. (Adapted from Fidalgo e Costa, Sardá and Cancela da Fonseca, 1998)

adult specimens captured, measured, and opened, only 270 presented some sexual products in the coelomic fluid. Of these, 78 were obtained from ODX, 115 from ALZ, and 77 from CAR.

At ODX, the beginning of oogenesis occurs in May; although the earliest stages have been missed, a gradual but significant increase in oocyte numbers and diameters were observed between July and September (figures 4 and 5). In August, no oocyte-carrying females were observed since spawning occurred at the end of this month and early in September, which is reflected in the sharp larval recruitment observed in September (figure 6). In the

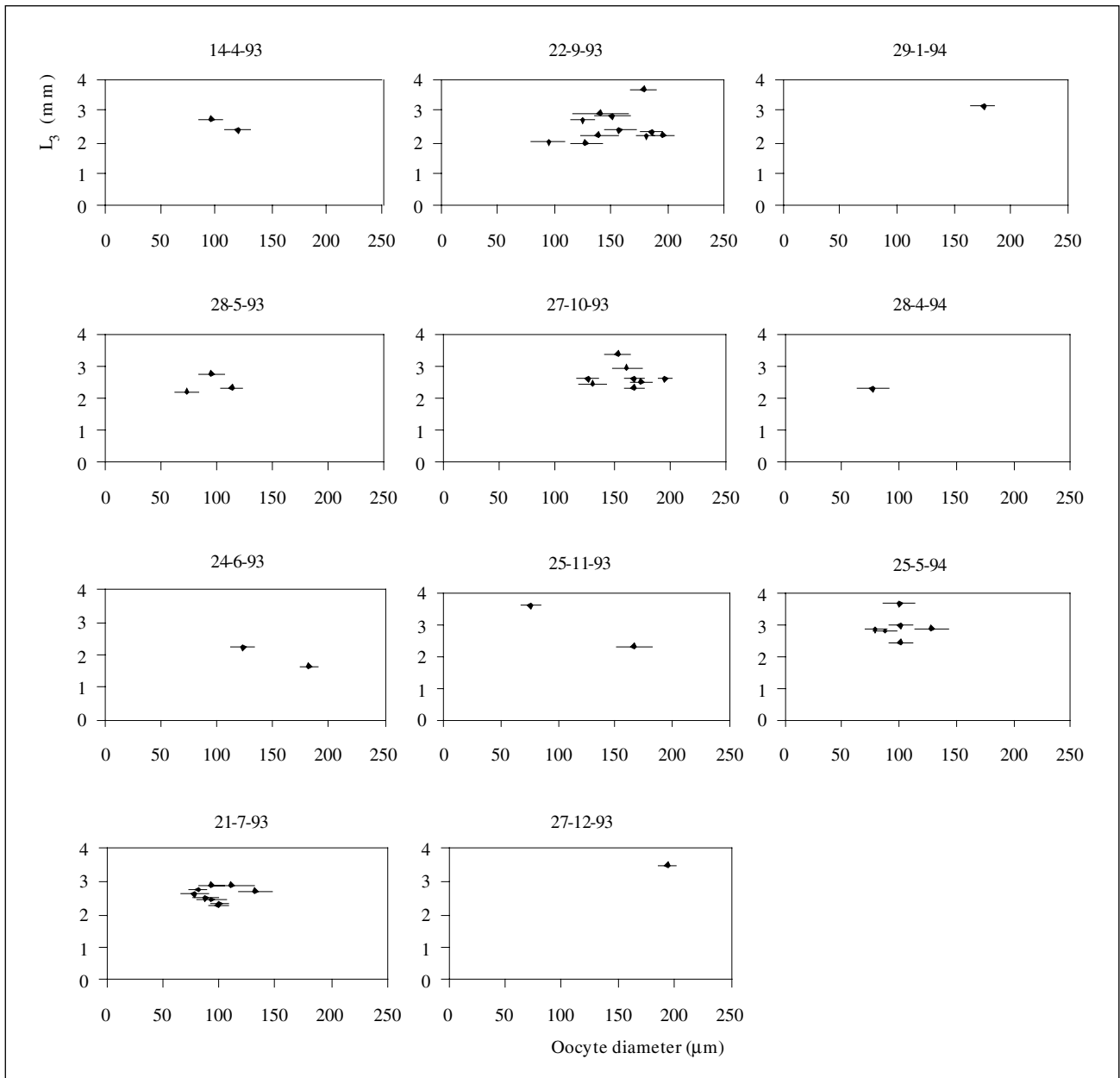


Figure 4. Relation between L_3 length (mm) and average diameter (μm), with standard deviation of oocytes in each individual of *N. diversicolor* sampled monthly at Odeceixe (ODX)

following months the presence of oocyte carrying females decreased. The number of larvae and juveniles also decreased due to interspecific and intraspecific predation as confirmed through gut content analysis (personal observations) and the