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**ASSISTENTE CONVIDADO DO DEPARTAMENTO DE ZOOLOGIA E ANTROPOLOGIA
DA FACULDADE DE CIÊNCIAS DA UNIVERSIDADE DE LISBOA**

BIO-ECOLOGIA E PRODUÇÃO DO POLIQUETA

***Nereis diversicolor* O.F. MÜLLER, RECURSO ZOOLOGICO DE
AMBIENTES COSTEIROS**

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AMBIENTES COSTEIROS

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de Ciências da Universidade de
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NOTA PRÉVIA

Na presente dissertação, foi feito o aproveitamento total dos resultados de trabalhos já publicados, submetidos ou a submeter para publicação, que constituem capítulos desta Tese. Para efeitos no disposto do nº 1 do Artigo 15º, do Regulamento de Doutoramentos da Universidade de Lisboa, publicado no Diário da República – II Série Nº 194, de 19-08-1993, o candidato esclarece que participou na obtenção, execução do trabalho experimental, análise e discussão dos resultados, bem como redacção dos manuscritos.

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**Aos meus pais
À Jú**

**“The study of polychaetes used to be a
leisurely occupation, practised calmly
and slowly.”**

(in The Polychaete Worms, 1977 - Kristian Fauchald)

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Resumo

A presente dissertação teve como objectivo investigar alguns aspectos da biologia e ecologia do poliqueta *Nereis diversicolor* na costa Sudoeste de Portugal, nomeadamente nas ribeiras de Odeceixe, Aljezur e Carrapateira, durante o período de Abril de 1993 até Maio de 1994, bem como a tentativa de produção deste recurso em pequena escala e em condições controladas.

Esta espécie de poliqueta muito comum e frequentemente dominante em substratos móveis de ambientes estuarinos, apresentou algumas flutuações temporais das suas densidades. Estas oscilações estão associadas à própria biologia reprodutora da espécie, mas também às interacções inter- e intra-específicas e à dinâmica sedimentar provocada pela movimentação de massas de água de origem marinha e continental, que interagem e modificam conseqüentemente as condições ambientais destes sistemas.

A produção obtida para *N. diversicolor* em cada local de amostragem, bem como as taxas de renovação da biomassa (P/B) na costa Sudoeste, situam-se numa posição intermédia entre as encontradas para outros sistemas da costa Atlântica. No entanto a ribeira da Carrapateira apresenta-se como um caso à parte, verificando-se uma produção baixa com um P/B elevado. A fase de maior crescimento, seja em animais selvagens ou de laboratório, foi obtida nos primeiros 12 meses de vida. A partir do segundo ano de idade há uma redução no crescimento somático e um investimento na reprodução, nomeadamente na produção de gâmetas.

O estabelecimento do período reprodutivo baseou-se nos resultados da recolha de amostras de estados larvares, bem como da análise do conteúdo celomático dos adultos. Neste estudo registou-se a presença contínua de fêmeas maduras e estados juvenis ao longo do ano, com um evento de recrutamento intenso em Setembro, seguido de um segundo pico de menores proporções em Maio. Na Carrapateira, ao contrário de Odeceixe e Aljezur, foi observado um amadurecimento precoce de *N. diversicolor* e conseqüentemente indivíduos de menores dimensões. Em todas as estações a razão sexual estimada foi sempre favorável às fêmeas.

O estudo da ecologia alimentar de *N. diversicolor* revelou hábitos maioritariamente filtradores, embora na estação da Carrapateira esta espécie evidenciou tendências carnívoras e canibais. Não foram encontradas diferenças significativas nos conteúdos digestivos de machos e fêmeas, mas sim uma variação na composição da dieta consoante o local de estudo, época do ano e tamanho dos indivíduos.

Através do cultivo de juvenis *N. diversicolor* alimentados com variadas dietas e em diferentes condições controladas de salinidade, temperatura e luminosidade, pôde constatar-se que esta espécie parece não apresentar uma relação entre as taxas de crescimento e o tipo de dieta fornecida. A análise dos ácidos gordos realizadas aos indivíduos reflectiu o perfil das dietas testadas. Os indivíduos que foram alimentados com dietas ricas em DHA (ácido docosahexaenoico) podem metabolizar apenas 50%, por outro lado, se a quantidade for baixa ou ausente, podem biosintetizar *de novo*. *N. diversicolor* retém aproximadamente todo EPA (ácido eicosapentanoico) presente nas dietas, porém, se a quantidade de EPA for baixa, pode biosintetizar *de novo*. Este facto demonstra que *N. diversicolor* não necessita DHA em quantidade, contudo, dietas ricas em EPA e DHA permitem uma alta assimilação de HUFA (ácidos-gordos insaturados de cadeia longa) o que é essencial para um bom crescimento e uma alta sobrevivência.

Os estudos de cultivo realizados, também demonstraram que *N. diversicolor* não apresenta diferenças significativas quanto à preferência dos sedimentos testados. Sob condições de salinidade de 15 ‰ e temperatura de 25°C esta espécie atinge as mais altas taxas de crescimento e uma sobrevivência de 100%, alcançando em poucos meses o “tamanho comercial”. Em todas as combinações testadas, os indivíduos atingiram um elevado estado de maturação em somente três meses. Estas condições de cultivo, podem ser aplicadas em futuros estudos para a obtenção intensiva de larvas, no entanto será necessário um estudo pormenorizado acerca da viabilidade destes gâmetas e a certificação de que a maturação extremamente acelerada dos progenitores não tenha sido prejudicial à gametogénese.

“Bio-ecology and production of the polychaete *Nereis diversicolor* O.F. Müller, zoological resources of coastal environments.”

Abstract

The goal of the present dissertation was to investigate some aspects of the biology and ecology of the polychaete *Nereis diversicolor* in the south-western coast of Portugal, specifically in the Odeceixe, Aljezur and Carrapateira streams, from April 1993 to May 1994. Simultaneously, it was attempted to produce this resource in a small scale under controlled conditions.

This very common polychaete species, which is frequently dominant in mobile substrates of estuarine environments, had shown some temporal fluctuations in its density. This variation is associated to the reproductive biology of the species, but also to the inter and intraspecific interactions and to the sediment dynamics due to water movement of both marine and continental origin, which interact and consequently modify the environmental conditions in these systems.

The production of *N. diversicolor* at each sampled site, as well as the biomass turnover rates (P/B) in the south-western coast, are within average values of those found in other systems in the Atlantic coast. However, the Carrapateira stream stands aside because it has revealed a low production with a high P/B . The major growth phase in both wild and laboratory-held animals was obtained during the first twelve months of life. During the second year, the somatic growth decreases and the investment for reproductive purposes increases, namely in gamete production.

The reproductive period was assessed based in the collection of samples of larval stages, as well as through the analysis of the coelomic content of adults. This study showed that mature females and juveniles are found year-round in the sampled locations. However, an intense recruitment event was recorded in September 1993 followed by a second peak, of lower intensity, in May 1994. A premature maturation, and consequently a reduced dimension of the reproductive group, was observed at Carrapateira. At all stations, the sex ratio was always biased towards females.

The study on the feeding ecology of *N. diversicolor* revealed a predominance of a filter-feeding habit, although at Carrapateira this species showed both carnivore and cannibal tendencies. No significant differences were found in the digestive contents of males and females. A difference was found, however, in the diet composition among the study sites, year seasons, and according to size.

By rearing *N. diversicolor* juveniles fed with an array of diets and under different controlled conditions of salinity, temperature, and light, it can be observed that growth rate is not related to diet. Yet, the fatty acid analyses reflected the profile of the diets tested. The individuals fed with diets rich in DHA (docosahexaenoic acid) can metabolize only 50%, on the other hand, if the amount of DHA is low or absent, they can biosynthesize it *de novo*. *N. diversicolor* retains nearly all EPA (eicosapentanoic acid) from their diet, however, if the amount of EPA is low, they can biosynthesize it *de novo*. This fact demonstrates that this species does not require DHA in large quantities, nevertheless, diets rich in EPA and DHA allow a high incorporation of HUFA (High Unsaturated Fatty Acids), which is essential for a high growth and survival rates.

The studies on this species' culture also demonstrated that *N. diversicolor* does not show any preferences among the sediments tested. However, under salinity conditions of 15‰ and a temperature of 25°C, this species reaches the highest growth rates and a 100% survival rate, attaining in a few months the "commercial size". In all tested combinations, the individuals reached an advanced state of maturation in only three months. These rearing conditions may be applied in future studies as to obtain an intensive production of larvae. However, it becomes necessary to carry out a detailed study of the viability of the oocytes produced thereby, as to ascertain if such a fast maturation does not hamper the oogenic cycle.

Capítulo I

Introdução e Objectivos

Porquê Nereis diversicolor

Distribuição

Taxonomia

Primeira citação para Portugal

A presente dissertação

A gestão racional do recurso N. diversicolor

Referências Bibliográficas

Porquê *Nereis diversicolor* ?

O interesse por aprofundar os conhecimentos acerca do poliqueta *N. diversicolor* (O.F. Müller, 1776) ocorreu no Laboratório Marítimo da Guia (LMG) da Faculdade de Ciências da Universidade de Lisboa (FCUL) a partir dos trabalhos desenvolvidos por Dinis (1986) e Luís (1989). Estes versavam sobre a nutrição dos linguados *Solea vulgaris* e *Solea senegalensis* (Osteichthyes, Pleuronectiformes) e dos camarões *Palaemon serratus* e *Penaeus kerathurus* (Crustacea, Decapoda) em cativeiro e, particularmente o papel dos lípidos no respectivo crescimento e reprodução. Estes autores demonstraram claramente a importância da presença deste poliqueta nas dietas fornecidas, e a influência determinante na indução da maturação dos ovários e qualidade das posturas, e nomearam esta influência de “efeito Nereis”. Em 1992, com projecto de investigação financiado pela Junta Nacional de Investigação Científica e Tecnológica (JNICT) “Ecologia, biologia e produção da minhoca-da-pesca (*Hediste diversicolor*) recurso zoológico de ambientes salobros” deu-se início ao estudo que permitiu alargar consideravelmente os conhecimentos relativos a esta espécie de poliqueta. O impacto da captura desregrada de *N. diversicolor*, cujo valor ascende anualmente a vários milhares de contos, nos principais estuários portugueses continua por avaliar. A falta de conhecimentos de base existentes até então, faz com que esta dissertação apresente como principais objectivos: i) o conhecimento da bio-ecologia desta espécie, nomeadamente na ecologia alimentar, dinâmica populacional, recrutamento e assentamento larvar nos sistemas estuarino-lagunares da costa sudoeste de Portugal. ii) uma primeira abordagem no que se refere ao cultivo de poliquetas em nosso país, designadamente crescimento e sobrevivência com diferentes dietas, e em diferentes condições ambientais. Desta forma pretendemos fornecer informação de base necessária à gestão deste recurso, e tornar possível, num futuro próximo, o seu cultivo em sistemas artificiais.

Distribuição

N. diversicolor é uma espécie eurihalina e euritérmica, tipicamente estuarina e lagunar. Possui elevada tolerância fisiológica a variações extremas de muitos parâmetros ambientais crescendo e reproduzindo-se em diferentes tipos de substratos e em locais sujeitos a um elevado *stress* ambiental (Dales, 1950, Smith, 1956, 1977, Bartels-Hardege & Zeeck, 1990, Cheggour *et al.*, 1990, Miron &

Kristensen, 1993, Salinas & Zubillaga, 1997). Encontra-se, assim, largamente distribuída desde o Mediterrâneo, Norte de África, frio e salobro Báltico interior e até às quentes e hipersalinas lagunas do Mar Negro (Fauvel, 1923, Clay, 1967, Gillet, 1993, Mettam, 1979). Embora possa viver em áreas de salinidades tão baixas como 1‰, não pode desenvolver-se nem reproduzir-se em meios dulceaquícolas (Smith, 1956, Muus, 1967). A amplitude da sua distribuição vertical é diversa; ocorre em todo o mediolitoral e está presente nos primeiros metros do infralitoral. Nos estuários é considerada como frequentadora habitual dos sedimentos vasosos, aparecendo em galerias a partir do baixo sapal e nas amplas plataformas vasosas intertidais estuarinas (Mettam, 1979). Nos meios lagunares ocorre nas áreas mesohalinas, parecendo não estar dependente dos tipos de substrato (Cancela da Fonseca, 1989). A sua importância é ainda revelada pelas elevadas densidades populacionais que podem atingir (Heip & Herman, 1979, Cruz, 1989, Cancela da Fonseca, 1989, Fidalgo e Costa *et al.*, 1998). As variações geográficas e temporais da densidade de *N. diversicolor* reflectem a influência de diversos factores bióticos e abióticos. É conhecida a discriminação de substratos propícios por parte das larvas de muitos invertebrados bentónicos durante o assentamento (e.g. Thorson, 1966, Scheltema, 1974, Hadfield, 1986, Bhaud *et al.*, 1995), mas para os recrutas de *N. diversicolor* a composição do sedimento parece não ser um factor limitante (Jensen, 1988). Mas outros factores abióticos como a luz, salinidade, oxigénio dissolvido e temperatura condicionam frequentemente o comportamento larvar (Bhaud *et al.*, 1995). Devido a baixa agressividade, as interacções intra- e inter-específicas são igualmente importantes na distribuição desta espécie (Davey & George, 1986, Jensen, 1988, Kristensen, 1988).

Taxonomia

De acordo com estudos mais recentes (Rouse & Fauchald, 1997, Glasby *et al.*, 2000) o sistema de nomenclatura actual, ainda baseado no sistema linneano, tem demonstrado ser altamente inadequado para reflectir as relações filogenéticas entre os organismos. Por esta razão Rouse & Fauchald (*op.cit.*) não utilizam categorias linneanas (classe, ordem, etc.) para nomear as suas clades, sendo cada uma destas um grupo monofilético. Este estudo deve no entanto, ser tomado como uma aproximação ao que será a classificação filogenética dos Polychaeta, já que provavelmente irá ser modificada à medida que novos dados (anatomia interna dos

diferentes grupos, novas espécies, biologia molecular, etc.) sobre os Polychaeta sejam conhecidos.

Rouse & Fauchald (op.cit.) chamam a atenção para o facto de que as terminações latinas dos seus clades não devem ser interpretadas como tendo uma categoria Linneana; em vez disso fornecem simples nomes formais para evitar dúvidas com o uso vernacular dos mesmos. Do antigo sistema Linneano apenas utilizam as famílias.

A família Nereidae foi criada por Johnston (1865) a partir do nome Nereïdes, que eram as ninfas marinhas filhas de Nereus, segundo o poema de Spencer, que Johnston inclui no seu trabalho. Mais tarde Pettibone (1971) corrigiu o nome da família, já que a latinização correcta de Nereïdes seria Nereididae. Isto não afecta a autoria da família, já que se tratava apenas de um problema de latim.

Ainda no domínio da taxonomia, existe alguma falta de consenso na utilização do nome genérico (*Neanthes*, *Nereis* ou *Hediste*), como também, no uso ou não do sub-género entre o nome genérico e o específico. Esta falta de consenso reflecte-se a nível das citações frequentemente observadas na literatura existente. Assim esta espécie surge referida como *Hediste diversicolor* (Fong & Garthwaite, 1994, Fidalgo e Costa, 1994, Fidalgo e Costa & Cancela da Fonseca, 1995, Abbiati & Maltagliati, 1996), *Nereis (Hediste) diversicolor* (Olive & Garwood, 1981, Davey & George, 1986, Fidalgo e Costa & Cancela da Fonseca, 1998), *Nereis diversicolor* (Dales, 1950, Chambers & Milne, 1975, Kristensen, 1984, Essink *et al.*, 1985, Hughes *et al.*, 2000). Refira-se ainda uma estranha citação de Ozoh & Jones (1990), em que *Nereis* aparece como sub-género *Hediste (Nereis) diversicolor*. Instituições como a Agência Escocesa de Protecção Ambiental (SEPA) e o Departamento Ambiental do Reino Unido, utilizam a designação específica *Hediste diversicolor*, por ser esta nomenclatura rotineiramente aceite pela maioria dos ecologistas e citada no “MCS Species Directory”. Recentemente, foi lançada uma segunda edição desta publicação que atribui o estatuto de género a *Hediste* e *Neanthes*, reafirmando no entanto a sinonímia com *Nereis*, já citada anteriormente por Chambers & Garwood (1992). No presente trabalho optámos por simplificar e utilizar apenas *Nereis diversicolor*, omitindo o sub-género *Hediste* entre parêntesis, correctamente utilizado por alguns autores.

*Primeira citação de *N. diversicolor* para Portugal*

A citação mais antiga de *N. diversicolor* para Portugal é a de Carvalho (1929) publicado na antiga revista Memórias e Estudos do Museu Zoológico da Universidade de Coimbra. Constava de uma lista da colecção de invertebrados de Portugal existente no Museu Zoológico desta mesma instituição. Mas os estudos que abordam a biologia e a ecologia desta espécie em Portugal no início da presente dissertação ainda eram escassos (Almeida, 1977, Gonçalves & Ribeiro, 1987, Cruz, 1989, Lopes da Silva, 1993), não abordando aspectos fundamentais do ciclo de vida. A necessidade de alargar estes estudos a diferentes áreas geográficas e ambientes salobros era evidente, face às divergências encontradas na literatura no que respeita à reprodução, ao ciclo de vida e à produção secundária. Considerando o determinismo ecológico da reprodução dos poliquetas (Bhaud, 1972), afigurava-se pouco provável que *N. diversicolor* se reproduzisse ao longo da costa portuguesa em épocas semelhantes às definidas para outros locais, sendo mesmo de esperar uma variabilidade acentuada de um local para outro.

A presente dissertação

O estudo da bio-ecologia de *N. diversicolor* foi realizado entre Abril de 1993 e Maio de 1994, na costa Sudoeste Portuguesa, mais precisamente na secção sob influência marinha das ribeiras de Odeceixe, Aljezur e Carrapateira. Estas ribeiras inserem-se na área do Parque Natural do Sudoeste Alentejano e Costa Vicentina, e fazem parte do conjunto genericamente denominado “Ribeiras do Algarve” que engloba os pequenos cursos de água com orientação Oeste ou Sul e que desaguardam directamente no Atlântico (Loureiro & Nunes, 1986). Devido às suas características físicas e diversidade geomorfológica, estas ribeiras podem ser classificadas como sistemas lagunares (cf. Stewart, 1972, Colombo, 1977) ou estuarino-lagunares (cf. Day *et al.*, 1987). Por apresentarem uma elevada riqueza específica, e algumas peculiaridades ecológicas extremamente interessantes e de importância indiscutível (Silva e Costa *et al.*, 1983), estes sistemas foram já objecto de estudo em outros trabalhos (Magalhães *et al.*, 1987, Magalhães, 1988, Costa *et al.*, 1989, Duarte *et al.*, 1990, Costa, 1993). A escolha dos locais de amostragem nos três sistemas, teve por base as estações onde, nos estudos anteriores, *N. diversicolor* se tinha revelado mais abundante. Por este motivo optámos por manter a nomenclatura das estações

anteriormente utilizada, tentando desta forma, dar alguma continuidade aos estudos dos referidos sistemas. Ao fim dos trabalhos de amostragem, triagem e determinação das abundâncias, chegou-se à conclusão que o número de indivíduos capturados por local de amostragem nas três ribeiras, embora expressivo, era insuficiente, principalmente para uma identificação precisa das diferentes coortes e cálculos de produção, mas também para estudo do ciclo gametogénico. Devido a estes factos optámos por uma nova abordagem: i) somente na análise dos parâmetros abióticos (Artigo 1) se consideraram as diferentes estações de amostragem estabelecidas inicialmente para cada local; ii) sendo provável que em cada um dos sistemas estudados se esteja na presença da mesma população de *N. diversicolor*, optou-se por considerar todas as estações de amostragem dentro de cada um dos sistemas prospectados como uma única estação. Deste modo, em todos os capítulos do presente trabalho são feitas análises comparativas da bio-ecologia desta espécie nos três sistemas costeiros, abdicando-se de uma abordagem comparativa entre diferentes locais dentro do mesmo sistema. Desta forma, com a escolha de três sistemas, aparentemente distintos, que proporcionam uma extensa variabilidade, tentamos estabelecer se as características inerentes a cada um dos locais apresentam diferenças importantes que influenciem o ciclo de vida da espécie em questão.

Com o intuito de alicerçar os conhecimentos relativos ao ciclo de vida de *Nereis diversicolor*, estudou-se seu ciclo gametogénico (Artigo 2), em conjunto com amostragens exclusivamente direccionadas para os primeiros estádios larvares, nomeadamente a reprodução e assentamento larvar. Este tipo de trabalho jamais fora realizado em Portugal, pelo que era fundamental esta abordagem conjunta, já que esta espécie é ovípara, não hermafrodita e estritamente monotélica - os indivíduos reproduzem-se uma única vez e morrem após a emissão de gâmetas (Dales, 1950, Olive & Clark, 1977, Olive & Garwood, 1981, Mettam *et al.*, 1982, Fischer, 1984, Humpheys, 1985, Möller, 1985). Desta forma, a análise oocitária em consonância com a amostragem direccionada aos novos efectivos, permitiu-nos estabelecer com exactidão o seu período reprodutivo nos referidos sistemas.

Os cálculos de biomassa e produção secundária, bem como a identificação de coortes e determinação do crescimento (Artigo 3), apresentam algumas dificuldades práticas no estudo dos macropovoamentos bentónicos, e em particular o de *N.*

diversicolor, devido à fácil autotomia que estes animais apresentam. Durante o processo de crivagem muitos indivíduos partem-se ou ficam danificados pelo contacto com conchas partidas e arestas das partículas sedimentares. Por este motivo, ao invés do comprimento total utilizado nos primeiros estudos da dinâmica de *N. diversicolor* (Dales, 1951, Muus, 1967), optámos por medir parcialmente os indivíduos, recorrendo a estruturas mais rígidas para os referidos cálculos de biomassa. Este procedimento tem sido utilizado com sucesso por diversos autores que elegem diferentes estruturas do corpo, tais como: largura do 1º setígero (Davey & George, 1986), largura do 10º setígero (Heip & Herman, 1979, Sola & Ibañez, 1990, Garcia-Arberas, 1998), comprimento da mandíbula (Chambers & Milne, 1975, Mettam, 1979, Olive & Garwood, 1981, Mettam *et al.*, 1982, Essink *et al.*, 1985, Möller, 1985), comprimento do prostómio, peristómio e 1º segmento Gillet (1990).

No momento da escolha de um parâmetro biométrico para os estudos referidos no parágrafo anterior, elegeu-se o comprimento L_3 (prostómio, peristómio e 1º segmento setígero), como a medida mais adequada. Embora o comprimento da mandíbula seja o parâmetro biométrico mais utilizado por outros autores, este foi desde o início descartado pelo facto de ser pouco prático o processo de extracção, face à presença de um grande número de juvenis nas nossas amostras (e.g. Bachelet, 1987). Para a biometria, seleccionou-se um número determinado de indivíduos em bom estado de conservação, e representantes de todas as classes de tamanho, pertencentes aos locais em estudo. Em seguida, estes exemplares foram medidos, secos (48h a 60°C) e pesados. Utilizando os dados do comprimento somático representado por L_3 (mm) vs peso seco (mg) foi calculada uma regressão, com o intuito de converter as medidas somáticas de todos os indivíduos encontrados em biomassa (Fidalgo e Costa *et al.*, 1998).

O estudo da dinâmica populacional de *N. diversicolor*, confrontou-se com a dificuldade no processo de identificação das diferentes coortes, pela entrada contínua de novos indivíduos na população e diferenças nas dimensões entre indivíduos da mesma idade, o que levou à sobreposição das modas etárias. Desta forma, e tal como em diversos estudos de ecologia marinha, assumiu-se que a distribuição de frequência das dimensões dos indivíduos de uma mesma classe etária, apresenta uma distribuição normal. O histograma geral de frequências de tamanhos pode ser decomposto em diversas curvas de distribuição normal,

representando cada uma delas uma coorte. Recorrendo ao método de Bhattacharya (1967), a decomposição gráfica das frequências é feita através das diferenças logarítmicas, que permite estabelecer a média e desvios típicos das componentes gaussianas, calculando assim o efectivo teórico de cada uma das coortes e determinando as respectivas curvas de crescimento (Lepetit, *et al.*, 1991). A sobreposição de classes etárias tem sido observada frequentemente em outras populações desta espécie (Gillet, 1990, Dueso & Sardá, 1994, Nithart, 1998, Abrantes *et al.*, 1999). Devido a esta característica, a produção secundária teve que ser determinada por dois métodos distintos: i) o método de Crisp (1984) com uma ligeira modificação, baseado no aumento em peso das coortes calculado a partir das medidas de L_3 de todos os indivíduos, ao invés do peso seco. Este método foi utilizado aquando da identificação das coortes, sendo aplicado apenas às mais velhas; ii) e o método baseado na coorte única anual (Hynes & Coleman, 1968, Hamilton, 1969, Benke, 1979) seguindo a fórmula elaborada por Cornet (1986). Este método foi aplicado somente aos efectivos do primeiro ano, que se misturam mais facilmente com os novos efectivos frequentemente incorporados à população. Este facto deve-se às diferenças de tamanho existentes a nível individual sobretudo nos indivíduos mais jovens.

Esta espécie de poliqueta pode considerar-se como um dos recursos naturais relevantes nos ambientes salobros por constituir uma peça fundamental nas teias tróficas dos sistemas que integra. É presa habitual de espécies piscícolas de elevado interesse económico, como *Anguilla anguilla*, *Dicentrarchus labrax*, *Solea solea*, *S. senegalensis* e crustáceos como *Carcinus maenas* e *Penaeus kerathurus* (e.g. Costa, 1982, Davey & George, 1986, Dinis, 1986, Luis & Passos, 1989, Gillet, 1990, Bernardo, 1990, Cabral, 1998, Cancela da Fonseca, 1989, Cabral, 2000), mas também é frequentemente predado por aves limnícolas (e.g. Triplet, 1989, Zwartz & Esselink, 1989). Todavia, quanto à alimentação, pode considerar-se uma espécie ecléctica, sendo múltiplos os seus hábitos alimentares: predadores, filtradores, herbívoros, detritívoros e suspensívoros (Goerke, 1971, Reise, 1979, Rönn *et al.*, 1988, Riisgård, 1991, Smith *et al.*, 1996, Lucas & Betru, 1997, Vedel, 1998, Hansen & Kristensen, 1998). Os conteúdos digestivos de *N. diversicolor* nos locais por nós estudados (Artigo 4), confirmam hábitos maioritariamente filtradores (e.g. Riisgård, 1991, Vedel, 1998), mas também, como no caso da Carrapateira, tendências

carnívoras e canibais. Trabalhos antigos como os de McIntosh (1907) e Bogucki (1953) já atribuíam a esta espécie uma predação intra-específica.

Para além da importância ecológica de *N. diversicolor* nas cadeias tróficas dos principais estuários portugueses e europeus, tem-se verificado a sua importância como presa viva em sistemas de cultivo artificial. As características nutricionais de *N. diversicolor* parecem estar relacionadas com os lípidos, mormente com o seu perfil em ácidos gordos. No entanto, embora esse perfil não exiba, do ponto de vista quantitativo, as características em ácidos gordos poli-insaturados de cadeia longa (C>20) patenteadas por muitos alimentos naturais utilizados em aquacultura, é com *N. diversicolor* que se têm obtido os melhores resultados em Portugal no crescimento, maturação e na indução da postura em camarões penaeideos e linguados (Dinis, 1986, Luis, 1989, Luis & Ponte, 1993, Dinis *et al.*, 1999). Pensa-se que tais factos estejam relacionados com a proporção de ácidos gordos com cadeia em série n6, de que esta espécie de poliqueta apresenta uma proporção invulgarmente elevada, particularmente em ácido linoleico - C18:2n-6 (Luis, 1989, Luis & Passos, 1995). No presente trabalho (Artigo 5) tentamos compreender melhor a influência da alimentação no crescimento, nas taxas de sobrevivência e na composição dos ácidos gordos em *N. diversicolor*. Os estudos de Giese (1966), Morris & Sargent (1973) e Holland (1978) estabeleceram a importância dos lípidos na fisiologia dos invertebrados marinhos, funcionando como reservas metabólicas. Outros trabalhos realizados com poliquetas (*e.g.* Pocock *et al.*, 1971, Farrington *et al.*, 1973, Bradshaw *et al.*, 1990, Lytle *et al.*, 1990) serviram também como base impulsionadora determinante para que o cultivo de poliquetas passasse a ter maior destaque em aquacultura alcançando um alto valor comercial.

Em Portugal, o cultivo de poliquetas em pequena escala, desde larva até adulto, foi desenvolvido no âmbito desta Tese (Artigos 6 e 7) e restrito a uma única espécie (*N. diversicolor*). Esta espécie, embora possua menores dimensões quando comparada com *Nereis virens*, uma das poucas espécies actualmente cultivadas e comercializadas (Olive, 1994), tem um óptimo ritmo de crescimento e uma apetência por diferentes dietas em condições laboratoriais.

A gestão racional do recurso N. diversicolor

Na Europa, os poliquetas têm sido apanhados nas zonas estuarinas, por pescadores, para uso próprio, e cada vez mais para o comércio de isco vivo. Em Itália, onde há uma intensa apanha de poliquetas, não existe legislação, nem estudos prévios do ciclo de vida e da dinâmica populacional nos locais onde as espécies comercializadas são exploradas (Gambi *et al.*, 1994). Em Espanha, França e Itália encontram-se à venda no mercado poliquetas provenientes da Coreia e mesmo de Portugal (Sardá, 1989, Castro, 1993). Nomeadamente no Reino Unido não há estatísticas oficiais nem uma lei específica que regule a apanha do isco, existindo uma economia “subterrânea” que movimentou em 1992 cerca de 600 toneladas (Olive & Cowin, 1994). Iversen (1976) refere que as receitas anuais do total dos apanhadores de poliquetas nos EUA ultrapassam um milhão de dólares. Na Ásia, o mercado está voltado fortemente para a exportação, sendo o Japão um dos países que importa anualmente da China e da Coreia quantidades substanciais de poliquetas (Choi, 1985).

Em Portugal, o volume real das capturas deste recurso é, geralmente, subestimado ou desconhecido, embora o desenvolvimento da sua exploração comercial para consumo interno e exportação tenha aumentado, devido ao incremento do número de apanhadores. Este aumento da apanha e a sua importância económica, levou à publicação do “Regulamento da Cultura e Apanha de Isco do Tipo Minhoca” (Portaria nº 254/79 de 31 de Maio). Este documento tem por finalidade pôr cobro à exploração desordenada deste recurso, conferir uma maior estabilidade na comercialização e facilitar a fiscalização. No entanto, apenas as espécies *Marphysa sanguinea* e *Diopatra neapolitana* foram alvo desta protecção, ficando à margem deste regulamento o poliqueta *Nereis diversicolor*, uma espécie, frequentemente capturada e que se encontra à venda no mercado de isco para pesca. Recentemente nova portaria do Ministério da Agricultura, do Desenvolvimento Rural e Pescas (Portaria nº 1102-B/2000 de 22 de Novembro) passa a incluir poliquetas do género *Nereis* no Regulamento da Apanha. A actividade de apanha de poliquetas para a pesca é efectuada por apanhadores credenciados, em diversos estuários portugueses, sendo o do Sado e o do Tejo os mais procurados. Segundo Castro (1993) existem diversas transgressões realizadas pelos apanhadores do “Minhocão” (*Marphysa sanguinea*) e do “Casulo” (*Diopatra neapolitana*) no estuário do Sado,

que vão desde o tamanho permitido por lei aos instrumentos de captura (Edital nº2/82) e à restrição da apanha na margem direita do Esteiro da Marateca (Edital nº3/83). *Nereis diversicolor* é apanhado indiscriminadamente durante todo o ano, embora seja mais intensa nos meses de Primavera e Verão, quando o número de pescadores desportivos é maior, provocando efeitos negativos sobre o seu ciclo reprodutivo. Esta espécie, e as outras duas citadas anteriormente, são cada vez mais procuradas pelos apanhadores, tendo *N. diversicolor* rendido no estuário do Sado 3 milhões de escudos ao ano por apanhador (Lopes da Silva, 1993). É primordial estabelecer, tal como para as outras espécies, uma época de defeso que minimize o impacto desregrado sobre este recurso. Importa também aumentar o controlo na atribuição de licenças de captura e a fiscalização nas áreas de apanha. São conhecidos casos de exploração comercial de anelídeos poliquetas em alguns países do Norte da Europa, EUA, Canadá e Austrália que quase levaram ao esgotamento de alguns *stocks*, tendo sido necessária a adopção de medidas legais rigorosas (Klawe & Dickie, 1957, Pope, 1965, Forbes, 1984, Castro, 1993).

Actividades como a apanha de algas, a pesca artesanal de organismos sedentários e de distribuição muito restrita, a colheita indiscriminada de moluscos e crustáceos têm levado a um empobrecimento crescente do litoral rochoso português (Ré e Boaventura, 1998). Nas zonas de substrato móvel do nosso litoral, a situação não é mais animadora, pois além da apanha pouco fiscalizada de diversos organismos nas zonas estuarinas, verifica-se um aumento da poluição orgânica e industrial (Castro, 1990). Apesar do esforço de consolidação legislativa ocorrida em 1971, a legislação, hoje contribui de forma agravada para uma longa tradição de fragmentação de competências entre instituições. O actual quadro legislativo relativo à gestão da zona costeira está, pois, demasiado disperso e necessita claramente de um esforço de coordenação e integração (CNADS, 2001). Um outro agravante, é que as leis existentes, por muito positivas que sejam e por muito determinada que seja a intenção da sua aplicação, sofrem do grave problema do seu não cumprimento (Dias *et al.*, 1997).

Na realidade, a exploração e o comércio de poliquetas têm aumentado em todo o mundo, com um impacto provocado pelo pisoteio e revolvimento de grandes extensões de sedimento. Outras actividades humanas provocam uma forte entropia nas zonas costeiras, o que torna imperativo uma gestão e ordenamento destas

áreas, aliada a uma legislação actualizada e a uma fiscalização actuante. São conhecidas as alterações físicas do substrato, devido a acções mecânicas de revolvimento dos sedimentos causadas pelo pisoteio e a apanha do isco. Esta perturbação influencia de forma significativa os ciclos biogeoquímicos, bem como o estabelecimento dos organismos bentónicos e a recolonização dos sedimentos perturbados (Olive *et al.*, 1991, Castro, 1993, Gambi *et al.*, 1994, Warren, 1994, Fidalgo e Costa & Cancela da Fonseca, 1995). Esta recolonização parece variar com a estação do ano, as características locais e a extensão da sobreexploração em áreas adjacentes (McLusky *et al.*, 1983, Olive, 1993). Outro aspecto importante é o facto da sobreexploração de poliquetas levar a uma diminuição destes recursos, os quais são presas fundamentais para certas espécies de aves migradoras (Warren, 1994).

A apanha do isco com intuito de comercialização não é um “direito” dos apanhadores, e é razoável assumir que os *stocks* de poliquetas necessitam de uma legislação apropriada e uma fiscalização contínua. Com esse efeito é interesse dos apanhadores que o recurso se mantenha a longo prazo, isto é, que a sua exploração seja sustentável. O cultivo de poliquetas em Portugal poderá abranger apenas uma pequena cota do mercado de isco vivo, mas será um contributo para a redução do impacto provocado pela apanha. Do ponto de vista conservacionista, a produção de juvenis pode ser direccionada para o repovoamento de áreas que apresentem uma redução significativa dos seus *stocks* (Fidalgo e Costa & Cancela da Fonseca, 2000).

Uma parte considerável dos poliquetas apanhados para comercialização nas zonas estuarinas do nosso país, provêm de áreas protegidas ou reservas naturais. A necessidade de conservação do nosso litoral deve ter em atenção aspectos sócio-económicos relacionados com as actividades das populações. A proibição da apanha do isco devido ao defeso durante um largo período de tempo, poderá ter implicações catastróficas para a economia familiar das populações que dependem desta actividade (Castro, 1996). A redução do impacto provocado pela apanha do isco no meio natural e o combate à depauperação dos *stocks* de poliquetas exige uma melhor gestão e conservação das áreas costeiras. O primeiro passo para a conservação será a implementação de um sistema de rotação de áreas de apanha, tal como na agricultura, onde os juvenis da área em descanso podem colonizar a

área que acabou de ser explorada (Olive, 1994). Este sistema rotativo é utilizado com sucesso na Coreia, um dos maiores exportadores de poliquetas a nível mundial (Choi, 1985). Uma hipótese, embora não posta em prática, é manter uma área não explorada que possa servir de reserva populacional para fornecer juvenis destinados ao povoamento das áreas exploradas.

O cultivo de animais marinhos em Portugal é uma actividade ainda pouco desenvolvida, e baseia-se principalmente, na produção de algumas espécies de peixes e crustáceos (P. Pousão, com.pess.) O cultivo de anelídeos poliquetas com objectivos comerciais não existe no nosso país (Castro, 1993). Noutros países, a procura por isco vivo tem crescido devido ao aumento nos últimos vinte anos da aquacultura de crustáceos (Olive *et al.*, 1991) e de pescadores desportivos e profissionais. O cultivo artificial de anelídeos poliquetas ainda não satisfaz a procura deste mercado em expansão. Na Formosa, cerca de dez aquacultores, produzem cerca de 25 toneladas de *Perinereis brevicirrus* que exportam para o Japão, um país onde também há produção desta espécie mas que recorre às importações (Chen, 1990). Na Europa, este tipo de cultivo resume-se à Holanda e ao Reino Unido; este último país, iniciou em 1986, o cultivo de *Nereis virens* a partir de transferência de tecnologia adquirida por investigadores da Universidade de Newcastle upon Tyne (UK) para o sector privado. O sucesso desta ligação entre a investigação universitária e o sector privado traduziu-se na produção de 15 toneladas em 1992/3. Esse intercâmbio mantém-se, pois é fundamental a investigação contínua e a melhoria da técnica de cultivo, bem como o controlo de patogenias inerentes à aquacultura intensiva (Olive, 1994). Na vizinha Espanha, um país com grande tradição na aquacultura e um dos principais produtores mundiais, o comércio de isco vivo é um negócio em expansão, embora ainda necessite de recorrer à importação (Sardá, 1989). O cultivo de poliquetas começa a desenvolver-se à escala piloto, com o cultivo de *Arenicola cristata*, embora não exista bibliografia a este respeito (Aranda, comunicação pessoal).

A monocultura intensiva, causa frequentemente problemas de natureza ambiental tendo vindo a ser substituída pelo cultivo integrado de espécies de diferentes níveis tróficos (Tenore *et al.*, 1974, Folke *et al.*, 1998). Esse tipo de cultivo, poderá ser o ponto de partida para a aquacultura comercial de poliquetas em Portugal, com a vantagem deste minimizar o impacte orgânico dos efluentes no ambiente, através da

utilização por parte do poliquetas, das fezes, pseudo fezes e restos de ração não ingeridos.

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Capítulo II

Caracterização da área de estudo

Artigo 1

PHYSICAL CHARACTERISATION AND MICROPHYTOBENTHOS BIOMASS OF ESTUARINE AND LAGOON ENVIRONMENTS ON THE SOUTHWEST COAST OF PORTUGAL

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ABSTRACT

The purpose of this study was to compare three lagoon-estuarine systems of the Southwest coast of Portugal, on the basis of their physical characteristics and microphytobenthos biomass. During a period of 14 months (April 1993 to May 1994), monthly samples of sediment were collected intertidally at low tide in order to characterize the sediment based on organic matter content, granulometry, chlorophyll *a* and phaeopigments. Water parameters from the bottom layer (temperature, salinity, dissolved oxygen and pH) were also measured *in situ*. Principal Component Analysis (PCA) was used to explain and define trends between sampling sites and parameter distribution. Sediment dynamics, caused by tidal currents and runoff during periods of rainfall, seems to be the main driving force influencing the behaviour of abiotic parameters in these systems.

Keywords: Estuarine systems; microphytobenthos; organic matter; granulometry; salinity

INTRODUCTION

In intertidal estuarine ecosystems, particularly shallow aquatic environments, benthic fauna and flora are intimately associated with bottom sediment and exposed to large environmental fluctuations determined by the alternation between emersion and immersion conditions (Day et al., 1987). Wind and, especially, tidal currents are usually

the most important causes of mixing in estuaries, and help determine the distribution and abundance of several species of macrofauna and microphytobenthos (Colijn & Dijkema, 1981, Tamaki, 1987, Delgado, 1989, De Jonge & Beusekom, 1995). Other factors that influence the spatial distribution of different organisms are light intensity and granulometric composition, directly affecting many species by limiting vertical distribution and burrowing performance (Asmus, 1982, Riaux-Gobin et al., 1993). Microphytobenthos production has a significant importance on the estuarine food web, representing potential food sources for a variety of benthic invertebrates, ranging from micro- to macrofauna (Fauchald & Jumars, 1979, Montagna, 1984, Plante-Cuny & Plante, 1986, Bianchi et al., 1988). Rainfall, on the other hand, causes a sudden decrease in temperature and salinity and also a strong leaching of the sediment surface (Rasmussen, et al., 1983). These frequent changes in environmental conditions provide an intricate network of biotic and abiotic factors that affect the spatial distribution of different organisms and physical disturbance of the sediment on different spatial scales. Due to their physical properties and geomorphological diversity, the systems considered in this study cannot be considered true estuaries, but rather lagoonal systems (Colombo, 1978) or lagoon-estuarine ecosystems (Day et al., 1987). In fact the three studied sites are examples of temporarily closed small systems, ranging from a period of summer months (Odeceixe and Aljezur) to most part of the year (Carrapateira).

The main objectives of the present research were to: i) study the spatial and temporal distribution of the physical and chemical parameters in the water column and surface sediment; ii) determine which local factors within these three systems are more relevant to their characterisation; and iii) evaluate the variation of microphytobenthic biomass on both spatial and temporal scales.

MATERIAL AND METHODS

Study area

The streams of Odeceixe (ODX), Aljezur (ALZ) and Carrapateira (CAR) (Fig. 1) drain relatively small watersheds (250, 200 and 110 km², respectively). In spite of that, together with a few other small estuaries, their drainage basins cover most of the SW area of Portugal (Magalhães et al., 1987). The mouths of these estuaries are frequently

obstructed by sand bars, resulting sometimes in small temporary coastal lagoons (Colombo, 1978, Heliotis, 1988). While the estuaries of Odeceixe and Aljezur were connected with the sea throughout the whole sampling period, the small estuary of Carrapateira was a coastal lagoon, remaining closed most of the year and opening only during winter storm events.

All the samples were collected between April 1993 and May 1994. Sampling was performed during low tide, in the intertidal area of ODX (stations 7A and 7V) and ALZ (stations 9A, 9V and 18) and in the subtidal area of CAR (stations 1, 3 and 5). The choice of sampling sites and stations, located at the terminal part of these three different systems, was based on a previous study (Magalhães et al., 1987). Due to the proximity between the two sampling sites in ODX, water parameters were only collected at station 7V.

Sampling and laboratory procedure

Salinity (psu) temperature ($^{\circ}\text{C}$), dissolved oxygen (ppt), and pH were measured *in situ* on the bottom layer, in the vicinity of sediment sampling sites approximately 15 cm above the bottom, using sensors (YSI Model 33, Engineered Systems and Designs-Model 600, Philips W9424). Sediment samples for granulometric purposes were collected with a hand corer (0.02 m², 30 cm deep), and separated through wet sieving in to: gravel (<-1 ϕ fraction), sand (from -1 to 4 ϕ) and mud (> 4 ϕ) using the Wentworth scale and phi notation. For water content, organic matter and photosynthetic pigment analysis, the top 1 cm of sediment was sampled with a corer of 5 cm of diameter, and freeze-dried until analysis. Water content (%H₂O) was obtained as the difference between dry and wet weight of the sediment. Organic matter (OM) was obtained as the percentage of weight loss by ignition (24 h, 450 $^{\circ}\text{C}$) from the dried sediment. Microphytobenthos biomass was determined as chlorophyll *a* concentration (Chl *a*); pigments were extracted with 90% acetone during 24 h in darkness at 4 $^{\circ}\text{C}$, chlorophyll *a* and phaeopigments were determined spectrophotometrically by the method of Lorenzen (1967) and expressed as mg m⁻². Monthly data of precipitation and atmospheric temperature were obtained from the National Meteorological Institute and the General Administration of Natural Resources.

Multivariate analysis

Principal Component Analysis (PCA) was applied in this study to explore the relationships between the sampling sites and the distribution of environmental parameters. The analysis was run using NTSYS-pc package version 1.80, by Rohlf (1993).

RESULTS

Abiotic parameters

Precipitation was the same at all sites: the highest values occurred during October and November (Fig. 2). The water parameters measured at each station are presented in Table 1. Differences within each parameter were related mainly to the season and also with biochemical reactions occurring in the surface sediment. Water temperature showed a seasonal variation related to atmospheric temperature values. Differences in salinity between the three estuaries studied were observed (Table 1). At CAR, salinity values clearly show that the mouth of the estuary remained closed for most of the year.

During the sampling period it was possible to observe a clear variation in grain size distribution at ODX and ALZ (Fig. 3), which reflects the prevailing sediment source, whether predominantly marine, terrestrial (freshwater flow) or both. On the other hand, CAR, with lagoonal characteristics, presented just a small annual variation.

The organic matter content (Table 2) showed a marked variation between stations. At CAR, the southernmost estuary, all stations presented the lowest organic matter averages of the three systems. As expected, within each estuary, a sharp decrease from the head to the mouth was observed, associated with a corresponding gradient of increasing grain size sediment.

Phytopigments

Temporal variation of chlorophyll *a* at the sampling stations are showed in Figure 4, peaks of biomass tending to occur after periods of higher rainfall (see Fig. 2). At ODX both stations displayed the same temporal of biomass pattern. The stations ODX 7A

and ALZ 9A, which presented sand predominance, always showed the highest biomass values. At CAR, all stations were closed to the sea, resulting in a strong influence of aeolian and marine sediment deposition. At this site, a clear biomass fluctuation throughout the months at all stations was observed. Phaeopigments, (representing chlorophyll degradation), present higher values in stations with fine sediments, where the accumulation of inorganic and organic material is stronger (Table 2).