minimum values of biomass obtained (figure 7). In spring, there was another increase in biomass values, and in May 1994 maturing females and recruits were observed once again, indicating a second peak of reproduction, although weaker than in September (figure 6).

In ALZ, the presence of *N. diversicolor* with the development coelomic gametes began in May and peaked in July, but mature individuals were observed in September (figures 5 and 8). In August, there was a clear decrease in the mature population, as some adults began to spawn, and over the next two months biomass dropped to its lowest values (figure 7). This correlated with a sharp larval increase in September (figure 6) after the reproductive period, and the consequent death of the adults after releasing gametes.

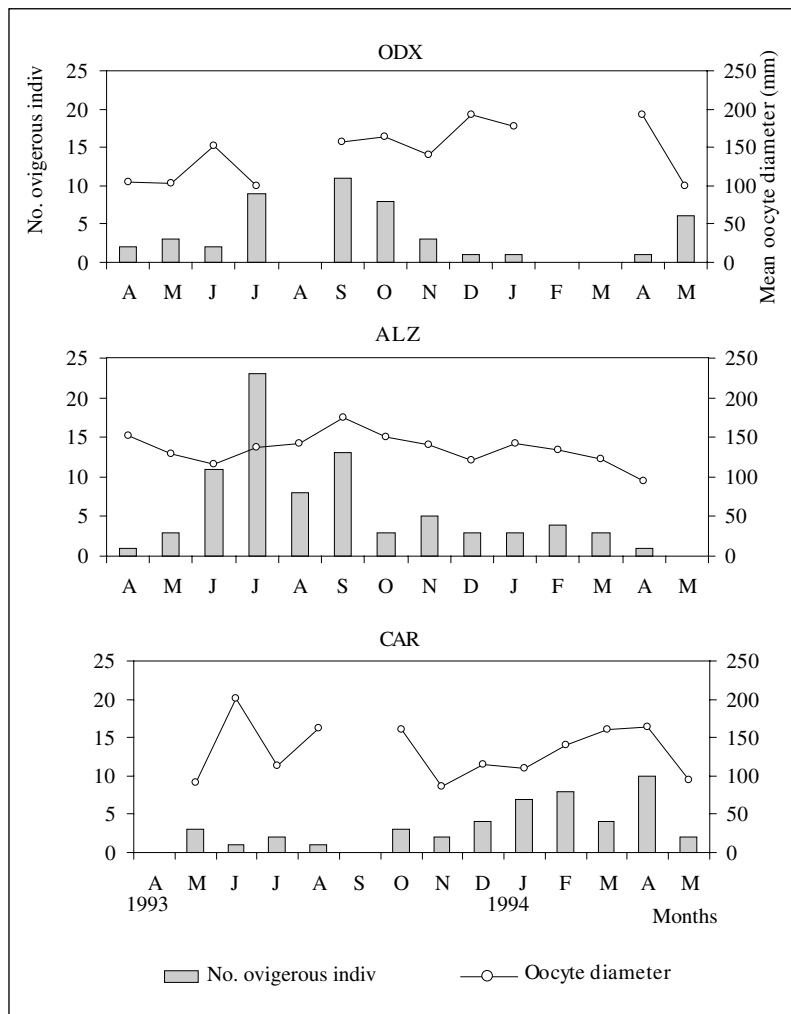


Figure 5. Number of ovigerous individuals *N. diversicolor* and mean oocyte diameters throughout the sampling period at Odeceixe (ODX), Aljezur (ALZ), and Carrapateira (CAR)

A progressive but distinct increase in oocyte diameter from June ($106 \pm 25 \mu\text{m}$) to September ($171 \pm 31.5 \mu\text{m}$) was recorded (figure 8).