Multivariate analysis

In order to establish site similarity during the whole sampling period, a PCA was performed using all descriptors from ODX, ALZ and CAR (Fig. 5 and 6). The first three axes explain 58.5 % of total variability. A good correlation ($r = 0.892$) was also obtained between the original matrix and the projection matrix. Axes 1 and 2 divide the sites in two groups: stations with predominantly sandy sediment (O7A, A9A, C1, 3 and 5) and stations with high percentage of silt (O7V, A7V and A18). These axes reveal the gradient of riverine/marine sediments. There was a clear opposition between 2 ϕ class of grain size, and organic matter, water percentage and the smallest grain sizes (4 and 5 ϕ class). In axes 1 and 3, salinity is opposed to temperature, reflecting stations with predominance of marine or freshwater influences.

DISCUSSION

The Portuguese coast is a high-energy coast, where the dominant wave directions are SW-W and E, and the most frequent storms are from WNW in the west coast, and from SW in the south coast (Costa, 1994). Its estuaries are subject to frequent tidal intake of marine sand, which may lead to obstruction of the inlet. During spring tides, the marine sand is carried further upstream, towards the head of the estuary. Another relevant aspect is the marked interannual variation of rainfall, common to Mediterranean climatic regions, which has important consequences on river flows (Magalhães et al., 1987).

Temperatures in shallow estuaries as CAR, change faster compared to ODX and ALZ, attaining the maximum average values encountered (see Table 1). As observed by McLusky (1994) the temperature of estuarine water is influenced by periodic inputs of water (marine or freshwater), as well as by seasonal and diurnal changes in air temperature. According to Day (1981), rivers are usually cooler than the sea in winter

(due to continental drainage of colder water) and warmer in summer (due to diurnal heating of the water mass).

Salinity variations in the three sites could be explained by the rainfall regime, tidal amplitude and distance from the sea (see Fig. 2). The biggest recorded range in salinity was observed in ALZ 18, which is the site located further upstream in this estuary. CAR estuary showed the lowest average salinity values, as a consequence of being closed to the sea throughout most of the year, except in November. However, the small distance from the beach and the relatively low elevation of the sand bar, allowed for periodic entrances of saltwater and marine sediments during high water spring tides. This marine water mixes with estuarine water, generating abrupt increases in salinity.

In ODX and ALZ, except in the station ALZ 18, seawater was the dominant water mass, transporting sediment particles of marine origin (personal observation), as evidenced by the strong presence of the 2Φ fraction. This size class was also characterised as marine origin by Buller & McManus (1979) in Scotland. Similar situations can also be found in Portugal, at the mouth of the Sado (Andrade & Cancela da Fonseca, 1982) and the Mira (Andrade, 1986, Andrade et al., 1991) estuaries. This material was carried into the estuary either as suspended sediment flux or as bedload, in the bottom inflowing currents that characterise salt wedges. Nevertheless, flocculation, adsorption and deposition processes are bound to take place in these estuaries, specially in ODX, as a consequence of the dominance of fine sediments carried from continental runoff. According to several works (Day, 1981, Andrade, 1986, Dyer, 1995,), the nature of the sediment and the geomorphology of the drainage basin have extreme importance in the flocculation process. Differences in fine settled sediment during the sampling period can be related to flood regime, which had an important influence on the transport and deposition processes. During periods of highest river discharge, the freshwater flow tends to flush sediment through and out of these systems.

The organic matter sources in these estuaries are the following: i) fragments of terrestrial and riverine vegetation carried by the river flow ii) detached seaweeds, sea grasses such as *Ruppia cirrhosa* and *Ruppia maritima* in CAR, and marine plankton iii) and autochthonous decomposed animal and plant material. The detected gradient of organic content in the sediments along the estuary in ODX and ALZ was observed previously for other lagoonal systems of the southwest coast of Portugal (Magalhães et

al., 1987, Cancela da Fonseca et al., 1987, Cancela da Fonseca, 1989). Sandy-muds are consistently richer in organic matter than muddy-sands and sands (Table 2), because clay particles tend to bind organic matter in greater quantities (Nichols, 1970, Cancela da Fonseca et al., 1987). Moreover, the effects of runoff are higher during the winter, contributing to the accumulation of allochthonous materials and dead organisms, displaced from other areas of the estuary with different ecological characteristics. The highest values of organic matter were found during the rainy season (when the lowest values of water temperature were recorded), probably due to a low mineralization rate of organic compounds.

Comparison of chlorophyll *a* results, with those reported by other authors for temperate tidal flats, indicates that microphytobenthos biomass in these small estuaries present the same order of values (Colijn & Dijkema, 1981, Davis & McIntire, 1983, Brotas et al., 1995). Higher benthic microalgal biomass is displayed in stations with medium sand as the predominant sediment, in comparison with stations with fine sand and mud (Fig. 4 and Table 2). The opposite situation is frequently found in the literature (Colijn & Dijkema, 1981, Davis & McIntire, 1983, Brotas et al., 1995). However, this outcome may be due to two different reasons. Firstly, chlorophyll *a* was analysed in the first centimetre, and the depth penetration of microphytobenthic cells is higher in sandy sediments (Delgado et al., 1991; Brotas & Serôdio, 1995). Secondly, a negative relationship between percentage of finer sediments and microphytic biomass suggests that in our systems the anthropogenic loadings of fine sediments may reduce biological productivity, as observed by Cahoon et al. (1999) for shallow-water ecosystems.

The chlorophyll *a* in these three systems revealed a distinct seasonal cycle, with an increase during the rainy season. The existence of a seasonal cycle for microphytobenthos communities with a biomass maximum during summer has been reported for northern temperate systems (Colijn & Dijkema, 1981, Sundbäck et al., 2000), but it was absent in milder climates (Varela & Penas, 1985, Brotas et al., 1995). The small scale ecosystems surveyed during this study, present a particular case within temperate coastal zones, as biomass peaks were strongly dependent on the input of terrestrial nutrients (from cattle-breeding and agriculture activities) into the estuarine basin brought by rainfall.

In conclusion, sediment dynamics promoted by tidal currents and runoff during the rainfall period, seem to be the driving forces that influence the abiotic parameters in these systems. The ecology of different organisms, namely the macrofauna present in these areas, could be affected through the dragging and redistribution of juvenile post-settlement (Fidalgo e Costa, 1994).

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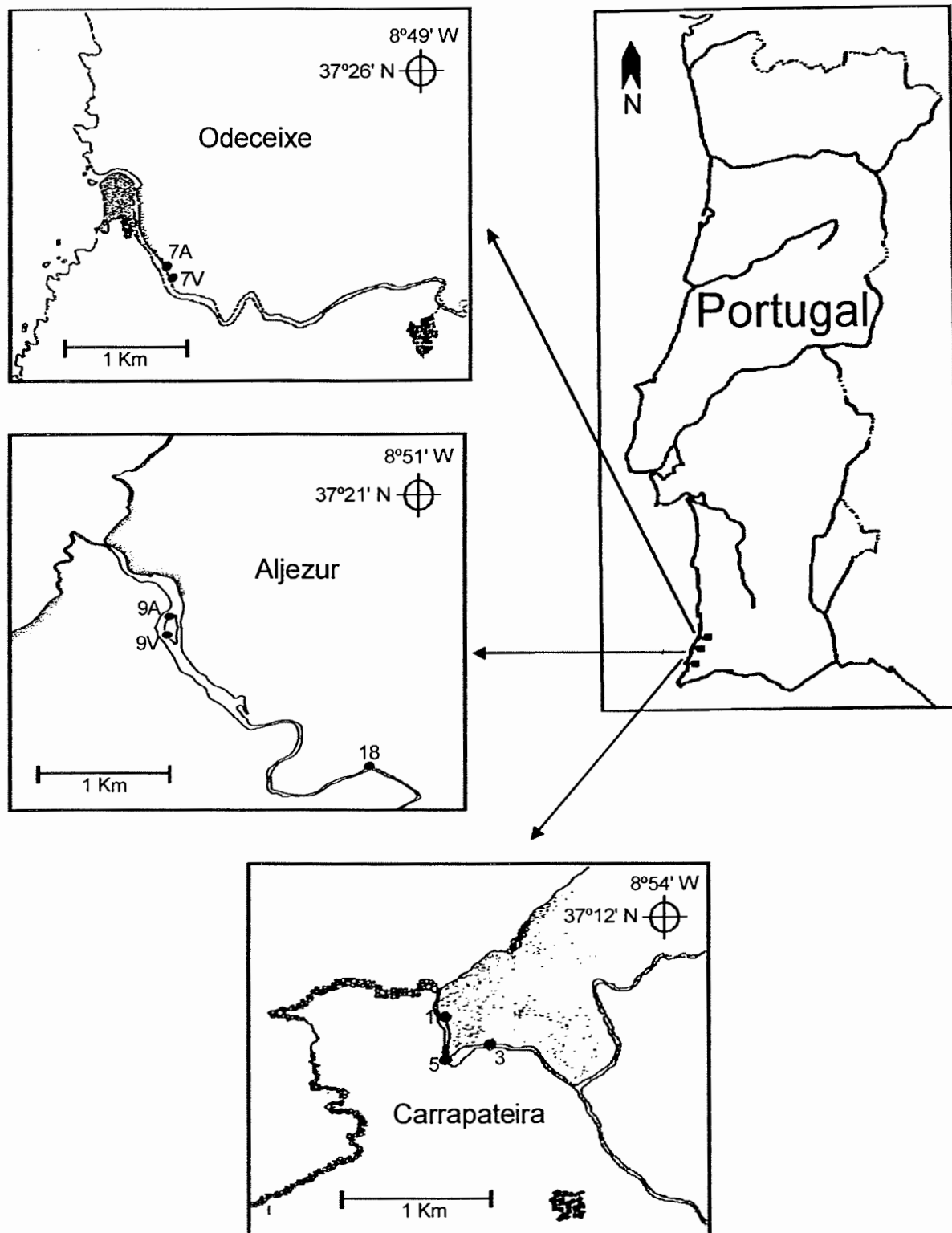


Figure 1 – Sampling sites and stations located on the southwestern coast of Portugal.

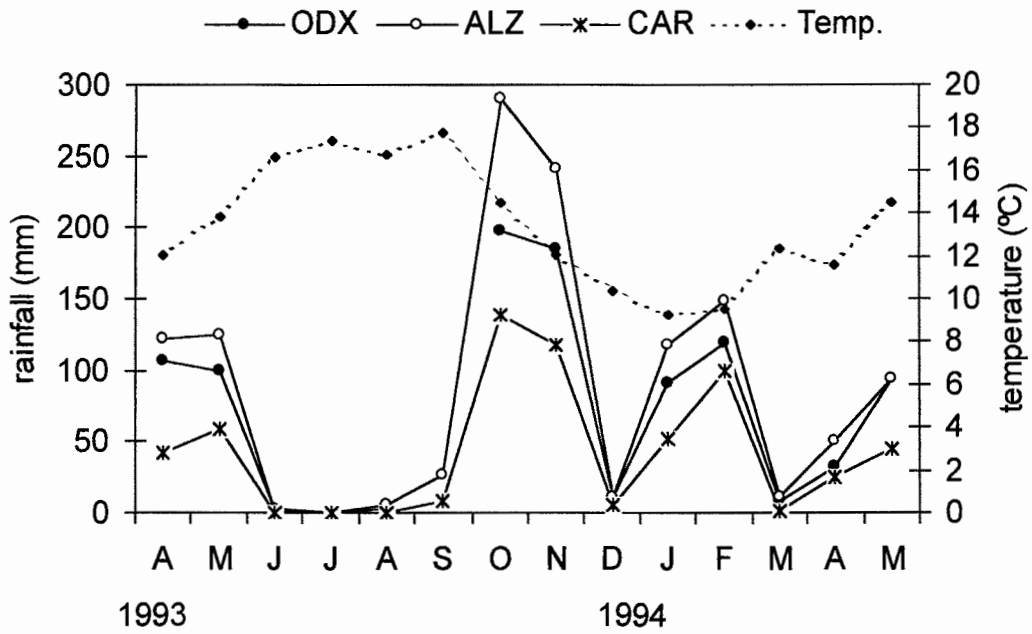


Figure 2 – Rainfall regime (mm) in ODX, ALZ and CAR, and atmospheric temperature (°C) attained for the region during sampling period.

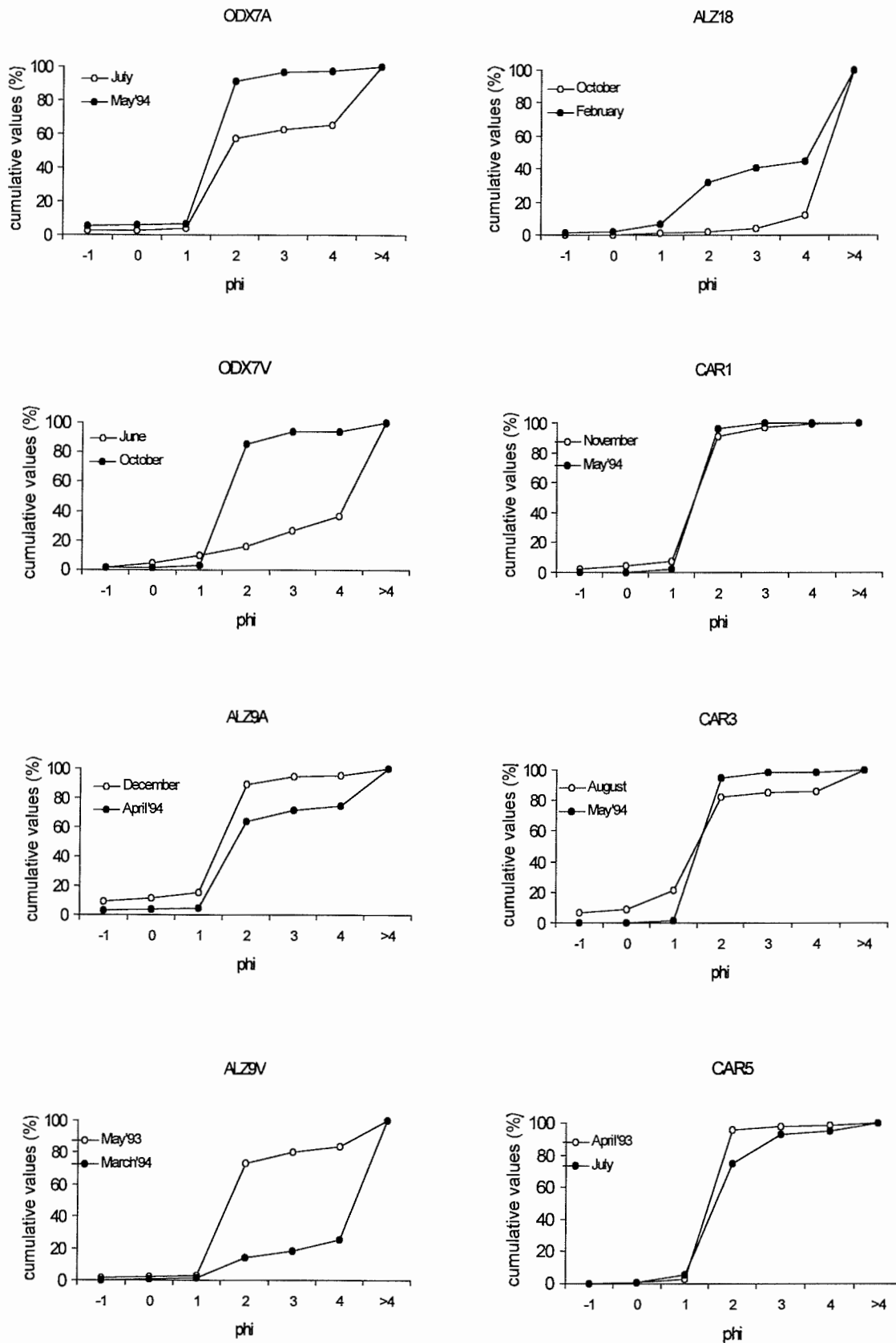


Figure 3 – Maximum variation of granulometric composition expressed as percentage of cumulative values in all sampling stations within ODX, ALZ and CAR.

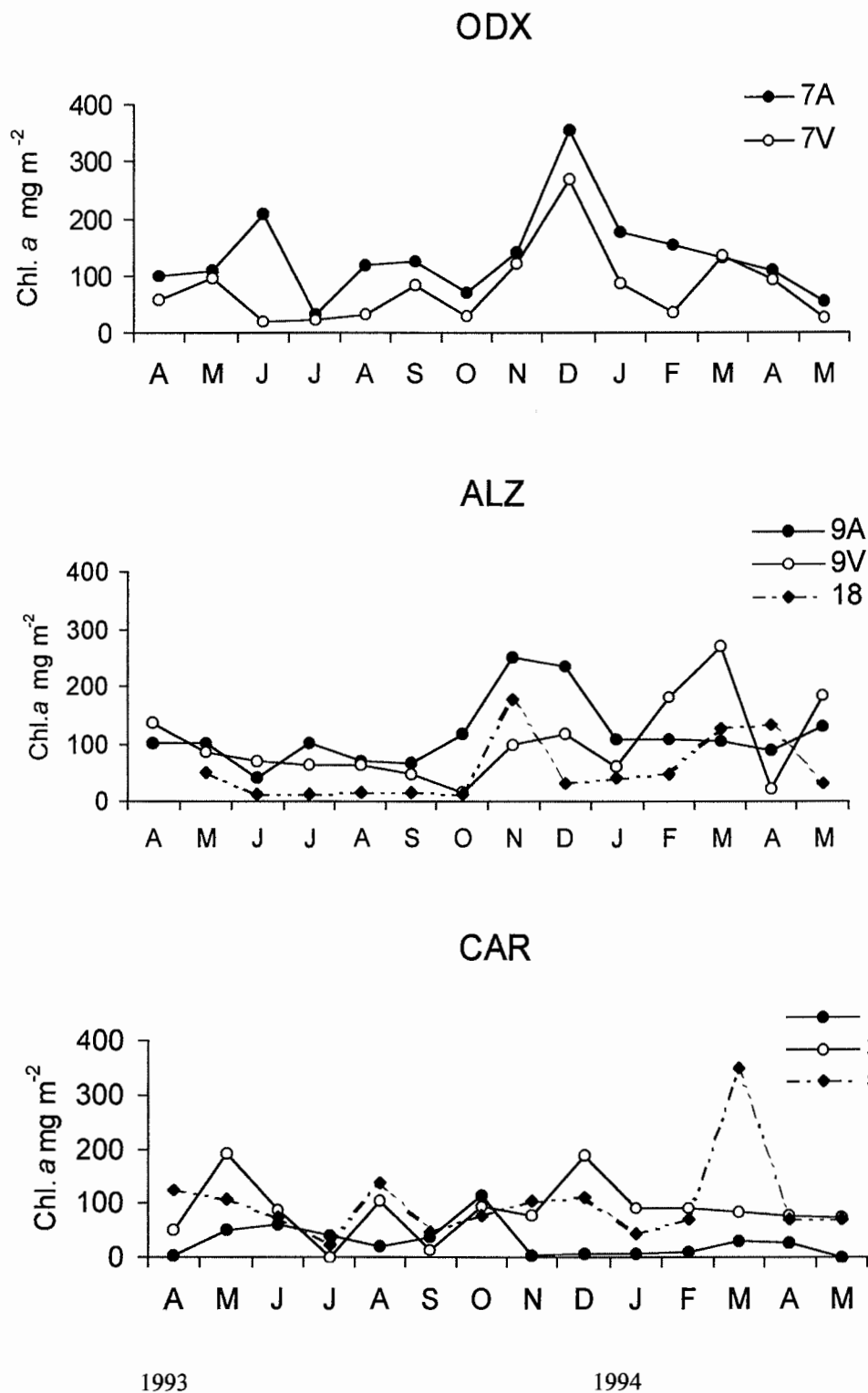


Figure 4 - Temporal variation of chlorophyll a in all sampling stations within ODX, ALZ and CAR.

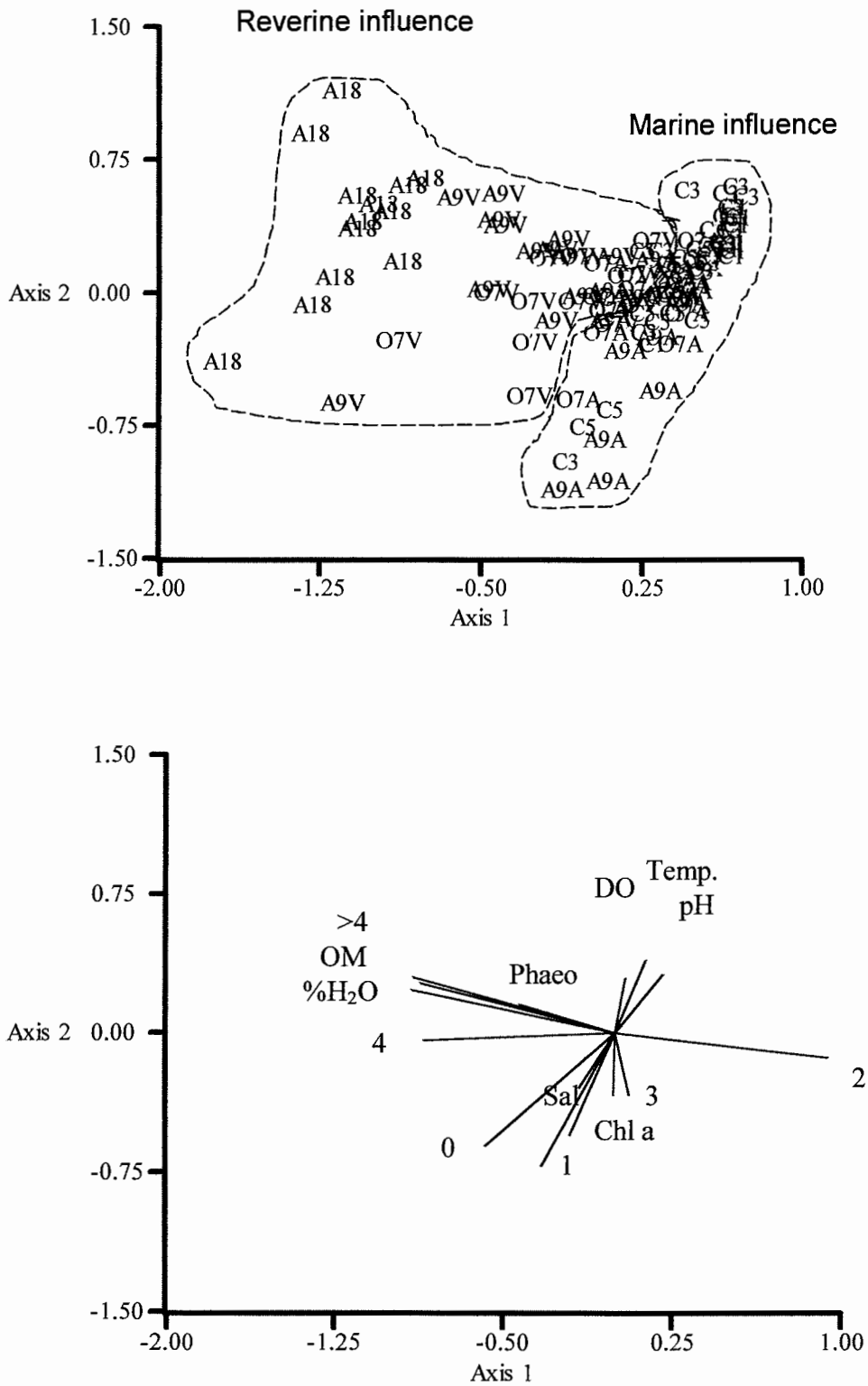


Figure 5 – Projection of water (temp., sal., pH, DO) and sediment (MO, Phaeo., Chl a, % H₂O and granulometry- Phi) parameters related with all different stations within ODX (O), ALZ (A) and CAR (C), in the space defined by axis 1 and axis 2.

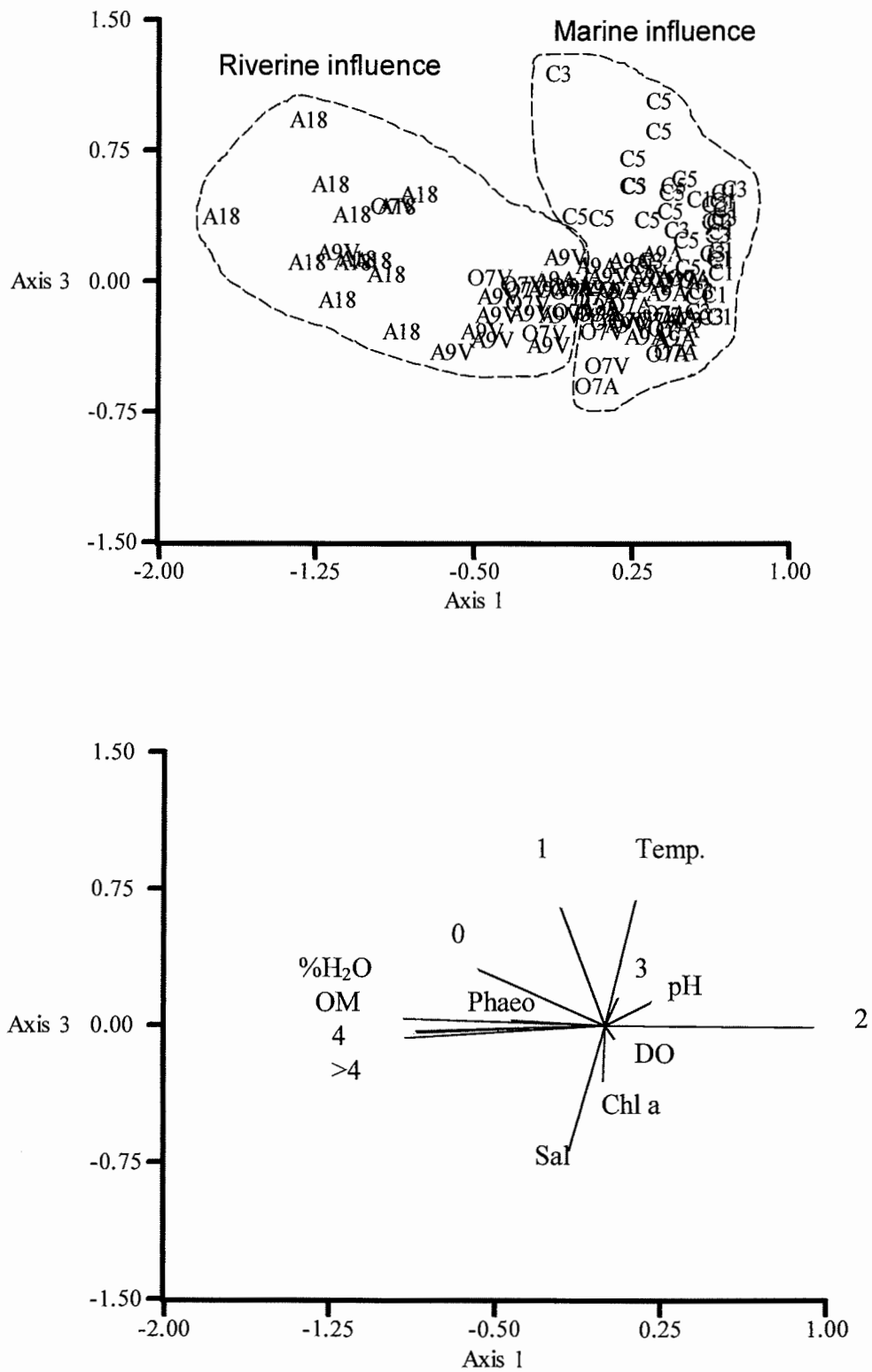


Figure 6 – Projection of water (temp., sal., pH, DO) and sediment (MO, Phaeo., Chl a, % H₂O and granulometry- Phi) parameters related with all different stations within ODX (O), ALZ (A) and CAR (C), in the space defined by axis 1 and axis 3.

	Temp. (°C)			Salinity (psu)			DO (pp)			pH		
	range	average	St. error	range	average	St. error	range	average	St. error	range	average	St. error
ODX7AV	21-12	15.6	± 0.72	35-18	31.8	± 1.23	14.5-34	8.6	± 0.7	8.2-7.2	7.8	± 0.07
ALZ9A	25.5-13	17	± 0.77	36.5-29	33.9	± 0.61	12.2-2.7	8.5	± 0.71	10.9-7.9	8.3	± 0.21
ALZ9V	23.5-13	16.8	± 0.75	36.5-26.5	32.9	± 0.81	14.2-2.7	8.7	± 0.81	10.1-7.7	8.3	± 0.16
ALZ18	27-11	18.9	± 1.34	34-0.5	19	± 3.51	15.5-3.8	9.7	± 1	9.1-7	8	± 0.16
CARI	25-13	18.8	± 1.02	34-5	16.5	± 2.26	10.6-7.5	9.5	± 0.46	8.6-8	8.3	± 0.04
CAR3	27-14	20	± 1.01	28-5.4	19.3	± 1.86	14.5-6.2	10.3	± 0.53	8.6-8.1	8.3	± 0.04
CAR5	27-13	19.8	± 1.11	32-5-5	15.6	± 2.07	11.8-3	10	± 0.58	8.7-7.8	8.3	± 0.06

Table 1 - Range and average values of temperature, salinity, dissolved oxygen and pH with respective standard error.

	Chl. <i>a</i> (mg.m ⁻³)			Phaeo. (mg.m ⁻³)			O.M (%)		
	range	average	St. error	range	average	St. error	range	average	St. error
ODX7A	31.9-356.0	134.6	±20.26	8.6-239.9	71.1	±14.74	0.5-2.8	1.22	±0.15
ODX7V	18.1-267.3	78.9	±17.14	8.8-159.1	64.1	±10.76	1.8-5.8	3.16	±0.31
ALZ9A	40.0-250.7	115.9	±15.01	18.6-94.5	49.8	±5.68	0.3-2.3	1.04	±0.12
ALZ9V	21.0-271.0	101.3	±18.4	20.6-166	95.2	±12.04	1.1-5.8	2.85	±0.36
ALZ18	11.2-177.6	57.7	±14.71	31.1-230.5	110.2	±13.88	4.8-10.8	6.96	±0.42
CAR1	0-112.7	29.1	±7.95	0-48.5	17.8	±4.18	0.2-0.8	0.51	±0.05
CAR3	0.8-191.9	87.6	±13.69	1.8-133.5	36.9	±8.51	0.2-1.8	0.71	±0.10
CAR5	24.2-350.1	100.4	±20.27	16.4-133.4	54.4	±7.59	0.5-1.6	0.91	±0.08

Table 2 - Range and average values of chlorophyll *a*, phaeopigments and organic matter content with respective standard errors.

Capítulo III

Bio-ecologia de *Nereis diversicolor* na costa sudoeste de Portugal

Artigo 2

*THE OOGENIC CYCLE OF *Nereis diversicolor* ON ESTUARINE AND LAGOON ENVIRONMENTS IN THE SOUTHWEST COAST OF PORTUGAL.*

Pedro Fidalgo e Costa

(Artigo submetido à revista International Journal of Invertebrate Reproduction and Development)

Artigo 3

*LIFE CYCLE, GROWTH AND PRODUCTION OF THE POLYCHAETE *Nereis diversicolor* O.F. MÜLLER IN THREE LAGOONAL ESTUARINE SYSTEMS OF THE SOUTHWESTERN PORTUGUESE COAST (ODECEIXE, ALJEZUR AND CARRAPATEIRA).*

Fidalgo e Costa, P., Sardá, R. & Cancela da Fonseca, L.

Écologie, 29 (4), 523-533 (1998).

Artigo 4

*FEEDING ECOLOGY OF THE POLYCHAETA *Nereis diversicolor* ON ESTUARINE AND LAGOON ENVIRONMENTS IN THE SOUTHWEST COAST OF PORTUGAL.*

Fidalgo e Costa, P., Oliveira, R.F. & Cancela da Fonseca, L.

(Artigo submetido à revista Marine Ecology – P.S.Z.N.)

THE OOGENIC CYCLE OF *Nereis diversicolor* (ANNELIDA: POLYCHAETA) IN ESTUARINE AND LAGOON ENVIRONMENTS IN THE SOUTHWEST COAST OF PORTUGAL

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Keywords: Polychaeta, *Nereis diversicolor*, oogenic cycle.

SUMMARY

This study presents data on the gametogenesis and sex ratio of *Nereis diversicolor* from April 1993 to May 1994 at the mouth of three small estuaries of the southwestern coast of Portugal: Odeceixe (ODX), Aljezur (ALZ) and Carrapateira (CAR). The development of oocytes in each individual was correlated with the measure of L₃ (length of prostomium, plus peristomium and 1st chaetiger segment) and the reproductive period was defined through the average diameter of oocytes in each population and larval recruitment peaks. The results obtained showed that the populations of *N. diversicolor* studied presented, during the entire period of study, mature females as well as a constant presence of juveniles. The most important recruitment period was in September 1993, although in May 1994 a second peak of smaller size occurred. The average (\pm SD) oocyte diameter and average (\pm SD) L₃ length in reproductive individuals were respectively 136 \pm 39 μ m and 2.64 \pm 0.45mm for ODX; 140 \pm 37 μ m and 2.36 \pm 0.43 μ m for ALZ; and 130 \pm 49 μ m and 2.08 \pm 0.38mm 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 a premature maturation, a reduced size and a strong female-biased sex ratio was observed.

INTRODUCTION

The study of aspects of reproductive biology, and more specifically of gametogenesis in populations of *Nereis diversicolor* O. F. Müller, started with the detailed work of Dales (1950) at the Thames estuary. More recent papers such as those of Chambers

and Milne (1975), Heip and Herman (1979), Mettam (1979), Olive and Garwood (1981), Möller (1985), Ozoh and Jones (1990), and Zubillaga and Sallinas (1997) focused on aspects of population structure, gametogenic cycle and influence of environmental factors and pollution on oocyte development of these polychaeta.

The life cycle of *N. diversicolor* is particularly interesting, considering that during the 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 the ovaries (Olive, 1983, Grémare and Olive, 1986). As a result of this great investment in gametogenesis, a reduction of somatic body mass occurs and growth ceases (Mettam, 1979; Olive, 1983; Fischer, 1984). This fact is not surprising as this species reproduces only once, dying a few days after releasing the gametes (Dales, 1950; Olive and Garwood, 1981; Olive et al., 1981; Mettam et al., 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. The 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. The method most frequently used in oocyte growth analysis is 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*: Ria de Aveiro - Abrantes et al. (1999); Mondego estuary - Gonçalves and Ribeiro (unpublished); Stº André lagoon - Cruz (unpublished); Mira estuary - Lopes da Silva (unpublished); southwest coast – (Fidalgo e Costa and Cancela da Fonseca, 1998 and Fidalgo e Costa et al., 1998). Specifically concerning oocyte development, Gonçalves and Ribeiro (unpublished) and Abrantes et al. (1999) have determined the oogenic cycle for this species, but only to the north part of this country.

In the present work we studied the oogenic cycle and sex ratio of *N. diversicolor* in three small estuaries in the southwest coast of Portugal. The development of oocytes in each individual was correlated with the measure of L₃ (length of prostomium, plus peristomium and 1st chaetiger segment) and the reproductive period was also determined through the analysis of larval recruitment peaks.

MATERIAL 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 were established at the terminal part of the Odeceixe (ODX), Aljezur (ALZ) and Carrapateira (CAR) estuaries which form temporary lagoons (Fig. 1). These water basins extend through about 250, 200 and 110 km² respectively, and partially drain the western part of the southwest Portugal. This brackish environments, although representing a great geomorphological diversity are, from an ecological point of view, lagoon-estuarine systems (Day et al., 1987).

Environmental conditions

The water temperature during the sampling period in all sites followed closely the atmospheric temperature values (Fidalgo e Costa et al., 1998), oscillated between 12-21°C (ODX), 12.7-24.7°C (ALZ) and 13.3-26.3°C (CAR). The average were respectively 15.6, 17.5 and 19.5°C.

Changes in salinity at several parts per thousand were obtained in this habitats clearly reflecting the distinct water entries (Fidalgo e Costa et al., 1998). The obtained annual average values were 31.4 ‰ (ODX), 29 ‰ (ALZ) and 15 ‰ (CAR).

Sampling techniques

All samples were collected between April 1993 and May 1994 at three sites: at low tide in the sublittoral fringe of ODX and ALZ, and in the upper sublittoral area of CAR within a small lagoon formed by the partial closing of the river mouth by a sand

barrier. 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 and sieved through a 0.2 mm square mesh.

Size Determination

All specimens, except the small ones (approximately less than 10 mg dry weight or L₃=1.5mm) were measured, based on the value of L₃ (length of prostomium, plus peristomium and 1st 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; $p < 0.001$), being a valuable conversion factor (Fidalgo e Costa et al., 1998) and 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. For each female a minimum of 10 oocytes (for small specimens), and a maximum of 36 oocytes (for specimens in late stages of maturation), were measured. This procedure follows the ones used by other authors in similar studies (Mettam, 1979; Olive and Garwood, 1981; Mettam et al., 1982; Möller, 1985; Heffernan and Keegan, 1988; Zubillaga and Sallinas, 1997, Abrantes, et al., 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

Oocyte cycle

Nereis diversicolor presented, at all studied sites, females with large oocytes during the entire study period. From a total of 950 presumed mature specimens captured, measured, and opened, only 270 presented some sexual products in the coelomic fluid. From these, 78 were obtained from ODX, 115 from ALZ and 77 from CAR.

At ODX the beginning of oogenesis occur in May, although the earliest stages have been missed, a gradual but significant increase in oocyte diameters were observed between July and September (Fig. 2). Spawning occurred at the end of August and early September, which is stressed by a sharp larval recruitment observed in September (see Fig. 5). In the 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). In May 1994 there was once again an increase of maturing females and recruits, indicating a second peak of reproduction although weaker than in September.

In ALZ the beginning of oocyte development and maturation occurred in June with a peak of mature individuals in September (Fig. 3). In August there was a clear decrease of the mature population, as some females begin to spawn. This was also correlated to a sharp larval increase in September (see Fig. 5). A progressive but distinct increase in oocyte diameter from June to September was recorded ($106 \pm 25 \mu\text{m}$ in June and $171 \pm 31.5 \mu\text{m}$ in September). As breeding progressed oocyte sizes became more uniform (Fig. 3).

In CAR, the distribution of oocyte carrying females throughout the sampling period was very different from the other two sites (Fig. 4). At CAR mature females were mainly found between December and April. One female captured in December presented the highest oocyte diameter ever observed in our samples ($221 \pm 10 \mu\text{m}$). However, the larval recruitment peak was also observed in September with a less intense peak than at ODX and ALZ (Fig. 5). There was also a gradual increase in average oocyte diameter ($107 \pm 51 \mu\text{m}$ in January and $160 \pm 35 \mu\text{m}$ in April).

Size frequency analysis

The investment in reproduction was clearly shown by the size (L_3) of the observed females in ODX, which was basically the same during July and September, the pre-spawning period (Fig. 2). While oocyte growth showed a marked increase during this period. This pattern was also found in ALZ and CAR (see Fig. 3 and 4). In these months the reproductive females had an average L_3 length of 2.55 ± 0.38 mm (ODX), 2.43 ± 0.45 mm (ALZ) and 2.08 ± 0.38 mm (CAR). These reproductive groups comprised individuals 18-24 months old in ODX and ALZ, and 12-18 months old in CAR (see Fidalgo e Costa et al., 1998).

Due to continuous recruitment and to the constant capture of oocyte carrying 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 during the maturation process, the coelomic oocytes become heterogeneous in size. At the end of development, the size range is again reduced just before spawning (Fig. 6). These results are similar to those found by other authors (e.g. Olive and Garwood, 1981; Mettam, et al., 1982; Sola, 1994; Abrantes et al., 1999).

Sex ratio

The sex ratio in the three studied sites was always favourable to females (Table 1). The number of individuals for which the sex could be clearly determined was relatively low caused by high presence of immature juveniles. Due to this characteristic the sex ratio variation throughout the year was not analysed in detail.

DISCUSSION

The breeding season of *Nereis 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) for 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 was 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; Zubillaga and Sallinas, 1997), and so showing a similar dynamics as the one presented in the present work. From the north of Portugal, Abrantes et al. (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. Distinct life span and overlapping of cohorts in populations of *N. diversicolor* seem to be an important biological aspect, which help in the prevention of genetic isolation between groups of different years (Olive and Garwood, 1981; Olive et al., 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.

In ODX, *N. diversicolor* showed a reproductive dynamics similar to that found by Olive and Garwood (1981) from North East England. The gametogenesis did not begin during the first year of life and, as mentioned earlier, the reproductive group comprised older individuals. In ODX, there was an increase of the average L_3 in all individuals responsible for the second recruitment (May 1994), in comparison with those of the first one (September 1993). This means that small part of the females thought to be involved in the first recruitment did not spawn, and will constitute part of the individuals which participate in the second one. This could be due to several causes: i) these females have missed their reproductive period probably due to a scarce food supply, or to environmental changes (temperature, salinity, etc.), or a failure in finding fertile males, because an unfavourable sex ratio could result in a reproductive failure. In the next breeding period, these females in which a reabsorption of oocyte cells has occurred develop again oocytes and reproduce; ii) a part of the females of the 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 (see Fig. 5). The oocyte dynamics of this

site was similar to that of ODX, although it starts and ends one month earlier (see Figure 3). This difference could be due to a mean water temperature + 2°C higher than in ODX.

In the southern site (CAR) there was a reduced density of adults and larvae (see also Fidalgo e Costa et al., 1998). In this site where the temperature was in average 19.5 °C, this species matures very quickly and did not attain large dimensions (when compared to the other sites) due to its short life time. A similar life span was observed at the Thames estuary (Dales, 1950) and at the Norsminde Fiord (Kristensen, 1984), however with lower temperature values. Our observations on feeding ecology of *N. diversicolor* show that this species has been characterized as an opportunist and a generalist species which adapts its foraging behaviour to biotic and abiotic variations. This eclectic feeding behaviour influence its gamete production and life span, and seems to be 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 Blyth (Olive and Garwood, 1981) and of Severn (Mettam, et al., 1982) and also in the Bidasoa estuary in Spain (Sola, 1994). In Portugal, more specifically at the Ria de Aveiro (Abrantes et al., 1999) and the Mondego estuary (Gonçalves and Ribeiro, unpublished), 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 relation with temperature will bring on synchronization of oocyte growth at a population level during the pre-spawning phase (Olive, 1980).

Our study pointed out that the average L_3 decreases in the southern and the warmest site (CAR), where temperature was 19.5°C. Under this feature, the individuals will attain maturity earlier and consequently reach reduced size ($L_3=2.1$ mm) than in 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 CAR *N. diversicolor* presents a similar development to that observed by Zubillaga and Sallinas (1997) in polluted areas. In both cases, this worm shows an adaptation typical of opportunist species, reaching maturity as rapidly as possible. Since pollution does not compromise the quality of the waters at CAR, there is here another reason for such adaptation. The rapid and premature oogenesis, observed at this site, could then be attributed to: i) high temperatures caused by the low depth of the water column (\pm 50 cm); ii) a different feeding behaviour adapted to the lagoonal conditions and especially to the constant submersion (personal observation).

The 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 this study we analysed the stage of maturation of individuals throughout the year. Our results are in agreement with most studies where the predominance of females is the rule (e.g. Dales, 1950; Olive and Garwood, 1981; Mettam et al., 1982).

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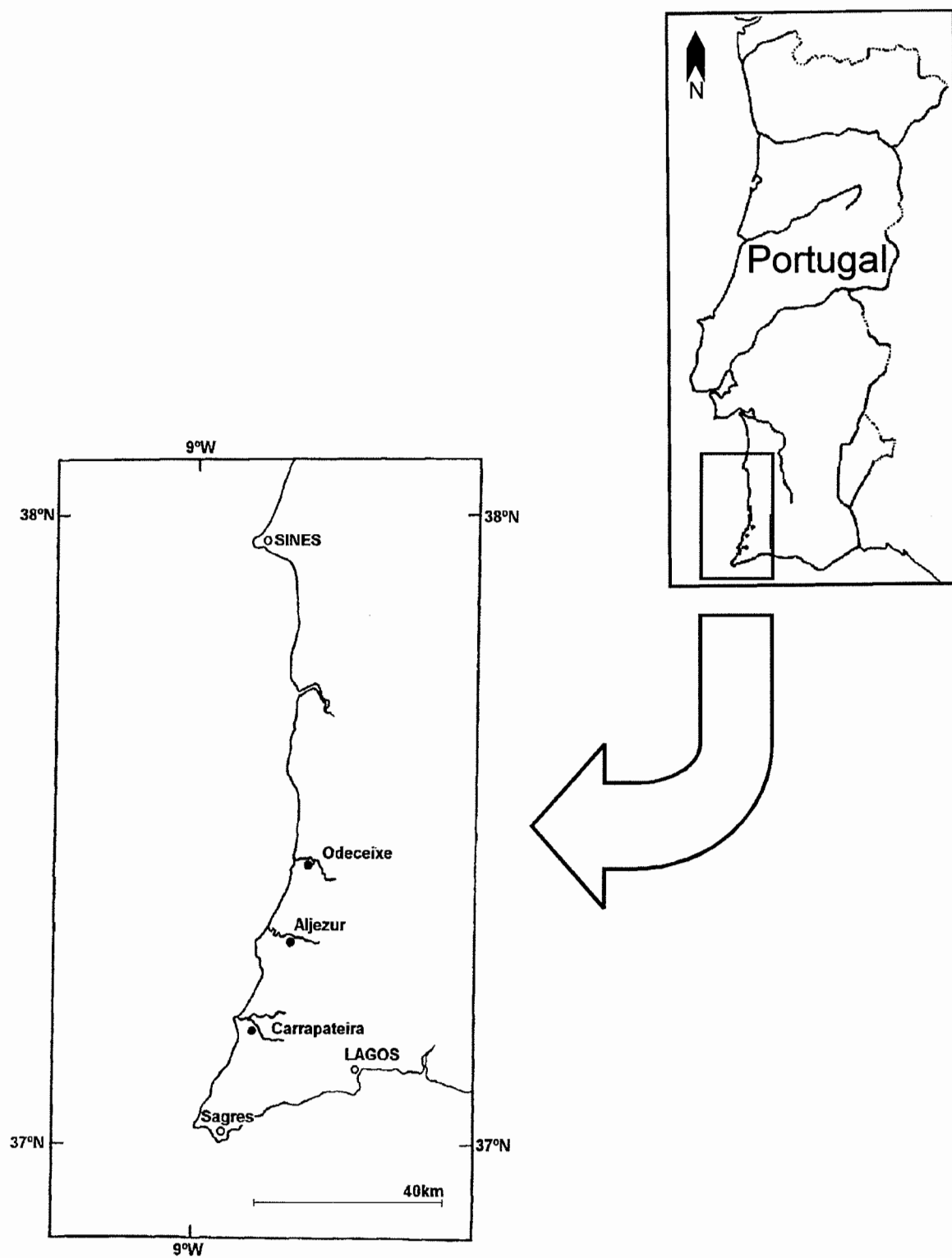


Figure 1 – Sampling locations on the SW coast of Portugal.