In CAR, the distribution of oocyte-carrying females throughout the sampling period was very different from the other two sites (figures 5 and 9). At CAR, females with coelomic gametes were found mainly between December and April (figure 9). However, the larval recruitment peak was also observed in September, with a less intense peak than at ODX and ALZ (figure 6). There was also a gradual increase in average oocyte diameter, from $107 \pm 51 \mu\text{m}$ in January to $160 \pm 35 \mu\text{m}$ in April (figure 9).

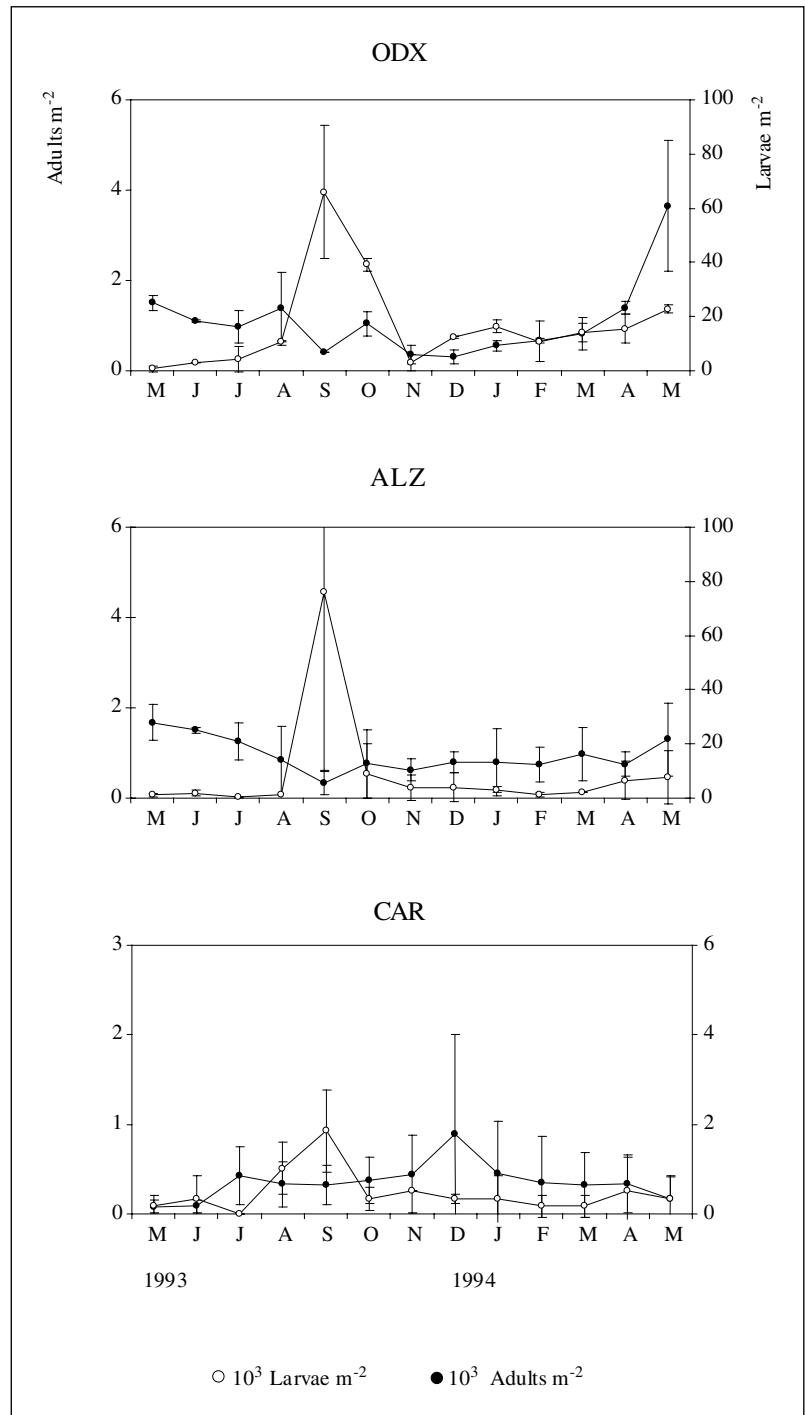
Size frequency analysis

The species' investment in reproduction was clearly shown by the size (L_3) of the observed fe-

males in ODX, which was basically the same during July and September, whilst oocyte growth showed a marked increase during this period (figure 4). This pattern was also found in ALZ and CAR (figures 8 and 9). During these months the reproductive females had an average L_3 length of $2.55 \pm 0.38 \text{ mm}$ (ODX), $2.43 \pm 0.45 \text{ mm}$ (ALZ) and $2.08 \pm 0.38 \text{ mm}$ (CAR). At CAR, the worms could be sexually mature as early as 12 months of age; almost all worms had reached their reproductive period by 18 months. On the other hand, in ODX and ALZ, this species reached sexual maturity at 18 months, and most of the individuals reproduced at the end of the second year of life (figure 3).

Due to continuous recruitment and to the constant presence of oocyte-bearing females in different stages of development, some variation of oocyte diameter was visible in all sites. In early oogenesis, oocytes presented a uniform size distribution, while

Figure 6. Abundance of adults and larvae of *N. diversicolor* at the three studied sites over the sampling period



during the maturation process, the coelomic oocytes become heterogeneous in size. At the end of development, the size range was again reduced just before spawning. These results are similar to those found by other authors (e.g. Olive and Garwood, 1981; Mettam, Santhanam and Havard, 1982; Sola, 1994; Abrantes, Pinto and Moreira, 1999).