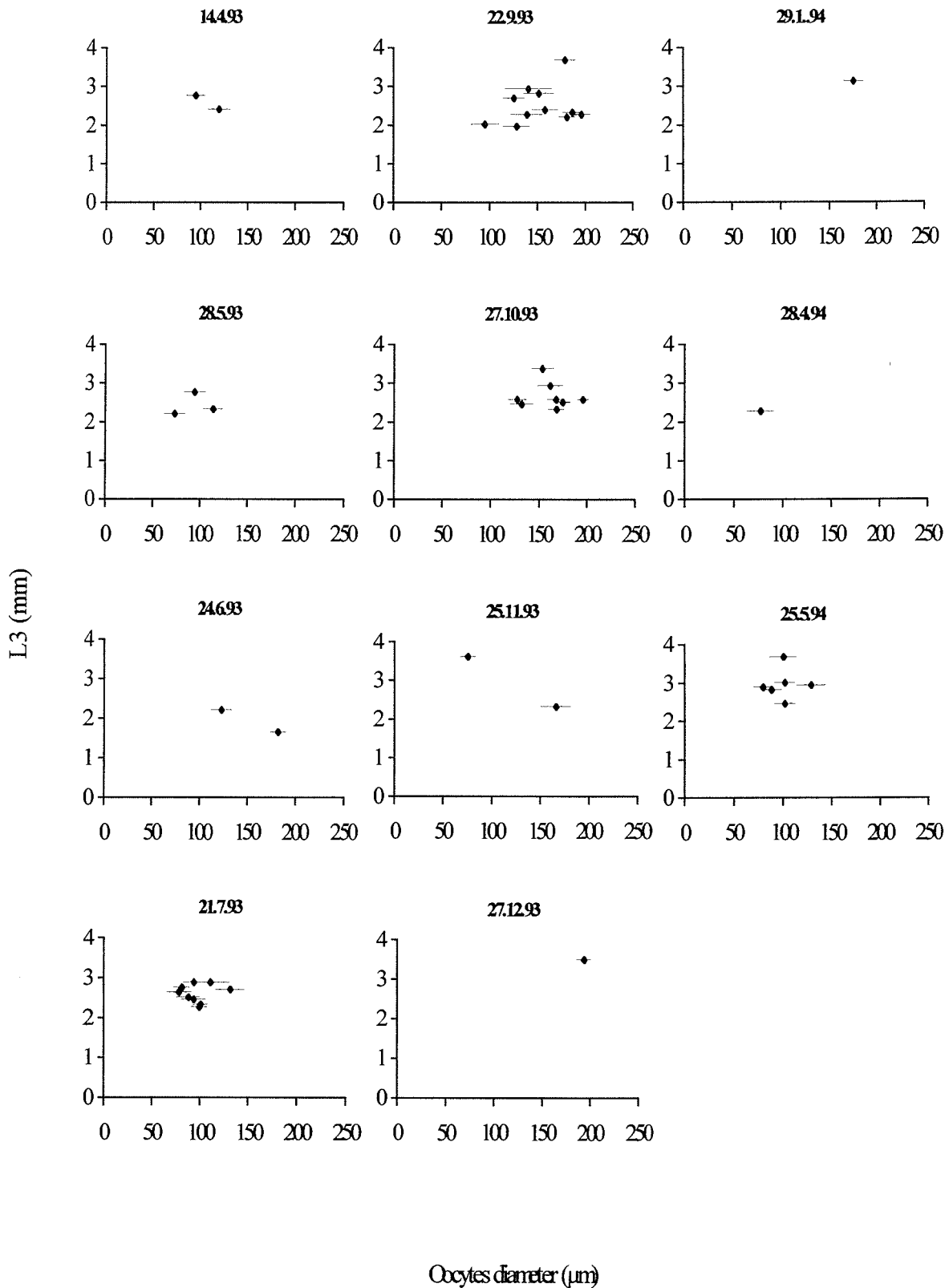


Figure 2 – Relation between L₃ length (mm) and average diameter (µm) with standard deviation of oocytes in each individual of *N. diversicolor* sampled monthly in Odeceixe (ODX).

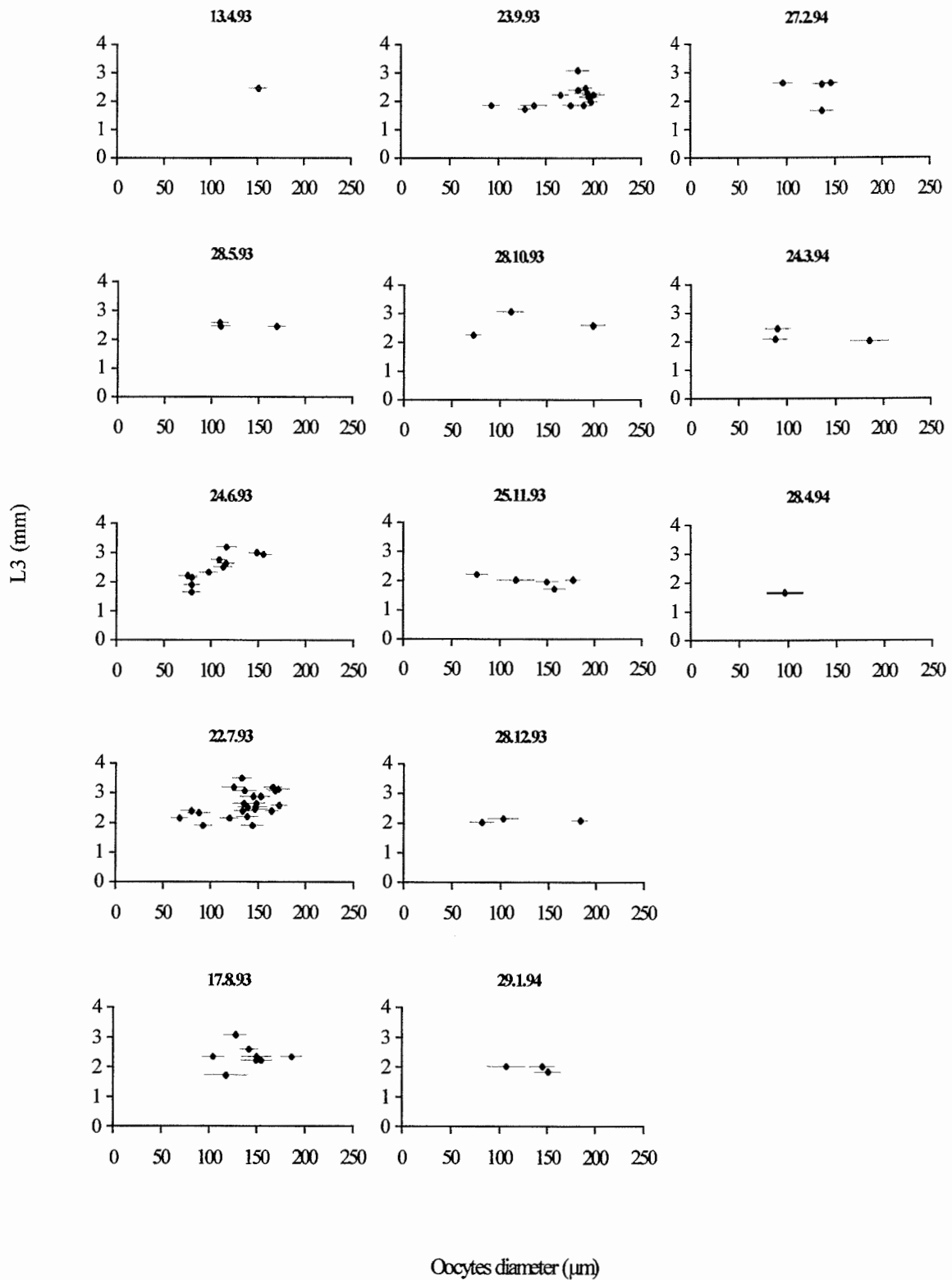


Figure 3 – Relation between L₃ length (mm) and average diameter (μm) with standard deviation of oocytes in each individual of *N. diversicolor* sampled monthly in Aljezur (ALZ).

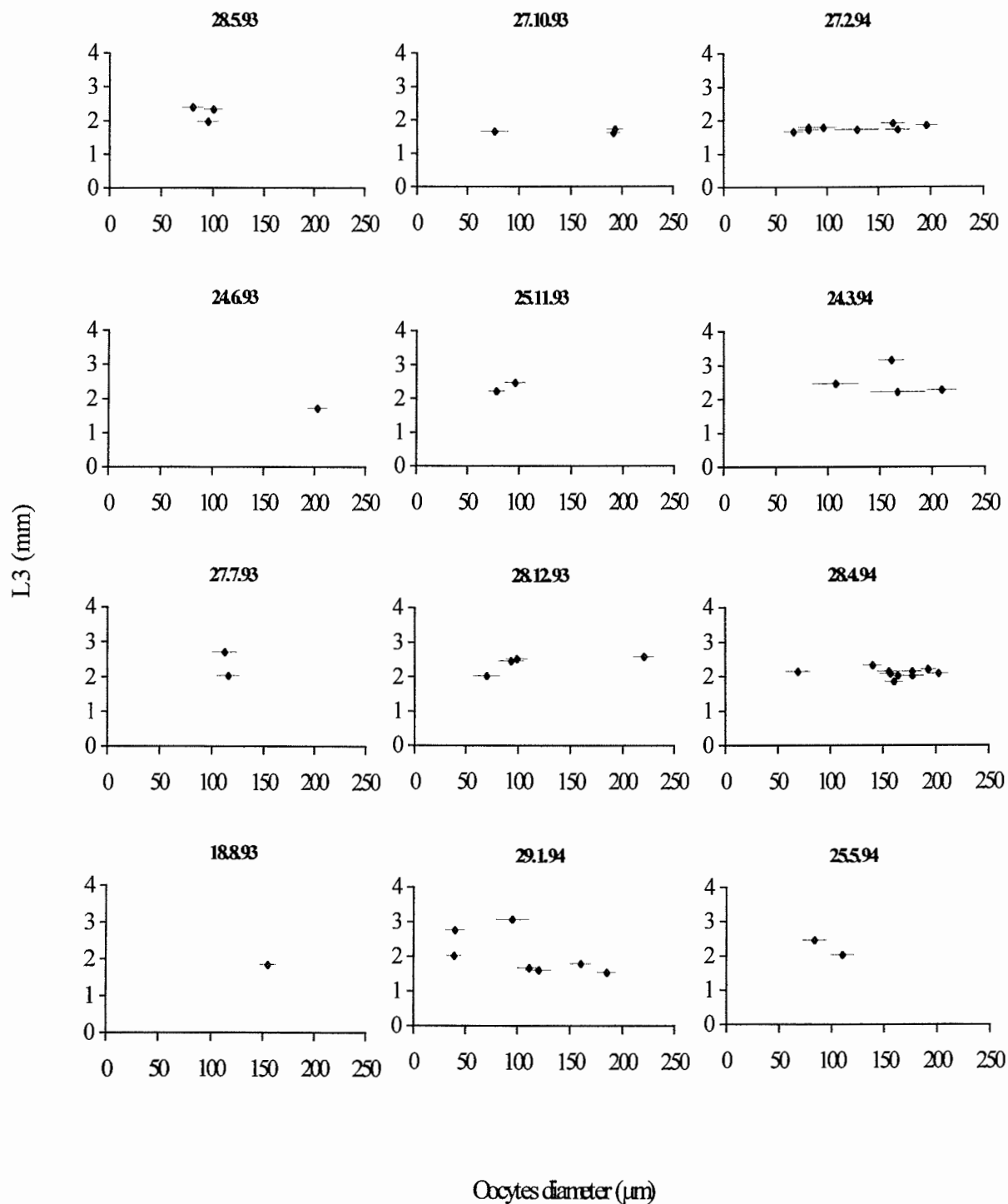


Figure 4 – Relation between L₃ length (mm) and average diameter (µm) with standard deviation of oocytes in each individual of *N. diversicolor* sampled monthly in Carrapateira (CAR).

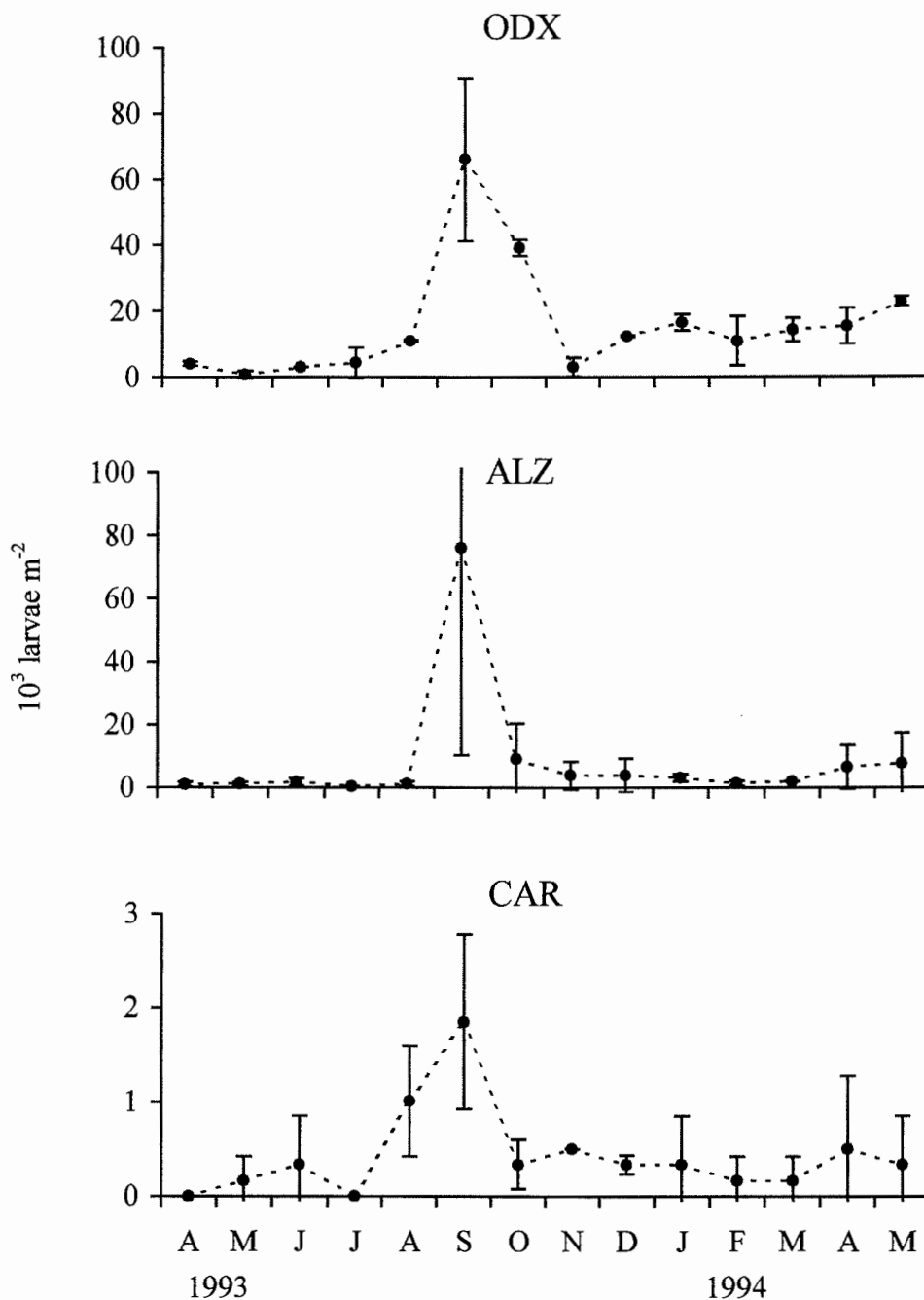


Figure 5 – Density of larvae in Odeceixe (ODX), Aljezur (ALZ) and Carrapateira (CAR) throughout the sampling period.

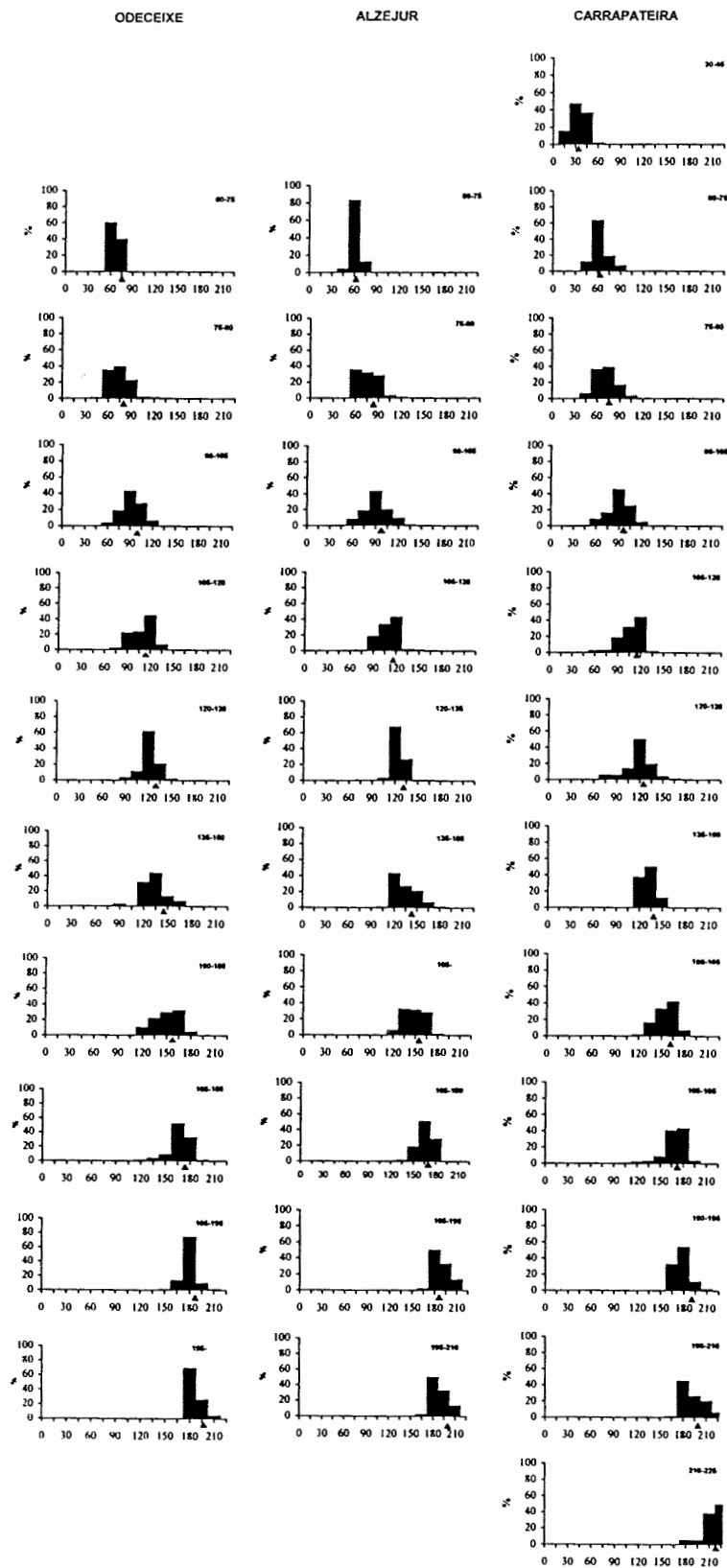


Figure 6 – Oocyte development sequence of *N. diversicolor* in Odeceixe (ODX), Aljezur (ALZ) and Carrapateira (CAR). Each histogram represents frequencies (%) and diameters of oocytes (μm), grouped into 15 μm classes by mean oocyte diameter (upper right on each graph). The arrow (\blacktriangle) presented on axis x indicates the mid-point of each class.

Table 1 - Number of males and females of *N. diversicolor* present in the three systems studied 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	

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**LIFE CYCLE, GROWTH AND PRODUCTION
OF THE POLYCHAETE *NEREIS DIVERSICOLOR* O. F. MÜLLER
IN THREE LAGOONAL ESTUARINE SYSTEMS OF THE
SOUTHWESTERN PORTUGUESE COAST
(ODECEIXE, ALJEZUR AND CARRAPATEIRA)***

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SUMMARY

(original scientific paper)

*LIFE CYCLE, GROWTH AND PRODUCTION OF THE POLYCHAETE *Nereis diversicolor* O. F. MÜLLER IN THREE LAGOONAL ESTUARINE SYSTEMS OF THE SOUTHWESTERN PORTUGUESE COAST (ODECEIXE, ALJEZUR AND CARRAPATEIRA).*

*During a period of 14 months (April/1993 to May/1994), monthly samples of *Nereis diversicolor* (adult and larvae) were taken in three coastal sites of the Portuguese southwest coast (Odeceixe, Aljezur and Carrapateira streams). The average of measured environmental parameters (temperature (°C), salinity (S‰) and dissolved oxygen (O.D.)) obtained for each site was: 15.6°C, 31.7‰ and 8.6ppm for Odeceixe, 17.5°C, 29‰ and 8.8ppm for Aljezur, 19.5°C, 15‰ and 9.9ppm for Carrapateira. Densities of *N. diversicolor* obtained were: 15927, 8538 and 433 ind. m⁻² for adult individuals and 1126, 957 and 337 ind. m⁻² for the larva. Separation by cohorts using the BHATTACHARYA method allowed determination of the specific growth rate (d⁻¹), the linear growth rate (mm/day) and the production by cohort. The population of Odeceixe presented an annual biomass of 85.65 g m⁻², an annual production of 12.83 g m⁻² and a P/B index of 1.95. In Aljezur the annual biomass was 99.96 g m⁻², the annual production 23.68 g m⁻² and the P/B index 3.08. Carrapateira presented an annual biomass of 40.14 g m⁻², an annual production of 12.31 g m⁻² and a P/B index of 4.0.*

KEY WORDS: Life-cycles - Production - Growth - Polychaeta - Lagoonal estuarine systems - Benthos.

RÉSUMÉ

(travail original)

*Pendant une période d'environ 14 mois (avril/1993 jusqu'à mai/1994), nous avons effectué des échantillonnages mensuels de *Nereis diversicolor* (des adultes et des larves) dans trois stations côtières localisées sur la côte sud-ouest du Portugal (Odeceixe, Aljezur et Carrapateira). Les moyennes des paramètres environnementaux "température (°C)", "salinité (S ‰)" et "oxygène dissous (O.D.)", obtenues dans chaque station côtière étaient : 15,6 °C, 15 ‰ et 9,9ppm pour Odeceixe, 17,5 °C, 29 ‰ et 8,8 ppm pour Aljezur, 19,5 °C, 15 ‰ et 9,9 ppm pour Carrapateira. Les densités obtenues ont été égales à 1126, 957 et 337 ind. m⁻² pour les individus adultes, 15927, 8538 et 433 ind.m⁻² pour les larves. La répartition par cohortes en utilisant la méthode de Bhattacharya nous a permis de déterminer le taux de croissance spécifique (d⁻¹), le taux de croissance linéaire (mm/jour), ainsi que la production par cohorte. Odeceixe présente une biomasse annuelle de 85,65 g m⁻², une production annuelle de 12,83 g m⁻² et un index P/B de 1,95. Aljezur présente une biomasse annuelle de 99,96 g m⁻², une production annuelle de 23,68 g m⁻² et un index P/B de 3,08. Carrapateira présente une biomasse annuelle de 40,14 g m⁻², une production annuelle de 12,31 g m⁻² et un index P/B de 4,0.*

MOTS CLÉS : Cycle de vie - Production - Croissance - Polychètes - Systèmes d'estuaires lagunaires - Benthos.

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INTRODUCTION

Nereis diversicolor (O.F. Müller) has been studied under different ecological aspects. It has high physiological tolerance to extreme variation of many environmental parameters such as temperature, salinity, and dissolved oxygen (DALES, 1950; SMITH, 1956, 1977; BARTELS-HARDEGE & ZEECK, 1990). This species is commonly present in estuaries and coastal lagoons (JENSEN, 1988; OZOH & JONES, 1990), dominating in the sandy and muddy sediments of brackish environments, where it plays an important part on the trophic estuarine chain, as predator of other benthic organisms (RÖNN *et al.*, 1988), and as prey to fish and birds (CHAMBERS & MILNE, 1975; HEIP & HERMAN, 1979; SANTOS, 1979; TRIPLET, 1989; ZWARTS & ESSELINK, 1989).

Nereis diversicolor has a wide geographic distribution and can be found as far south as Morocco (GILLET, 1993) and as far north as Scandinavia, in the cold and brackish Baltic Sea as well as in the hot and hypersaline lagoons of the Black Sea (METTAM, 1979). In the Caspian Sea the presence of this species has been attributed to human transport (SMITH, 1963).

There is a large number of papers dealing with the life cycle and production estimates of *N. diversicolor* all over its area of distribution (DALES, 1950, 1951; SMITH, 1963; CLAY, 1967; CHAMBERS & MILNE, 1975; HEIP & HERMAN, 1979; OLIVE & GARWOOD, 1981; METTAM *et al.*, 1982; KIRSTENSEN, 1984; ESSINK *et al.*, 1985; MÖLLER, 1985; HUMPHREYS, 1985; GILLET, 1990; NITHART, 1998).

On the Iberian Peninsula, the biology and ecology of *N. diversicolor* populations have been extensively studied i) along the coast of Portugal: the estuary of Mondego (GONÇALVES & RIBEIRO, 1987); the lagoon of Stº André (CRUZ, 1989); the estuary of Mira (LOPES DA SILVA, 1993); the swamp of Aveiro (ABRANTES, 1995); streams of Odeceixe, Aljezur, Carrapateira (FIDALGO E COSTA & CANCELA DA FONSECA, 1995), and ii) along the Spanish coast: the estuary of Bidasoa (SOLA & IBAÑEZ, 1990); the Vasc coast (GARCIA-ARBERAS & RALLO, 1993); the lagoon of Stª Margarita and the bay of Rosas, (DUESO & SARDA, 1994); Bay of Cadiz (ARIAS & DRAKE, 1995).

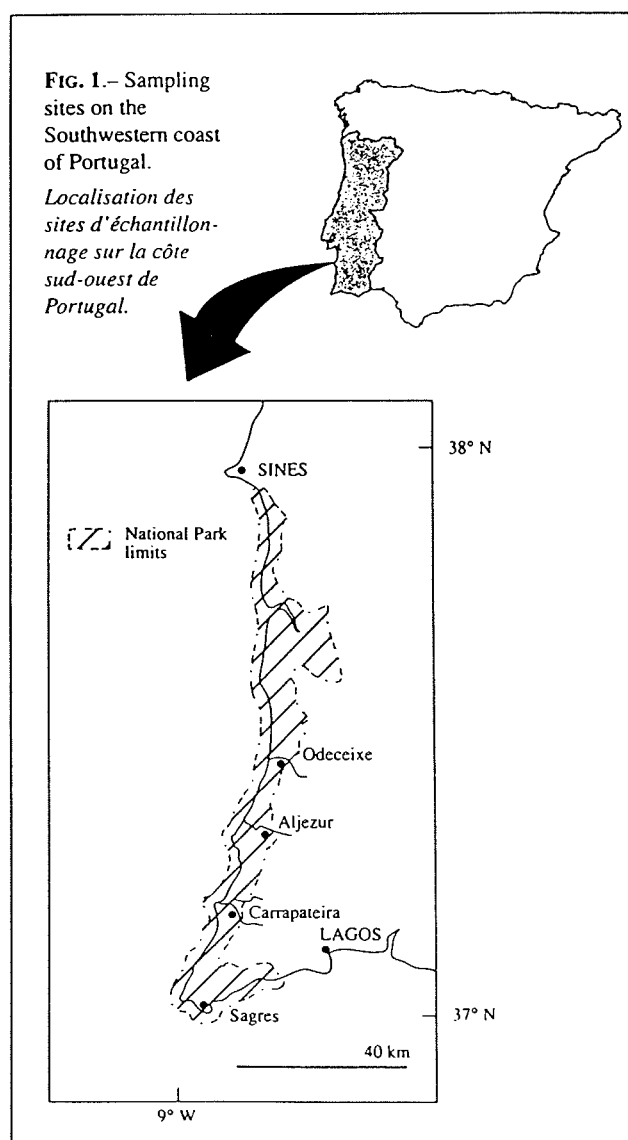
The main goal of this study was to follow the temporal variation of *N. diversicolor* in the SW coast of Portugal. Three different sites were selected to provide a wide range of regional variability and to determine if local factors within these three sites are more important to set the conditions for the life-cycle of the species than regional factors. One-year seasonal cycles and the secondary production for populations are described. Finally a comparison between the response of the species in these shallow sites and its behaviour in other European areas is presented.

MATERIAL AND METHODS

STUDY AREA

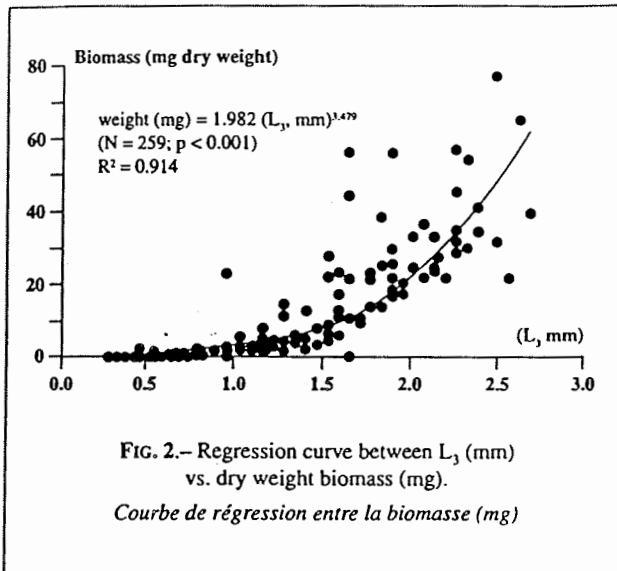
The SW coast of Portugal (FIG. 1) is one of the better preserved coastline in all Europe and its landscape and natural richness make it one of the most interesting coastal areas of Portugal (MAGALHÃES *et al.*, 1987). Different estuaries are located in this coast. They are beautiful and productive areas and constitute vital sites for many coastal species (SILVA E COSTA *et al.*, 1983).

With watersheds of approximately 250, 200 and 110 km², the streams of Odeceixe, Aljezur and Carrapateira (FIG. 1), as well as a few others are responsible for the draining of the SW of Portugal (MAGALHÃES *et al.*, 1987). The mouths of these tidal estuaries are frequently obstructed by sand drift, resulting sometimes in small and shallow brackish water coastal lagoons (COLOMBO, 1977;



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HELIOTIS, 1988). While the estuaries of Odeceixe and Aljezur where connected with the open sea throughout all the sampling period, the small river of Carrapateira remained closed most of the year and was open only during the severe winter storms.

FIELD PROCEDURE

Monthly samples were taken from May'93 to May'94 at the three estuaries studied. Samples were obtained at low tide using a hand corer (210 cm² per 30 cm deep) and five cores were taken and sieved through a 0.5 x 0.5 mm screen every sampling date, giving a sample total of 0.1 m². To collect the larvae specimens, which present less than 19 segments according to DALES (1950), a small corer (6.6 cm² per 10 cm deep) was used (HEIP & HERMAN, 1979). Three cores, covering 19.8 cm², were obtained at each sampling point and sieved through a 0.2 x 0.2 mm screen (METTAM, 1979; 1981).

All samples were fixed in 10% formalin, buffered with borax and stained with Bengal rose. Samples were then rinsed to remove excess of formalin and sediment and the remaining material passed through a saturated solution of saccharose which, by fluctuation, separated the organic from the inorganic phase (ANCELA DA FONSECA, 1989). All samples were preserved in 70° alcohol (MÖLLER, 1985). At each sampling date, salinity (S%), temperature (T°C) and dissolved oxygen levels (O.D.) of the bottom layer were measured for each station.

LABORATORY PROCEDURES

The length of the prostomium, peristomium and the first setigerous (L₃) was taken as a somatic measurement (GILLET, 1990, 1993). This measure was used to calculate biomass, to identify cohorts and to measure growth, as

well as to compute secondary production. Measurements were obtained using a binocular microscope equipped with a camera lucida and digitising tablet (Houston Instruments HIPad) linked to a computer. Selected individuals from representative size categories were measured, then dried (48h at 60°C) and weighed. Using these data we regressed the somatic lengths in mm (SL) against the dry weight in mg (DW) (FIG. 2) to convert the routine somatic measurements to biomass.

The ELEFAN[®] program was used with the BHATTACHARYA method (1967) for the analysis of the different cohorts. To evaluate the secondary production of the species two methods were used: for the cohorts that could easily be followed, the production was estimated by adding the biomass increase from one sample to the next monthly sample. Cohorts were identified according to the CRISP methodology (1984) with a small modification, i. e., the increased weight of the cohorts was calculated using the measures of all the individuals instead of the average dry weight. When the identification of cohorts was not possible, secondary production was obtained by using the method of the single annual cohort (HYNES & COLEMAN, 1968; HAMILTON, 1969; BENKE, 1979) following the formula elaborated by CORNET (1986). Secondary production was calculated from May of 1993 to May of 1994.

The growth was expressed in two ways: i) Through the specific growth rate μ (d⁻¹) obtained by JØRGENSEN's formula (1990), also used for calculating the growth of *N. diversicolor* "in vitro" (NIELSEN, *et al.*, 1995):

$$\mu = \ln (W_t / W_0) t^{-1}$$

being W_0 and W_t the average biomass of the polychaeta on day 0 and day t respectively, divided by the total number of days of existence of the cohort and, ii) by the linear growth rate (mm/day) per cohort, obtained by dividing the difference between the final and the initial average lengths by the days of existence of the cohort. Thus, converting the value of the average length of L₃ (L) to the total length (TL) through the following regression:

$$TL = 29.1 * L - 10.355 (r^2 = 0,947, N = 122, p < 0.001)$$

RESULTS

ENVIRONMENTAL CONDITIONS

The water temperature followed closely the atmospheric temperature values (TAB. I). According to monthly estimates, the water temperature for these three estuaries oscillated between a maximum of 21°C (Odeceixe), 25°C (Aljezur) and 26°C (Carrapateira) in June-July and a minimum of 12°C (Odeceixe), 13°C (Aljezur, Carrapateira) in December.

TABLE I.- Environmental parameters measured at the three studied estuaries.
Paramètres de l'environnement mesurés dans les trois sites étudiés.

	TEMPERATURE (°C).		SALINITY (‰)		DISSOLVED OXYGEN (ppm)		GRAIN SIZE (mm)
	range	mean	range	mean	range	mean	
ODECEIXE	12-21	15.6	18-35	31.4	3.4-4.5	8.6	0.5-0.25
ALJEZUR	12.7-24.7	17.5	19-36.5	29	2.7-12.5	8.8	0.25-0.125
CARRAPATEIRA	13.3-26.3	19.5	4.7-31.7	15	4.7-11.6	9.9	0.5-0.25

The variation observed for the dissolved oxygen values was similar at the three estuaries ranging from 2 to 5 ppm (lower values) and from 12 to 15 ppm (higher values). Yearly average dissolved oxygen values were 8.6 ppm (Odeceixe), 8.8 ppm (Aljezur) and 9.9 ppm (Carrapateira) (TAB. I).

Important differences between the three studied estuaries were observed for the salinity values. Salinity is a conservative parameter, which transmits the information on the system's distinct water entries. Although changes in salinity at several parts per thousand could be observed in these habitats, the obtained annual average salinity values (31.4% at Odeceixe; 29% at Aljezur and 15% at Carrapateira) clearly reflected the fact that in the latest estuarine area the junction with the sea remained closed for most of the year (TAB. I).

POPULATION DENSITY AND BIOMASS

Odeceixe

Recruitment of *N. diversicolor* was continuously observed during the whole year. Although larvae and juvenile specimens were found during the entire sampling period (FIG. 3), this recruitment dynamics seemed to have two periods of higher intensity. The most important peak was detected in September'93 (65909 ind. m⁻²) corresponding to 29.6% of the total recruitment. Another less intense peak was observed in May'94 (22727 ind. m⁻²), accounting for 10.2% of the total recruitment.

The seasonal pattern of density reached a maximum during May'94 (3652 ind.m⁻²) as new recruiters were incorporated into the population. Spring densities of the previous year steadily dropped throughout summer, reaching its lowest values (413 ind.m⁻²) in September'93 (FIG. 3). A value of 1126 ind.m⁻² was found for the average year abundance and the mean annual biomass was 6.6 g dry weight m⁻². Seasonality in biomass followed abundance values and was also well marked (FIG. 4).

Aljezur

The recruitment pattern observed in this estuary mirrors the pattern found in Odeceixe, with a main recruit-

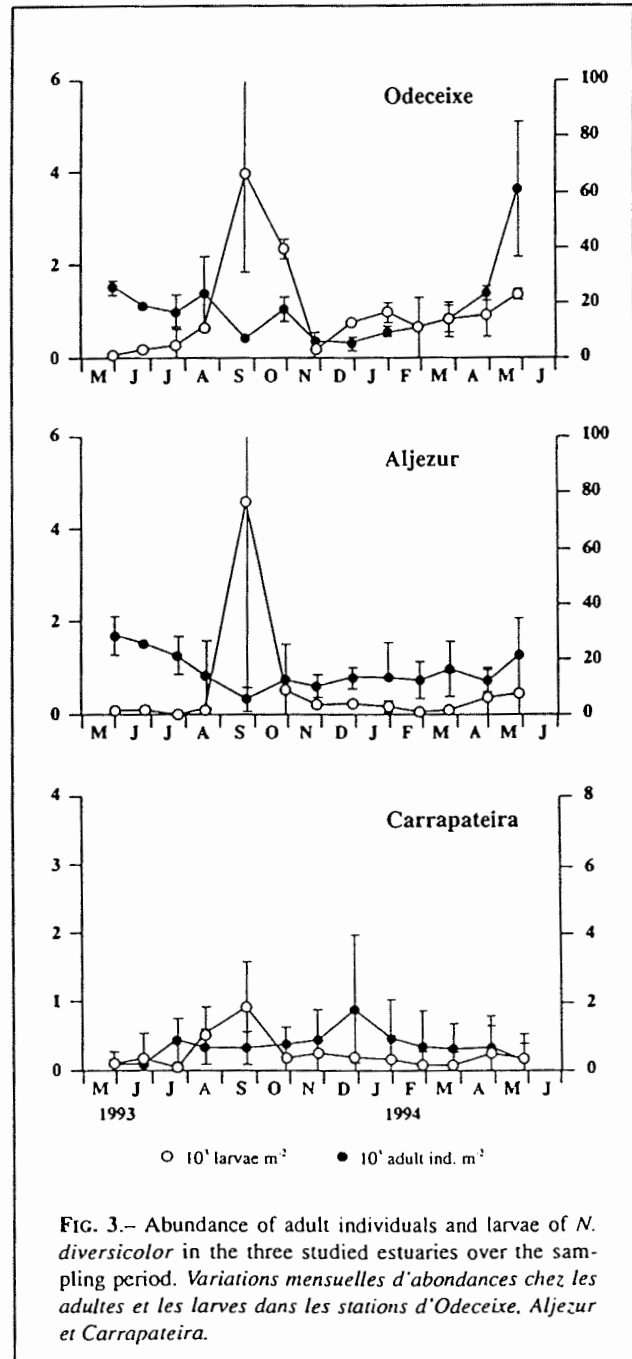
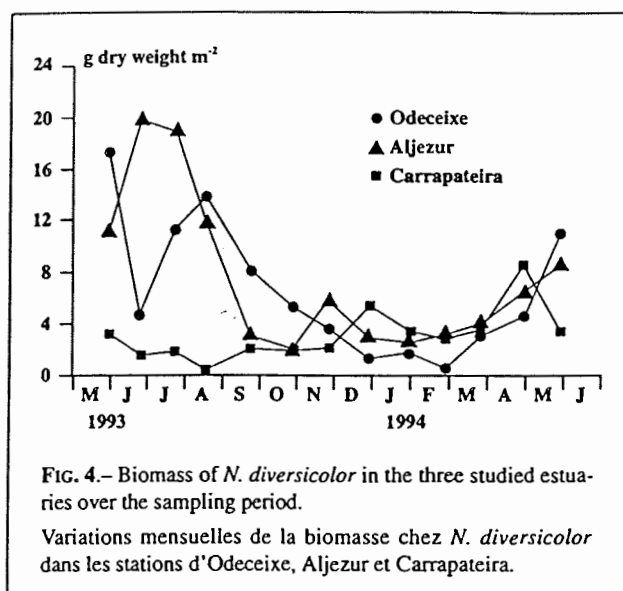


FIG. 3.- Abundance of adult individuals and larvae of *N. diversicolor* in the three studied estuaries over the sampling period. Variations mensuelles d'abondances chez les adultes et les larves dans les stations d'Odeceixe, Aljezur et Carrapateira.

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ment peak in September (76094 ind.m⁻²) which, in this case, was responsible for 64% of the total recruitment, followed by an extended season with a significant number of recruits, and a second small recruitment period in May '94, accounted for 6.5% of the total recruitment (FIG. 3). With an annual density of 957 ind.m⁻² adult individuals, the highest density was observed in May '93 (1678 ind.m⁻²), and the lowest (332 ind.m⁻²) in September (FIG. 3). It was further confirmed that the number of adult specimens falls significantly during the summer due to the reproductive activity and its associated high mortality. Mean annual biomass value was 7.69 g m⁻² dry weight, with a major peak in June '93 (19.84 g m⁻²) followed by July '93 (18.81 g m⁻²). These months had a high number of strong and well fed individuals. In October, biomass reached its minimum values (1.93 g m⁻²), probably influenced by the high mortality of the adults following the reproductive season (FIG. 4).

Carrapateira

In the estuary of Carrapateira, larval recruitment was slightly different from that found at the other two estuaries. Although the characteristic peak of recruitment was consistently found in September (1852 ind m⁻²), the recruitment period seemed to be initiated earlier and the number of recruits were far below the numbers found for the other estuaries (FIG. 3). The main peak accounted for 30.6% of the total recruitment with a second smaller peak in May '94 (505 ind m⁻²).

Except during the first three months, density values were quite similar throughout the year (FIG. 3). In December, an increase in the number of individuals was detected. This sudden increase of the population during the winter, where temperature values were the lowest (13.3°C),

could be related to the water draining due to the disruption of the sand barrier in the previous month (November '93). The following month (December '93) the lagoon was again full and showed a considerable increase in the pre-existent macrophytes (*Ruppia cirrhosa*). As a consequence, the productive and protective area was enlarged. It is believed that the increases in vegetation, besides providing nourishment, act through its roots as a protection against possible predators. Mean annual density value in this estuary was significantly smaller (337 ind m⁻²) than in the previous ones. Compared to the other two estuaries, mean annual biomass was low (3.1g dry weight m⁻²). Biomass values remained very low from May to November '93 then they increase to a first peak in December '93 (5.1g dry weight m⁻²), and later a second peak more pronounced (8.5g dry weight m⁻²) was observed in April '94 (FIG. 4).

GROWTH AND SECONDARY PRODUCTION

Odeceixe

The maximum life span of *N. diversicolor* in Odeceixe was 2 years. Although a large peak of recruitment was observed in September, the presence of newly-settled larvae every month did not allow us to clearly separate cohorts during the first year of life of these organisms (FIG. 5). The frequent affluence of new specimens determined that measured growth rates were very low (0.005 mm per day) and the specific growth rate was computed as 0.0053 d⁻¹. However, it was possible to identify two different cohorts in the population which was one year old (FIG. 5). The older cohort (1992-II) contained less effectives than the younger one. In this cohort, *N. diversicolor* presented a linear growth rate of 0.146 mm per day and a specific growth rate of 0.0047 d⁻¹. This cohort, besides being of influence in the larval recruitment of September, continued in the following months to have members that did not reproduce. These were the breeders that maintained the continuous recruitment after September. The second and younger cohort (1993-I) grouped mainly the spring recruiters and participated with some of the older organisms in the second peak of recruitment detected in the spring. This second cohort had a linear growth rate of 0.108 mm per day and a specific growth rate of 0.0097 d⁻¹. Finally the older worms combined the organisms coming from the 1992-I and 1991 cohorts and they were responsible for the peak of recruitment in September '93 (FIG. 6). The linear growth rate and the specific growth rate in this case were calculated as 0.003 mm per day and 0.001 d⁻¹ respectively.