Sex ratio

The sex ratio at the three study sites showed a higher proportion of females (table I). The number of individuals for which the sex could be clearly determined was relatively low, due to the high presence of immature juveniles. Because of this

Tabla I. Number of males and females of *N. diversicolor* present at the three sampling sites throughout the year

	Months	ODX		ALZ		CAR	
		♂	♀	♂	♀	♂	♀
1993	A	0	2	1	2	1	0
	M	0	3	1	3	1	5
	J	0	2	3	9	0	1
	J	0	9	2	23	1	2
	A	0	1	0	2	0	1
	S	10	11	6	9	2	0
	O	1	10	2	4	1	6
	N	4	4	0	10	2	5
	D	0	2	0	3	3	5
1994	J	2	4	3	6	1	9
	F	0	0	2	6	0	8
	M	1	1	2	8	0	5
	A	0	3	1	1	3	10
	M	2	6	5	1	0	5
Total		20	58	28	87	15	62
Sex ratio		1:2.9		1:3.1		1:4.1	

characteristic, the sex ratio variation throughout the year was not analysed in detail.

DISCUSSION

The breeding season of *N. diversicolor* has been the subject of several papers. The work of Chambers and Milne (1975) on the Ythan estuary (Scotland), determined two spawning periods, one in the summer and another between the end of winter and early spring. Mettam (1979) in the Severn estuary (England) observed larval recruitment only during the summer (May-July). On the other hand, Olive and Garwood (1981) for the Blyth estuary (England), limited spawning to a short period, between March and April. Gillet (1993) in a work on the estuary of Bou Regreg (Morocco), found two recruitment periods, one in spring and another in autumn. For the Iberian Peninsula, namely in Spain, recruitment has been found to be continuous throughout the year, presenting a peak at the end of summer/beginning of autumn, and in some cases, another one during the spring (Arias and Drake, 1995; Francés Zubillaga and Saiz Salinas, 1997), thus showing a dynamic similar to the one described in the present work. From the north of Portugal, Abrantes, Pinto and Moreira (1999) observed two breeding peaks, one at the end of winter and another during spring

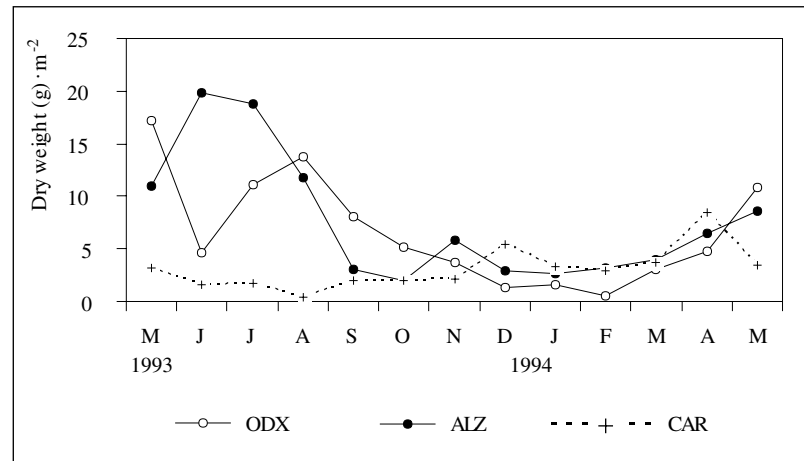
on the basis of the oogenic cycle. However, larval recruitment, based on samples was not considered by these authors. Different life spans and overlapping of cohorts in populations of *N. diversicolor* seem to be common, and are an important biological feature, which helps genetic isolation between groups of different years (Olive and Garwood, 1981; Olive, Garwood and Bentley 1981).

The duration of the coelomic phase of oogenesis at the three sites could not be precisely determined. Females carrying small oocytes were present throughout the entire sampling period, making it difficult to establish the actual duration of the coelomic phase of oogenesis.

Sex ratio is frequently determined by comparing the number of mature males and the number of mature females during the pre-spawning period, when both sexes are easily recognizable (Olive and Garwood, 1981). In the present study, we analysed the stage of maturation of individuals throughout the year. Our results are in agreement with most other studies, in which the predominance of females is the rule (e.g. Dales, 1950; Olive and Garwood, 1981; Mettam, Santhanam and Havard, 1982).

At ODX, *N. diversicolor* showed reproductive dynamics similar to those found by Olive and Garwood (1981) from northeast England. Gametogenesis did not begin during the first year of life and, as mentioned earlier, the reproductive group comprised older individuals (figure 3). At ODX, the average L_3 was higher in all individuals responsible for the second recruitment (May 1994), in comparison with those of the first (September 1993). This means that a passive migration of individuals from other parts of the estuary to this site could have occurred (Davey and George, 1986; Jensen, 1988). A more acceptable possibility, is a small fraction of the females thought to be involved in the first recruitment did not spawn, and would join the individuals participating in the second one. There are several accounts of reproductive failure in Polychaeta, as observed by different authors (Olive, Garwood and Bentley, 1981; Olive, 1983; Hardege, 1999). In our case, the discontinuance in reproduction could be because: 1) these females had missed their reproductive period due to a scarce food supply, or abrupt environmental changes (e.g. temperature and salinity); 2) they failed to find fertile males, because of an unfavourable sex ratio; 3) part of the females in the

Figure 7. Biomass of *N. diversicolor* at the three studied sites over the sampling period. (Adapted from Fidalgo e Costa, Sardá and Cancela da Fonseca, 1998)



September cohort were not mature, and continued to grow until the next breeding period (May 1994).

In ALZ, as in ODX, the recruitment peak was in September, and a second weaker recruitment also occurred in April/May 1994 (figure 6). The oocyte dynamics of this site were similar to those at ODX, although the cycle starts and ends one month earlier (figures 4 and 8). This difference could be due to a mean water temperature 2 °C higher than at ODX.