To evaluate the contribution of each cohort to the secondary production in this estuary the identified cohorts were studied separately. However, since the continuous recruitment of individuals throughout the first year of life did not allow us to separate cohorts, the average cohort

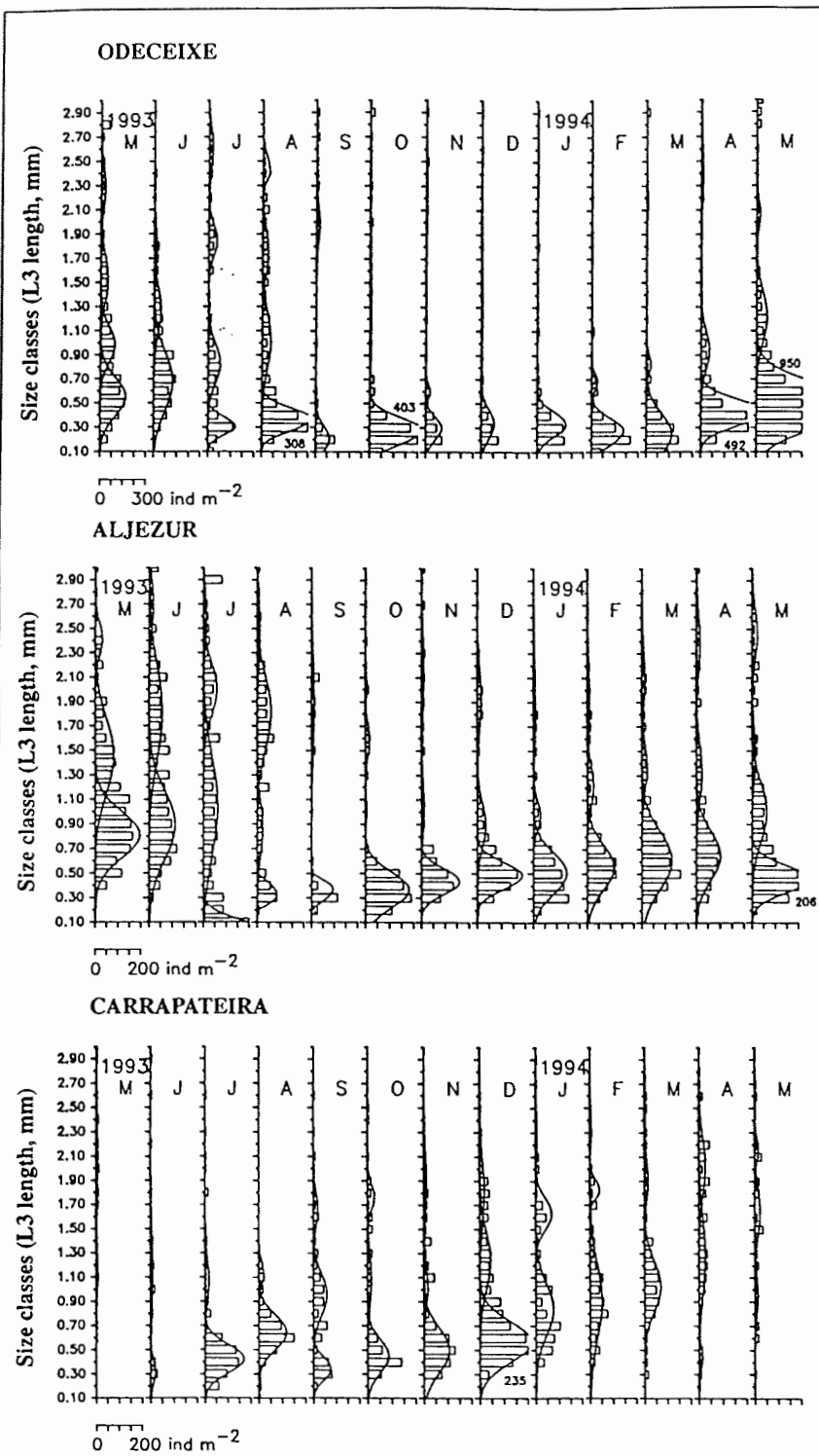


FIG. 5.— Size frequency histograms (ind m⁻²) of *N. diversicolor* population in the three studied estuaries over the sampling period. Curved lines are normalised expected frequencies.

*Histogrammes des fréquences de taille (ind m⁻²) de la population *N. diversicolor* dans les trois sites étudiés.*

method was used to obtain production in this first year-class (TAB. II).

Aljezur

The pattern of Aljezur mirrors again the described pattern in the Odeceixe estuary. The 1993-1994 younger class was also strongly influenced by the constant income of new individuals (FIG. 5). Nevertheless, the growth rate of this classes (0.026 mm per day) and the observed specific growth rate (0.212 d⁻¹) were higher than the measured rates in the previous estuary.

The 1992-II and the 1993-I cohorts with growth rates of 0.130 mm and 0.149 mm per day, and specific growth rates of 0.0059 d⁻¹ and 0.0107 d⁻¹, respectively, were basically responsible for the reproduction and recruitment during spring '94 (12% of the entire yearly recruitment). The older cohort was responsible for almost 50% of the biomass and the large peak of recruitment observed in September (FIG. 6). Its growth rate was measured as 0.142 mm per day with a specific growth rate of 0.0040 d⁻¹. The recruitment peak of September showed the highest densities observed in these three estuaries, which made us believe that there were a high number of individuals reproducing and contributing to this strong recruitment at the same time.

Total secondary production in Aljezur was computed as 23.7g m⁻². Most part of this production was attributed to medium-size class organisms (TAB. II).

Carrapateira

In the site of Carrapateira there were less adult and larval organisms. However, the youngest worms were able to grow quickly. As a consequence, growth rates (0.055 mm per day) and measured secondary production (7.35 g m⁻²) was higher than in the other estuaries (FIG. 5).

On the other hand, there was also very few large individuals belonging

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TABLE II.— Annual production of *N. diversicolor* at the three studied estuaries.
Production annuel de N. diversicolor pour chacune des zones étudiées.

Cohorts	ODECEIXE		ALJEZUR		CARRAPATEIRA	
	Production g m ⁻² y ⁻¹	P/B Class	Production g m ⁻² y ⁻¹	P/B Class g m ⁻² y ⁻¹	Production	P/B Class
1991 /1992 -I	1.58	0.29	2.94	0.62	0.03	0.04
1992-II	7.36	2.71	8.87	3.90	0.53	0.76
1993-I	1.02	1.33	9.46	4.15	4.43	3.85
1993 II/ 1994	1.45	6.61	2.41	5.03	7.35	5.09
	Σ (my93-my94) 12.83	P/B y ⁻¹ 1.95	Σ (my93-my94) 23.68	P/B y ⁻¹ 3.08	Σ (my93-my94) 12.31	P/B y ⁻¹ 4.0

to the older cohorts that contributed with low production values (TAB. II - FIG. 6). The 1993-I class with a production of 4.43 g m⁻² (TAB. II) was seen during the all studied months, being the main source of biomass for the adult peak in December. This cohort, after the collapse of the other two older ones in August and December, became the only supply of larvae to the system until May '94. Measured growth rates and specific growth rates were 0.137 mm per day and 0.0075 d⁻¹ respectively.

The population of *N. diversicolor* of the Carrapateira estuary was the least productive of the three populations studied in this work. In addition, most of the calculated production was due to the youngest worms (TAB. II).

DISCUSSION

The life span of *N. diversicolor* in the estuarine environments of the Southwest Portuguese coast seems to be close to two years. Similar life span has been observed in the majority of the previous studies conducted in European temperate waters (DALES, 1950; CHAMBERS & MILNE, 1975; KRISTENSEN, 1984; ESSINK *et al.*, 1985; GILLET, 1990; DUESO & SARDA, 1994). The adult population of this species was maintained in a rather constant number throughout the year but it showed a clear decrease through summer. This general pattern was also observed in other Iberian sites (SPRUNG, 1994; DUESO & SARDA, 1994). The summer decline can be attributed either to migration, since increased water temperature may force the worms to move towards shallower sediment layers (Esselink & Zwarts, 1989) and thus to a higher risk of predation, or to the fact that this species is monothelic, i.e. individuals reproduce once in a lifetime, dying after spawning (DALES, 1950; CLAY, 1967; OLIVE & GARWOOD, 1981; HUMPHREYS, 1985; MÖLLER, 1985; ARIAS & DRAKE, 1995). The population increased rapidly after summer. Although we found at the three studied sites and all along the sampling period ripe females asso-

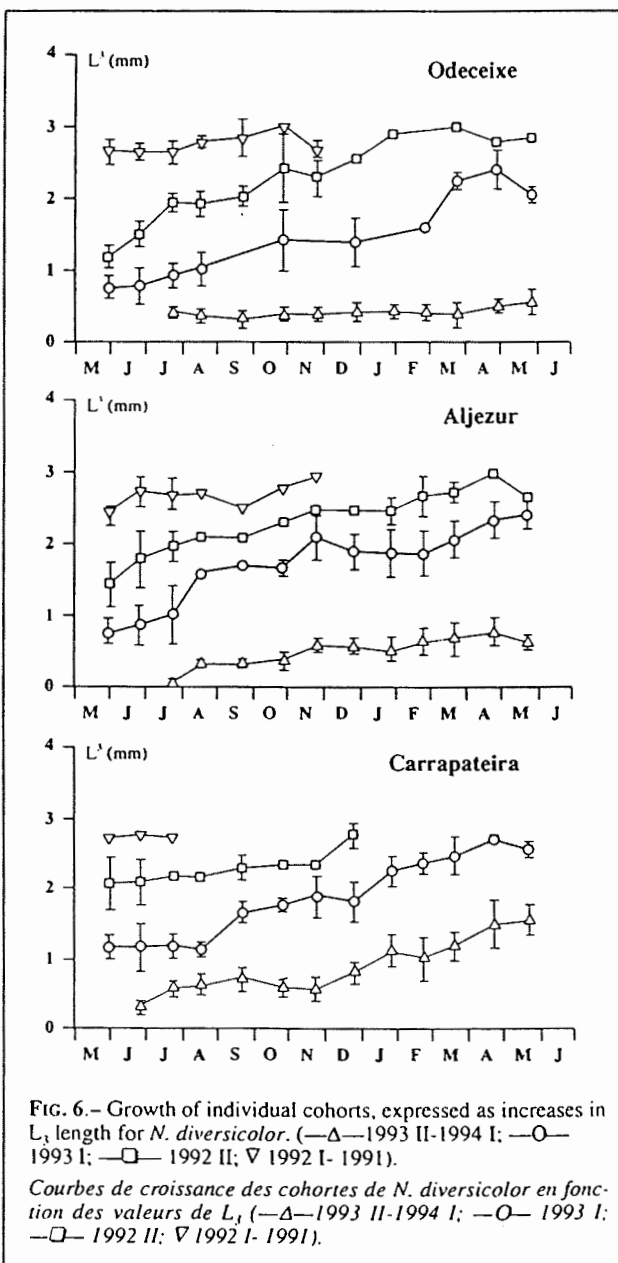


FIG. 6.— Growth of individual cohorts, expressed as increases in L₁ length for *N. diversicolor*. (—Δ—1993 II-1994 I; —○— 1993 I; —□— 1992 II; ▽ 1992 I- 1991).

Courbes de croissance des cohortes de N. diversicolor en fonction des valeurs de L₁ (—Δ—1993 II-1994 I; —○— 1993 I; —□— 1992 II; ▽ 1992 I- 1991).

ciated to the presence of larvae it was confirmed the presence of two main recruitment periods: a large recruitment peak at the end of summer, and another smaller peak during spring. Similar recruitment periods have been recorded in both, the South of Europe (SOLA & IBAÑEZ, 1990; GILLET, 1990; GARCIA-ARBERAS & RALLO, 1993; DUESO & SARDA, 1994; FIDALGO E COSTA, 1994; ARIAS & DRAKE, 1995) and the North of Africa (GILLET, 1993).

Seasonal dynamics of *N. diversicolor* varied similarly at the three estuaries. The dynamics of the species seemed to be controlled by regional factors and not by each particular set of local estuarine environmental constraints. However, the environmental conditions found in Carrapateira during the study "the estuary was always covered by water except during the storms of winter blocked off from the sea, and with no striking influences of tides" made this estuary more similar to a coastal lagoonal system. As a consequence some differences could be noticed in comparison with the other two estuarine zones (Odeceixe and Aljezur). The closed conditions of the Carrapateira allowed mean water temperature to be higher in it than in the other two sites. These higher temperatures increased the growth of juveniles and allowed them to be mature earlier than in the other two estuaries.

Adult individuals were larger in Odeceixe and Aljezur than in Carrapateira. This striking difference was related to the shorter life span for this species in this environment. In Carrapateira the worms could be sexually mature when they reached 12 months of age, however almost all worms reached their reproductive period by their first 18 months of life. On the other hand, in Odeceixe and Aljezur, this species reached sexual maturity at 18 months and most of the individuals reproduced at the end of the second year of life. The premature reproduction obtained in Carrapateira has been verified in the laboratory on experimental cultures accomplished at 15°C, in which *N. diversicolor* reached maturation in the first year of culture (OLIVE & GARWOOD, 1981; OLIVE, 1994). The same author in field experimentation and observation affirms the existence of an annual cycle in this species. Our data not yet published (work in process) reveals the existence of mature individuals after 3 months when we cultured the worms at 20°C in the laboratory.

Growth rates gradually decreased with age. During the first year of life growth rates were higher and varied the length of time needed for the individuals to reach 10 mm according to the study area and the environmental conditions. The population studied by CHAMBERS & MILNE (1975) reached this value in 11 months, while 5 to 6 months was the period calculated by DALES (1951) and 4 months by SMITH (1951), HEIP & HERMAN (1979) and MÖLLER (1985). Large differences were observed in our

estuaries, ranging from more than 6 months (Odeceixe) to less than 1 (Carrapateira). Environmental variability of the sites must be taken into account mainly because temperature is thought to be one of the most important factors influencing growth (DALES, 1950; HURYN, 1990). During the second year of the life, growth rates were considerably smaller. In general, these values were comparable with those reported for other estuaries, 0.07 mm per day (HEIP & HERMAN, 1979; LOPES DA SILVA, 1993) and 0.1 mm per day (DALES, 1951; CHAMBERS & MILNE, 1975; GARCIA-ARBERAS & RALLO, 1993).

The annual P/B ratio of a population usually decreases with age. Thus, a population dominated by older individuals will have a lower P/B than those comprising younger individual (WARWICK, 1979), and older cohorts will have lower P/B than younger ones (CHAMBERS & MILNE, 1975; ROBERTSON, 1979). The P/B obtained for the first year class in our three sites (6.6, 5.0 and 5.1) was similar to that obtained by MÖLLER (1985) for individuals of the same class (4.8). The entire population P/B (2.0 in Odeceixe, 3.1 in Aljezur and 4.0 in Carrapateira) clearly show a higher turnover associated to the highest average temperature (Carrapateira) and a lower turnover related to the lowest mean temperature (Odeceixe). According to Morin & Bourassa (1992) this P/B relation expresses the renovation rate of the biomass in a population, decreasing as the average individual biomass goes up and increasing with the elevation of the temperature.

Seasonal dynamics of *N. diversicolor* varies according to the area of study. In cooler sites of Northern regions, their populations exhibit short spawn and recruitment periods through the year and peaks of density are observed during the spring-summer period. In warmer sites of Southern regions, spawn and recruitment can be observed through large periods of the year and normally two peaks of recruitment can be noticed. The P/B ratio observed for *N. diversicolor* in European waters seems to be very constant between 2 and 3 being in the range of the studied populations of Odeceixe and Aljezur. The P/B obtained in Carrapateira, with warmer mean water temperatures and increased worm growth rates is similar to what was described by GILLET (1993) in Morocco.

ACKNOWLEDGEMENTS

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VERSION FRANÇAISE ABRÉGÉE

Nereis diversicolor occupe des sédiments sableux ou vaseux des milieux saumâtres; cette espèce est présente dans des estuaires et lagunes côtières (JENSEN, 1988; OZOH & JONES, 1990), où elle constitue une partie importante de la chaîne trophique estuarienne, comme prédateur d'autres organismes benthiques (RÖNN *et al.*, 1988).

En ce qui concerne sa distribution géographique, *N. diversicolor* peut être trouvée depuis le Maroc (GILLET, 1993) jusqu'en Scandinavie, aussi bien dans les eaux froides et saumâtres de la Mer Baltique que dans les lagunes chaudes et hypersalées de la Mer Noire (METTAM, 1979). Dans la Mer Caspienne, l'existence de cette espèce a été mise en relation avec la présence humaine (SMITH, 1963).

Dans la Péninsule Ibérique, plusieurs études ont porté sur les populations de *N. diversicolor* a) de la côte portugaise [GONÇALVES & RIBEIRO (1987) sur l'estuaire du Mondego; CRUZ (1979) sur la lagune de Saint-André; LOPES DA SILVA (1993) dans l'estuaire du Mira; ABRANTES (1995) dans la Ria de Aveiro; FIDALGO E COSTA & CANCELA DA FONSECA (1995) dans les rivières de Odeceixe, Aljezur et Carrapateira]; b) de la côte espagnole [SOLA & IBAÑEZ (1990) dans l'estuaire du Bidasoa; GARCIA-ARBERAS & RALLO (1993) sur la côte du Pays Basque; DUESO & SARDA (1994) dans la lagune de St. Margarita (Rosas); ARIAS & DRAKE (1995) dans la baie de Cadix]. Les rivières d'Odeceixe, Aljezur et Carrapateira, avec leur bassin hydrographique d'une surface respectivement égale à 250, 200 et 110 km², sont responsables du drainage dans le sud-ouest du Portugal. Pendant toute la durée de l'échantillonnage, les estuaires d'Odeceixe et Aljezur étaient en communication directe avec la mer, alors que la rivière de Carrapateira était fermée pendant la plus grande partie de l'année; cette dernière a été ouverte uniquement au cours de très forts orages dans le sud Portugal.

Des échantillons mensuels ont été prélevés en périodes de marée basse, entre mai 1993 et mai 1994, en utilisant un carottier de 201 cm² d'aire introduit à une profondeur d'environ 30 cm. Les cinq réplicats totalisent une aire de 0,1 m². Les contenus ainsi obtenus ont ensuite été passés à travers un tamis de 0,5 x 0,5 mm de vide de maille (OLIVE & GARWOOD, 1981; HUMPHREYS, 1985; GILLET, 1990; SARDA, communication personnelle). Pour les échantillons larvaires, (jusqu'à 19 segments d'après Dales, 1950), trois carottes plus petites (6,6 cm² par 10 cm de profondeur) couvrant 19,8 cm² ont été obtenues (HEIP & HERMAN, 1979). Les contenus ont été tamisés au travers d'une maille carrée de 0,2 x 0,2 mm (METTAM, 1979; 1981). Au moment de chaque échantillonnage et pour chaque station, la salinité (S ‰), la température (T °C) et la quantité d'oxygène dissous (ppm) ont été mesurées dans la couche la plus profonde.

CYCLE DE VIE DE *NEREIS DIVERSICOLOR*

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Les longueurs du prostomium, péristomium et premier sétigère (L_3) ont été utilisées comme mesures somatiques (GILLET, 1990 et 1993). Ces mesures ont ensuite été utilisées pour déterminer la biomasse, identifier des cohortes, mesurer la croissance et déterminer la production secondaire. Le programme ELEFAN® a été utilisé avec la méthode de BHATTACHARYA (1967) pour l'analyse des différentes cohortes. Pour évaluer la production secondaire de l'espèce, deux méthodes ont été utilisées : pour les cohortes qui pouvaient être facilement suivies, la production a été estimée en ajoutant l'incrément de biomasse obtenu pour un échantillon à celui obtenu le mois suivant. Quand l'identification des cohortes n'était pas possible, la production secondaire a été obtenue en utilisant la méthode de la cohorte annuelle unique (HYNES & COLEMAN, 1968 ; HAMILTON, 1969 ; BENKE, 1979) d'après la formule proposée par CORNET (1986).

Les moyennes des paramètres environnementaux "température" (en °C), "salinité" (en ‰) et "oxygène dissous" (en ppm), obtenues pour chaque station côtière, étaient : 15,6 °C, 31,7 ‰ et 8,6 ppm pour

Odeceixe, 17,5°C, 29 ‰ et 8.8 ppm pour Aljezur, 19.5°C, 15 ‰ et 9.9 ppm pour Carrapateira. Les densités obtenues sont égales à : 1126, 957 et 337 ind.m⁻² pour les adultes, 15 927, 8 538 et 433 ind.m⁻² pour les larves. Odeceixe présente un taux de biomasse annuel de 85,65 g m⁻², une production annuelle de 12,83 g m⁻² et un index P/B de 1,95. À Aljezur, le taux de biomasse annuel est de 99,96 g m⁻², la production annuelle de 26,68 g m⁻² et l'index de P/B de 3.08. Carrapateira présente un taux annuel de biomasse de 40,14 g m⁻², une production annuelle de 12,31 g m⁻² et l'index de P/B de 4.0.

L'objectif principal de cette étude était de suivre, au cours du temps, la variation de *N. diversicolor* sur la côte SW du Portugal. Trois localités différentes ont été sélectionnées, de façon à obtenir une variabilité régionale. Cette comparaison doit permettre d'évaluer si les facteurs locaux, spécifiques de chacun des trois sites choisis, sont plus importants que les facteurs régionaux pour conditionner le cycle de vie de cette espèce.

Errata

Após a publicação do presente artigo foram detectados os seguintes erros:

Página 523; linhas 6 e 7 (Summary); onde se lê “15927, 8538 and 433 ind. m⁻² for adult individuals and 1126, 957 and 337 ind. m⁻² for the larva” deve ler-se “15927, 8538 and 433 ind. m⁻² for the larva and 1126, 957 and 337 ind. m⁻² for adult individuals”

Página 523; e linhas 5 e 6 (Résumé); onde se lê “Les densités obtenues ont été respectivement 1126, 957 et 337 ind. m⁻² pour les individus adultes et 15927, 8538 et 433 ind. m⁻² pour les larves” deve ler-se “Les densités obtenues ont été respectivement 1126, 957 et 337 ind. m⁻² pour les larves et 15927, 8538 et 433 ind. m⁻² pour les individus adultes ”

FEEDING ECOLOGY OF THE POLYCHAETA *Nereis diversicolor* ON ESTUARINE AND LAGOON ENVIRONMENTS IN THE SOUTHWEST COAST OF PORTUGAL

RUNNING HEAD: Feeding ecology of *Nereis diversicolor*

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KEYWORDS: Polychaeta; *Nereis diversicolor*; feeding ecology; coastal environments.

ABSTRACT

The feeding ecology of *Nereis diversicolor* (Annelida: Polychaeta) was studied over 14 months in three estuary-lagoon systems of the Southwest coast of Portugal (Odeceixe, Aljezur and Carrapateira). The analyses of the digestive tract revealed that diet varies according to the site studied, time of the year and with the size of the individuals. There were no sex differences in the digestive contents. In all the sampling stations a total of thirty items were found, but only five revealed an occurrence superior to 1%. These were: mucus (56.3%), sand (17.6%), vegetable detritus (10.2%), Nereididae (7.7%) and *Corophium* sp. (1.8%). *N. diversicolor* was detected in all sampling stations. Filter-feeding was the usual feeding behaviour, although in Carrapateira there is evidence of carnivore habits, including cannibalism.

INTRODUCTION

The polychaeta *Nereis diversicolor* O.F. is widely distributed in estuarine and lagonal habitats from North Africa to the North of Europe (Mettam, 1979, 1981; Gillet, 1993). It has a high physiological tolerance to extreme variations in environmental factors. This species can grow and reproduce in different sediment types and in high stress environments (Bartels-Hardege and Zeeck, 1990; Cheggour et al., 1990; Miron and Kristensen, 1993; Zubillaga and Salinas, 1997).

It not only adapts well to a variety of environments, but also its feeding habits are quite generalist. *N. diversicolor* has a wide capacity regarding the size of food it feeds upon, which ranges from micro and macrozoobenthos, diatoms, to fragmented organic matter including detritus (Goerke, 1971; Reise, 1979; Witte and Wild, 1979; Smith et al., 1996; Lucas and Betru, 1997). This species has different strategies to capture its food: i) omnivorous - it captures its food on the sediment surface and around the gallery (Esselink and Zwarts, 1989; Esnault et al., 1990); ii) suspension feeding - it releases a web of mucus in the gallery and through dorsal-ventral movements it generates a continuous current capturing the phytoplankton in the web to be ingested afterward (Harley, 1950; Well and Dales, 1951; Riisgård, 1991; Vedel, 1998); iii) facultative filter-feeding – it adopts this feeding strategy when there is a high concentration of phytoplankton in the water (Vedel and Riisgård, 1993); iv) predatory – this species has an important role in the structure of brackish water ecosystems through the bioturbation and because it acts as a predator of different bottom fauna species (Rönn et al., 1988; Hansen and Kristensen, 1998). However, the variety of feeding strategies seems to be related to several variables, such as food availability and quality, presence or absence of predators, season and tidal height (Esselink and Zwarts, 1989; Masson et al., 1995).

The aim of this work was to establish the feeding patterns in the studied sites, through the analysis of the seasonal variation in digestive tract contents of *N. diversicolor*. In addition the type of food ingested by the different body sizes, as well as by different sexes was also investigated.