At the southern site (CAR), population densities of adults and larvae were lower (figure 6). At this site, where the temperature averages 19.5 °C, due to its short life span this species matures very quickly when compared with the other sites. A similar life span was observed in the Thames estuary (Dales, 1950) and the Norsminde Fiord (Kristensen, 1984), however with lower temperature values. Our observations on the feeding ecology of *N. diversicolor* show that this species is an opportunistic and generalist species which adapts its foraging behaviour to local conditions. This eclectic feeding behaviour influences its gamete production and life span, and is responsible for similar results in regions with different latitudes.

The amplitude and distribution of oocyte diameters in our populations were similar to those found in the British estuaries of the Blyth (Olive and Garwood, 1981) and the Severn (Mettam, Santhanam and Havard, 1982) and also in the Bidasoa estuary in Spain (Sola, 1994). In Portugal, more specifically at the Ria de Aveiro (Abrantes, Pinto and Moreira, 1999) and the Mondego estuary (Gonçalves and Ribeiro, 1987), identical dynamics were also found. Distribution patterns of

oocyte diameters seem to be similar in different populations of *N. diversicolor*, although some authors found more homogeneous distributions during the rapid oocyte growth phase (Clark and Ruston, 1963). If gamete growth rate is positively correlated with temperature, individuals for which oocyte development begins later will have a more rapid oogenesis under the influence of an increased temperature. This relationship with temperature will bring on synchronization of oocyte growth at the population level during the pre-spawning phase (Olive, 1980).

Our results indicate out that the average L₃ is lower at the southernmost warmest site (CAR), where the average temperature was higher. According to this feature, the individuals will attain maturity earlier and consequently reach a smaller size (L₃ = 2.1 mm), than at the other sites. ODX, the northern site, was the site that presented the lowest average temperatures and the highest L₃ (15.6 °C; L₃ = 2.6 mm) followed by ALZ (17.5 °C; L₃ = 2.3 mm).

At all sites, *N. diversicolor* presents a strong recruitment peak in September. This synchrony in the reproductive cycles implies a strong environmental input, and the most likely candidate is the photoperiod, as previously investigated for *Nereis virens* by Olive and his collaborators (Olive, Rees and Djunaedi, 1999). Nevertheless the development of this species at CAR was slightly different from other sites, showing an adaptation typical of opportunist species, reaching maturity as rapidly as possible, as observed by Francés Zubillaga and Saiz Salinas (1997) in polluted areas. Since pollution does not compromise the quality of the waters at

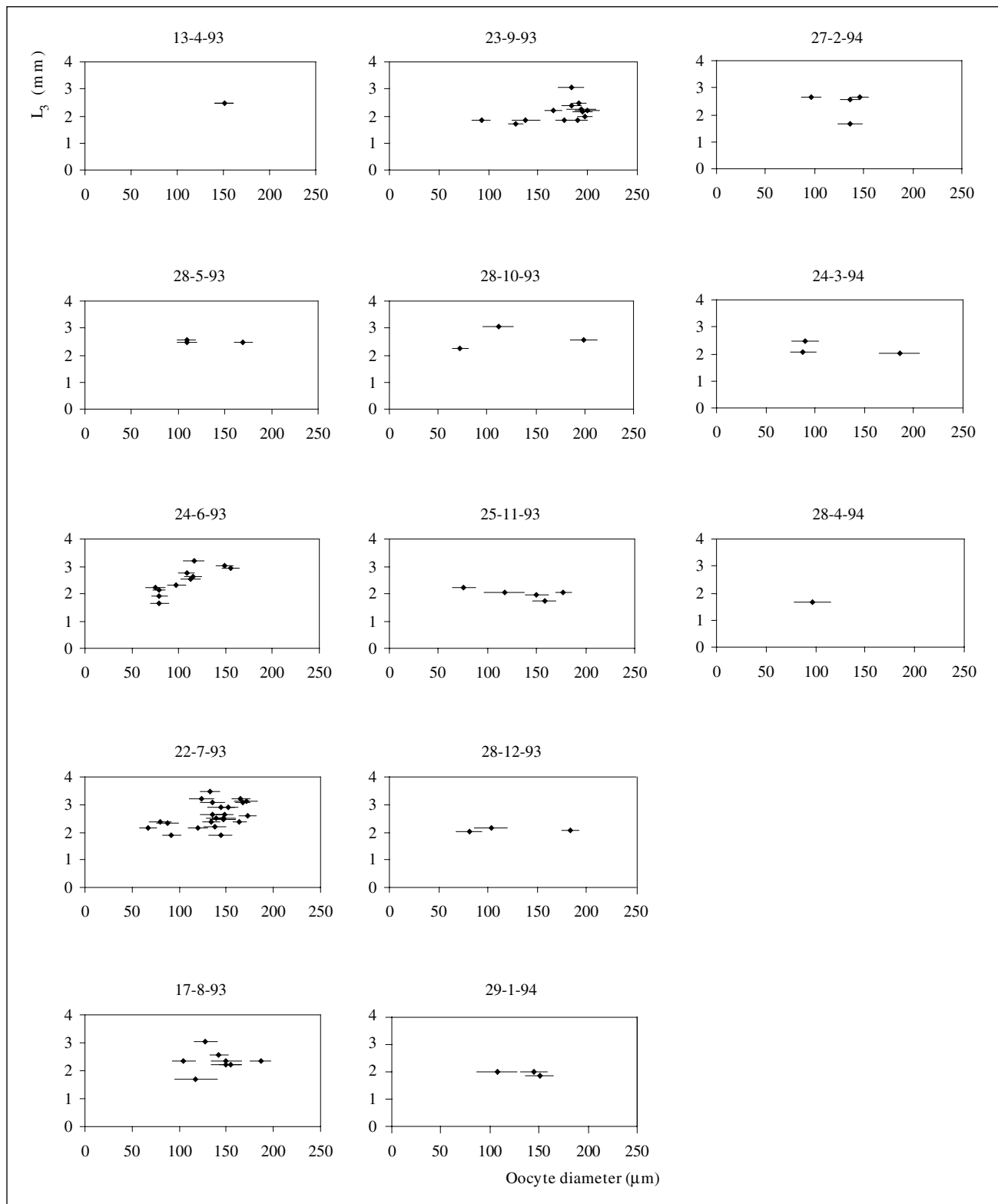


Figure 8. Relation between L_3 length (mm) and average diameter (μm), with standard deviation of oocytes in each individual of *N. diversicolor* sampled monthly at Aljezur (ALZ)

CAR, there must be another reason here for such adaptation. The rapid and premature oogenesis

observed at this site could be attributed to: 1) higher temperatures caused by the low depth of the wa-

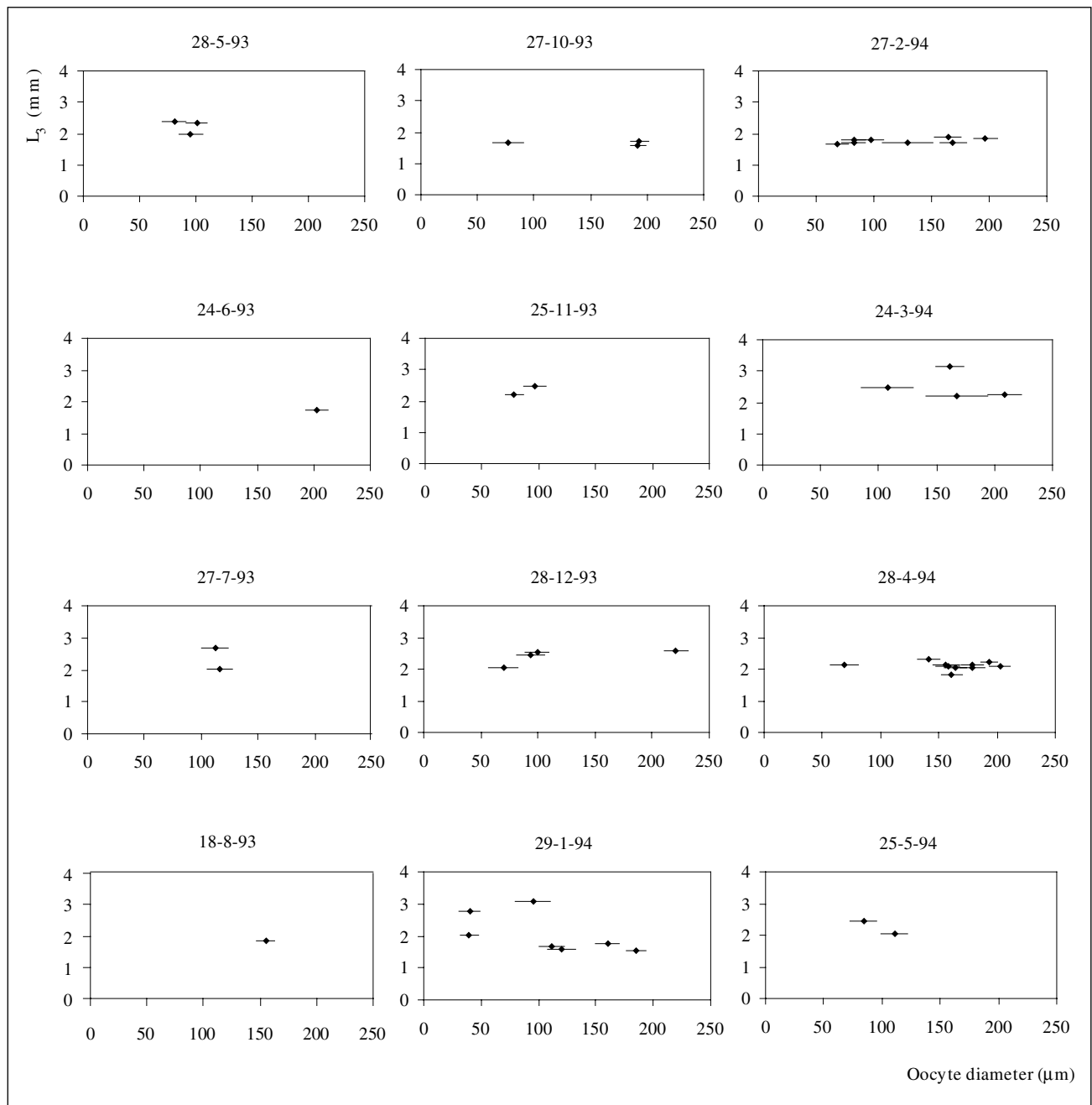


Figure 9. Relation between L₃ length (mm) and average diameter (µm), with standard deviation of oocytes in each individual of *N. diversicolor* sampled monthly at Carrapateira (CAR)

ter column (± 50 cm); 2) or the different feeding ecology of *N. diversicolor*. Our observations showed that filter-feeding was the usual feeding behaviour, although at CAR, due to lagoonal conditions and constant submersion, there is strong evidence of carnivore habits, including cannibalism.

At this site, a significant increase in individuals carrying oocytes occurred in autumn/winter (figure 5), where frequent storm events promoted pe-

riodic entrances of saltwater due to the low elevation of the sand bar. On the other hand, rainfall (figure 2) amplified freshwater flow, which produced the aperture of the sand bar and complete emptying of the lagoon in November 1993 (Fidalgo e Costa, Brotas and Cancela da Fonseca, 2002). An accumulation of allochthonous materials, such as fragments of terrestrial and riverine vegetation, was carried by the river flow. The increase in the pre-ex-

istent macrophyte meadows (*Ruppia* sp.), as well as detached seaweeds and marine organisms carried by tidal currents, provide protection against possible predators and a sudden increase in food availability for *N. diversicolor* (Fidalgo e Costa, Sardá and Cancela da Fonseca, 1998).

In conclusion, the change from an undisturbed environment with a strong lagoonal characteristics to a dynamic environment, promoted by the aperture of the sand bar, and all of the other modifications at this site after the aperture in November (Fidalgo e Costa, Brotas and Cancela da Fonseca, 2002), seems to be the driving force that influenced the different behaviour of *N. diversicolor* in CAR.

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