MATERIAL AND METHODS

Study area

The studied sites are within the Sudoeste Alentejano and Costa Vicentina Nature Park. These sites are in one of the better preserved coastal areas of Europe with high specific richness (Silva e Costa et al., 1983). Located on the Southwest coast of Portugal, its estuaries have an important role in the life cycle of several species of its fauna. The sampling stations studied were established at the end of the Odeceixe (ODX), Aljezur (ALZ) and Carrapateira (CAR) estuaries (Figure 1), with water basins of about 250, 200 and 110 km² respectively, which partially drain the Western part of Southwest Portugal. According to Day et al. 1987, these sort of brackish environments, although representing a great geomorphological diversity, are, from an ecological point of view, lagoon-estuarine ecosystems.

These sampling stations studied (two for Odeceixe and three for Aljezur and for Carrapateira) have the highest occurrences of *N. diversicolor* according to previous studies in those systems (Magalhães et al., 1987; Magalhães, 1988). To facilitate the statistical work and also to keep the initial goal of comparing the different brackish environments, it was decided to amalgamate and analyse all data per sampling site (ODX, ALZ and CAR) with no specific reference to the sampling stations, within each site. These are arranged in a North-South geographic position in which ODX is the sampling site located further North and CAR the sampling site further South. The distance between these two areas is approximately 35 km.

Field work

Monthly data on salinity, temperature, rainfall, organic matter, chlorophyll *a* and phaeopigments were obtained to characterize the environmental conditions of each site. Salinity (at the surface and at the bottom) and temperature (at the bottom) were measured *in situ* at each station. Rainfall values were obtained from the National Meteorological Institute and General Administration of Natural Resources. Organic matter, chlorophyll *a* and phaeopigments of sediment were determined on the top centimetre of sediment samples obtained in each station with a corer of 5 cm of diameter, and were freezed until analysis.

All the specimens of *N. diversicolor* used in this work were collected between April 1993 and May 1994: i) at low tide in the sublittoral fringe of the end part of two small estuaries, ODX and ALZ; ii) in the sublittoral area of CAR, in a small temporary lagoon formed by the partial closing of a sandy barrier near the beach. A 0.02 m² corer was used to obtain five sub-samples of a 30 cm depth, making a total sampling area of 0.1m². The samples were then sieved through a 0.5 mm square mesh, to capture small individuals, and the remainder fraction was kept in 10% formalin, buffered with borax. In the laboratory, samples were washed in order to extract the excess of formalin and sediment, the remain was dip into a saturated saccharose solution which separates the organic from the inorganic matter by flotation (Cancela da Fonseca, 1989; Caron et al., 1993). All samples were then kept in 70° alcohol (Möller, 1985).

Some of the captured animals were too small to be dissected while others had no digestive tract contents. Therefore the guts of 3000 animals were observed but, from these, only 907 (30%) had any content (220 in ODX, 460 in ALZ and 227 in CAR).

Laboratory work

All individuals found were measured, based on the length of L₃ (length of prostomium, plus peristomium and 1st chaetiger) according to Gillet (1990; 1993). This measure presented the best correlation (N=259, r²=0.914, p<0.001) with the biomass being a good conversion factor (dry weight (mg) = 1.982 (L₃, mm)^{3.479}). All measurements were performed using a camera lucida and digitising table (Houston Instruments HiPad) linked to a computer. Three basic sizes were established to this study (small (S1), medium (S2) and large (S3)) being represented by a L₃ measure, respectively, of [0.3, 1.5 mm[, [1.5, 3.0 mm [and [3.0, 4.5 mm]

The collection of digestive tract contents was effected after opening all the individuals with an iris scissors, from the first to the last chaetiger. The material was then placed in a Petri dish and observed with a binocular stereomicroscope and, when necessary, with a compound microscope. The identification of the diverse food items found was narrowed down to the lowest possible taxonomic level. The conditions of the material observed led us to choose to express the individual results in percentage

of occurrence. For each month the data were analysed as frequency of occurrence according to the following formulae:

$$\frac{\text{number of digestive tract with a certain item}}{\text{number of digestive tract with the observed content}} \times 100$$

Sex determination was carried out when the individuals were opened to extract the tract contents. Observation of the oocytes and the sperm plates was done using binocular microscopes and the total sex ratio found in ODX, ALZ and CAR was always female biased, being, respectively, 1:2.9, 1:3.1 and 1:4.1 (personal observation).

Pigments were extracted with 90% acetone during 24h in darkness at 4°C, and subsequently centrifuged. Chlorophyll *a* (surface Cl *a* is generally used as an index of microphytic biomass) and phaeopigments were determined spectrophotometrically by the method of Lorenzen (1967) and expressed as mg m⁻².

The organic contents of the superficial layer of sediment was estimated through the loss by ignition, a method that, according to Duck (1986), provides a close estimate of the total organic matter expressed in g m⁻².

Statistical analysis

The proportion of different food items ingested per individual were compared between the sexes using T-tests and among size classes (large, medium, small) using one-way ANOVA. In both cases the variables conformed to the assumptions of parametric statistics of normality and homogeneity of variances as investigated by normal probability plots and Levene's test respectively.

For the statistical analysis of seasonal effects, the data from the different months were merged into four seasons: Spring (April and May 1993; March, April and May 1994), Summer (June, July and August 1993), Autumn (September, October and November 1993) and Winter (December 1993; January and February 1994).

Spatial (Odeceixe vs. Aljezur vs. Carrapateira) and seasonal (Spring vs. Summer vs. Autumn vs. Winter) effects in the proportion of food items ingested were investigated using two-way ANOVA after transforming the data ($\arcsin\sqrt{x}$) to match the assumptions of ANOVA, followed by planned comparisons to identify the significant differences in the same site along different seasons or among the three sites in the same season. The same analysis (i.e. two-way ANOVA followed by planned comparisons) was performed for the abiotic factors measured. Of the abiotic factors only salinity and temperature values were log-transformed to meet the assumptions of ANOVA. All descriptive statistics were expressed as mean \pm standard error of the mean. All statistical tests were performed using software package STATISTICA for Windows v. 5.0 (StatSoft, 1995).

RESULTS

Abiotic factors and phytopigments

Water temperature showed a seasonal variation according to the atmosphere values of this parameter (Figure 2). The ANOVA revealed an effect of the sampling site ($F_{2,99} = 30.76$, $p < 0.001$) and of the season ($F_{3,99} = 77.50$, $p < 0.001$). The site effect is due to significant differences in temperature between ODX (15.6 ± 0.52 °C, $n = 42$), ALZ (17.5 ± 2.07 °C, $n = 42$) and CAR (19.5 ± 0.62 °C, $n = 28$) (post-doc HSD tests for samples with different sizes, $p < 0.05$). The effect caused by seasonality was determined by the differences between the seasons of the year: Spring (21.3 ± 2.1 °C, $n = 40$), Summer (21.6 ± 0.7 °C, $n = 24$), Autumn (15.7 ± 0.5 °C, $n = 24$) and Winter (13.7 ± 0.3 °C, $n = 24$) (post-doc HSD tests for samples with different sizes, $p < 0.05$).

Salinity values reflect a constant stratification throughout the year (Figure 2), specially in Spring and Winter and particularly in ODX and ALZ. The ANOVA clearly show an effect of the sampling site ($F_{2,99} = 10.14$, $p < 0.001$) due to differences in salinity between CAR (15.0 ± 1.2 ‰, $n = 42$) and the remainder sampling sites (ODX – 31.8 ± 0.89 ‰, $n = 28$; ALZ – 30.0 ± 2.8 ‰, $n = 42$) (post-doc HSD tests for samples with different sizes, $p < 0.05$).

The chlorophyll *a* reveals a seasonal cycle ($F_{3,100} = 3.46$, $p = 0.02$), associated with the amount of fresh water brought by rain and in particular by the continental runoff that penetrates in the system (Figure 3A). The significant variations occur between Summer ($59.8 \pm 9.90 \text{ mg.m}^{-2}$, $n = 24$) and Winter ($113 \pm 17.6 \text{ mg.m}^{-2}$, $n = 24$) (post-doc HSD tests for samples with different sizes, $p < 0.05$).

The chlorophyll degradation represented by the values of phaeopigments (Figure 3B), clearly show the areas where there was an accumulation of the remains. The ANOVA showed only a site effect ($F_{2,100} = 7.97$, $p < 0.001$).

The organic matter (Figure 3C), reflect basically the sediment grain size compositions in the different sampling sites, as well as a seasonal contribution of particulate organic matter brought by continental runoff. The ANOVA showed no significant differences between sites ($F_{2,100} = 2.19$, $p = 0.12$) and season of the year ($F_{3,100} = 0.60$, $p = 0.62$).

Spatial and seasonal diet variations

A total of 30 food items were found in all sampling sites has a result of the study of the digestive content (Table 1). However, from this total, only five showed a frequency of occurrence superior to 1%. These items were: mucus (56.3%), sand (17.6%), vegetable remains (10.2%), Nereididae (7.7%) and *Corophium* sp. (1.8%). Only these items were subsequently subject to statistical analyses. The ingestion of different food items in different sampling stations reflects the differences in occurrence of these items in the sampled sites (Figure 4).

The ANOVA showed an effect of the sampling site ($F_{2,895} = 4.65$, $p = 0.001$) and of the season of the year ($F_{3,895} = 3.10$, $p = 0.03$) on the percentage of mucus. There was also an effect of the interaction between the two independent variables ($F_{6,895} = 2.33$, $p = 0.03$). The effect of the sampling site is due to differences between CAR ($50 \pm 2.37 \%$, $n = 227$), with more lagonal properties, and the two remaining sampling sites (ODX: $61.3 \pm 2.17 \%$, $n = 220$; ALZ: $56.8 \pm 1.54 \%$, $n = 460$) (post-doc HSD tests for samples with different sizes, $p < 0.05$). On the other hand, the effect of the season was mainly due to the differences between Winter ($47.7 \pm 3.01 \%$, $n = 156$) and the other three seasons of the year (Spring: $58.4 \pm 1.68 \%$, $n = 383$; Summer:

59.5 ± 2.0 %, n = 205; Autumn: 58.6 ± 2.84 %, n = 163) (post-doc HSD tests for samples with different sizes, p<0.05). The interaction effect was due to differences between Autumn and Summer in CAR, and between CAR and ALZ in Winter (post-doc HSD tests for samples with different sizes, p<0.05).

There were no significant differences between sampling sites concerning the amount of ingested sand ($F_{2,895} = 1.60$, p = 0.20). However, season of the year ($F_{3,895} = 5.06$, p = 0.002) and interaction ($F_{6,895} = 4.64$, p<0.001) were significantly different in this variable. The effect of the seasonality is determined by the difference between Spring (15.0 ± 1.18 %, n = 383) and Summer (22.1 ± 1.79 %, n = 205) (post-doc HSD tests for samples with different sizes, p<0.05). The effect of the interaction is due to differences between ODX and ALZ in Autumn (p = 0.004), ALZ between Spring and Autumn (p = 0.004) and ALZ between Winter and Autumn (p = 0.002) (post-doc HSD tests for samples with different sizes, p<0.05).

Vegetable remains are also one of the items found in the contents of *N. diversicolor*, having the performed ANOVA revealed that there were no differences either away from the sampling sites ($F_{2,895} = 1.95$, p = 0.14) or, the season ($F_{3,895} = 0.35$, p = 0.79) or also interaction between them ($F_{6,895} = 1.23$, p = 0.27). However, it should be noticed that in the period Winter-Spring this item increases in the contents, specially in the sampling site CAR (Figure 4).

Larvae, parapods and bristles of *N. diversicolor* as well as some other Nereididae were found in the samples due to cannibalistic behaviour. The ANOVA showed a marginal non significant effect for the sampling sites ($F_{2,894} = 2.70$, p = 0.07) and a non significant effect for the season of the year ($F_{2,894} = 1.84$, p = 0.14). Although, the same analyses revealed a significant effect of the interactions between variables ($F_{6,894} = 2.77$, p = 0.01), due to differences between CAR in Spring and in Winter (post-doc HSD tests for samples with different sizes, p<0.05).

The amphipod *Corophium* sp. is another animal found in the digestive tract of *N. diversicolor*, and the analysis indicates that there was a significant effect of the sampling sites ($F_{2,895} = 20.7$, p<0.001) on the percentage of *Corophium* sp. found. This effect is due to the fact that CAR (5.4 ± 1.19 %, n = 227) is different from ODX and from ALZ (0.02 ± 0.02 %, n = 220; 0.8 ± 0.32 %, n = 460) (post-doc HSD tests for

samples with different sizes, $p < 0.05$). There was also an effect of the interaction between the sampling station and the time of the year ($F_{6,895} = 2.23$, $p = 0.04$), due to differences between CAR and ALZ in Winter (post-doc HSD tests for samples with different sizes, $p < 0.05$). Other animals were found in the samples in all sampling sites besides *Corophium* sp. and Nereididae, but only in small quantities (Table 1), with the tendency to increase in the rainy seasons (Winter and Spring). This rise can be seen in Figure 4, being represented for all sites by the item (others). Figure 5 shows the graphs of the contents for each site and also a graph formed by the whole of the sampling sites. As opposed to Figure 4, all crustacean, polychaetes and gastropods found were grouped to emphasise the consumption of these items. ODX is the sampling site where the presence of these items in the contents was more uniform throughout the sampling period.

In ODX the slight increase in the item “others” in November and in April 1994 (see Figure 4), was due to the presence of the isopod *Cyathura carinata* and the ostracod *Cyprideis* sp. The diversity in ALZ was slightly higher than the previous sampling site where, beginning in January, parts of crustaceans were found, which were not identified because they were practically digested, as well as samples from the family Anthuridae, possibly *Cyathura carinata*. Whole *Sphaeroma hookeri*, *Cyathura carinata* and Chironomidae larvae were also found. However, in the sampling site CAR, in December 1993, February 1994 and April 1994 (see Figure 4), a representative amount of non- identified parts of crustaceans were observed as well as *Cyprideis* sp., and Isopods such as *Cyathura carinata* and *Sphaeroma hookeri*, in comparison with other sampling sites. Also notable was the extensive presence of Chironomidae larvae in this sampling site.

Body size and sex effects on diet

In the study of food preference by size classes of *N. diversicolor*, priority was given to the five main items in the contents (Table 2). From these, only mucus ($F_{2,904} = 5.26$, $p = 0.005$) and sand ($F_{2,904} = 6.37$, $p = 0.002$) showed a significant difference among the three sizes studied (S1, S2 and S3) (post-doc HSD tests for samples with different sizes, $p < 0.05$). Small size individuals (S1) feed on a higher quantity of sand ($S1 < S2 = S3$) and on a lower quantity of mucus ($S1 > S2 = S3$). The feeding strategy changed specially between average size (S2) to larger size (S3) animals, which

usually begin feeding higher quantities of mucus (see Table II). There were no significant differences in food contents between males and females (Table 3).

DISCUSSION

In all studied sites, *Nereis diversicolor* samples with the digestive tract completely empty were found. This fact has been observed for this and others Nereididae by the time of sexual maturity, when the gut has been reabsorbed and feeding has ceased (Golding 1987; Golding and Yuwono, 1994; Last and Olive, 1999). It can also be an indicator of a predatory feeding strategy, in which digestion is done more rapidly, as opposed to digestion of vegetable matter rich in cellulose (Gaston, 1987).

Throughout the duration of this study, mucus was the most frequent digestive content (Figure 4). It is important to emphasise that mucus is not by itself a food item. It is a food complex formed by the aggregation of phytoplankton, organic matter, bacteria and fungi to mucus. The differences in the amount of mucus present in the contents was found between CAR and the remaining sampling sites. Possibly due to the fact that this sampling site had low average percentages of organic matter on sediment surface ($0.7 \pm 0.06\%$), in comparison with ODX ($2.2 \pm 0.32\%$) and ALZ ($3.5 \pm 0.14\%$), makes it a less eutrophic environment. Another explanation for this result may be related to the scarcity of phytoplankton in the water layer near the bottom caused by occasional stratification, which can be clearly seen in Figure 2, and by the insufficient renewal promoted by the tidal effect, since the connection to the sea in this site is reduced. It is known that *N. diversicolor* acts as an active filter-feeder when the amount of food particles in suspension is high, through the production of a mucus net. When it is submerged, this feeding strategy may be more effective because there is a higher predation risk (Harley, 1950; Wells and Dales, 1951; Esselink and Zwarts, 1989; Vedel, 1998). In CAR, where the tide effect is lacking, due to its lagoon properties (except in November 1993 when a rupture of the sand barrier occurred), a higher quantity of mucus in the gut contents is expected. In CAR *N. diversicolor* adopts, due to the apparent lack of phytoplankton, a carnivore strategy, partially abandoning filtering through the mucus net. This fact can be explained by the reduced quantity of particulate material in the water column, or by a favourable feeding option.

The fact that the ingestion of mucus and the aggregated food items increases with the size of the individuals, and the ingestion of sand decreases (Table 2), may be due to an ontogenetic change in feeding habits. Smaller individuals with concomitant smaller burrows, acquire detritivore habits, feeding more frequently on the surface of the sediment. Possibly this strategy is due to the inability of obtaining enough food through filtration due to the reduced dimensions of its mucus net or to difficulties to bring enough water inside the gallery through dorsal-ventral pumping movements. According to Jørgensen (1975), in order to obtain enough food to cover the minimum requirements, evaluated by consumed oxygen rates, filter-feeding animals need to filter more than 10 l of water per ml of consumed oxygen. On the other hand, larger individuals with a better physical condition are capable of building deeper galleries and pumping higher quantity of water (Esselink and Zwarts, 1989). Thus, as size increases, filtration becomes the main mechanism to obtain food and a mucus net is produced and ingested more frequently.

Sand was another item observed frequently in the digestive tract contents of *Nereis*, which swallows large amounts of sediment in sweep-and-plough food-searching strategy. Like mucus it shows micro algae glued to its surface, but also meio- and micro-fauna existing in the interstitial space (Lucas and Betru, 1997; Tita et al., 2000). From all sampling sites, the differences found can be narrowed down to seasonal differences that reflect the more or less torrential character of the studied areas and, consequently, a greater or lower sediment carriage from marine and terrestrial origins. These seasonal changes in the sediment dynamics induced by different degrees of hydrodynamism and even by bioturbation, have an effect on the superficial layers in which *N. diversicolor* feeds on (Tamaki, 1987; Meadows et al., 1990; Turner et al., 1995; McLachlan, 1996). Feeding on the surface of the sediment has been observed in the field and laboratory and it varies depending on the quantity of food available. This practice is considered as a complement to filter-feeding due to the risks that it involves (Masson et al., 1995). This non-selective feeding habit reduces significantly the abundance of diatoms which play an important role in this type of system as the first level in the trophic chain (Smith et al., 1996). When comparing the chlorophyll *a* and phaeopigment levels (Figure 3A), to the % of sand ingested (Figure 4B), a clear connection does not emerge. This is possibly due to the fact that the distribution and abundance of several species of micro algae is controlled by a number of inter-dependent factors such as: temperature, light

penetration, pH, redox potential, tide level, salinity, consumption by herbivorous, sediment type and stability (Cadée and Hegeman, 1974; Pomeroy et al., 1981; Zedler, 1982). This fact makes it difficult to interpret this result based only on phytopigment data, which was collected on one day each month. In fact the effect of the environmental variables on the microphytobenthos is much more predictable and easy to quantify than the biological variables (Brotas et al., 1995). Sand may have also been overvalued due to the fact that it was extremely persistent in the digestive tract, as it is not attacked by the digestive processes like the other mentioned items.

Detritus has an important role as a food source both in the freshwater and marine benthic communities. It is made up of all kinds of biogenic materials such as bacteria, protozoa, micro- and macro-algae, parts of vascular plants in various degrees of decomposition which may contain potential energy to be used by consumer species (Tenore, 1977; Hansen and Kristensen, 1998). In the Southwest coast of Portugal the polychaete *N. diversicolor* is one of these consumers, which also uses detritus as a food source in all sampling stations, especially vegetable detritus such as root parts, algae, and parts of the macrophyte *Rupia* sp. According to Tsuchiya and Kurihara (1979) in a study done on *Neanthes japonica*, which, like *N. diversicolor* presents detritivore feeding, bacteria together with other micro organisms are responsible for the decomposition of the remains which will be used as a food source. In a more recent study on *N. diversicolor*, the conclusion was reached that it is also capable of degrading the bacteria along the digestive track, using as an additional food source in its diet (Lucas and Bertru, 1997).

The sampling sites ODX and CAR revealed variations in the percentage of occurrence of ingested remains throughout the year, possibly as a consequence of greater or smaller hydrodynamism. In ODX, it was observed that in February the detritus in the contents increased and the occurrence of mucus decreases. This may be explained by the fact that in this month rainfall was abundant (Figure 2A), which may increase the remains in the system, with a more torrential character (Magalhães et al., 1987). In CAR, the rise in the consumption of vegetable remains started in November 1993 (Figure 4), the only month where occurred the total emptying of the lagoon remaining only a few puddles. During this month filter-feeding was difficult due to the small amount of water available and so *N. diversicolor* fed on remains and roots of *Rupia* sp. as an alternative. The decomposition of these macrophytes

occurred as a result of their emergence and exposure to the sun. The increase in the vegetable remains consumed in the winter and at the beginning of spring seems to be caused by their transport to the system by the frequent rains during these seasons (e.g. Figure 2A).

Nereis diversicolor showed continuous reproduction in the three systems studied, with a recruitment in September 1993 and in May 1994, reaching 1126 ind.m⁻² averages in ODX, 957 ind.m⁻² in ALZ and 337 ind.m⁻² in CAR (Fidalgo e Costa et al., 1998). For this reason the presence of larvae and juveniles in the contents was frequent at almost all sampling points and in particular in ALZ (Figure 4). The digestive contents of *N. diversicolor* in the sampling sites studied confirm the results of the previous studies such as those of McIntosh (1907) and Bogucki (1953) which suggest cannibalistic behaviour in this species. The larvae, juveniles and the parts of individuals found in the gut contents in CAR suggest that cannibalism was stronger in Winter, although the greater number of larvae in nature was found in early Autumn (Fidalgo e Costa et al., 1998). This feeding strategy may also be facilitated by the absence of predators of *N. diversicolor* such as *Carcinus maenas*. The lack of this species, as an active predator of *N. diversicolor* (Cancela da Fonseca, 1989), in this lagoon was mentioned by Magalhães (1988) and confirmed during the sampling period. For this reason *N. diversicolor* promotes prolonged feeding journeys outside of the burrows, which together with gallery digging, favours the sediment turnover making it easier to find prey. *Corophium* sp. was one of the prey frequently observed in the intertidal environment in all sampling stations. In ODX and ALZ there was no significant predation on crustaceans. However in CAR there was active predation on *Corophium* sp. during the period studied, which can be confirmed by the pieces and individuals found in the digestive content (Figure 4). According to Jensen and André (1993) even the juvenile *N. diversicolor* have a negative effect on the juvenile *Corophium volutator*, in particular in lagonal environments such as CAR.

From the trophic point of view, *N. diversicolor* is an opportunist and a generalist species which adapts its foraging strategy to abiotic and biotic variations in space and time. The choice of the type of strategy seems to be related to the food availability, to the predation risk, and to food quality. In the future it would be interesting to verify in laboratory a trade-off between food preferences and predation

risk. It would also be interesting to determine in the field how predation affects the population structure in this species.

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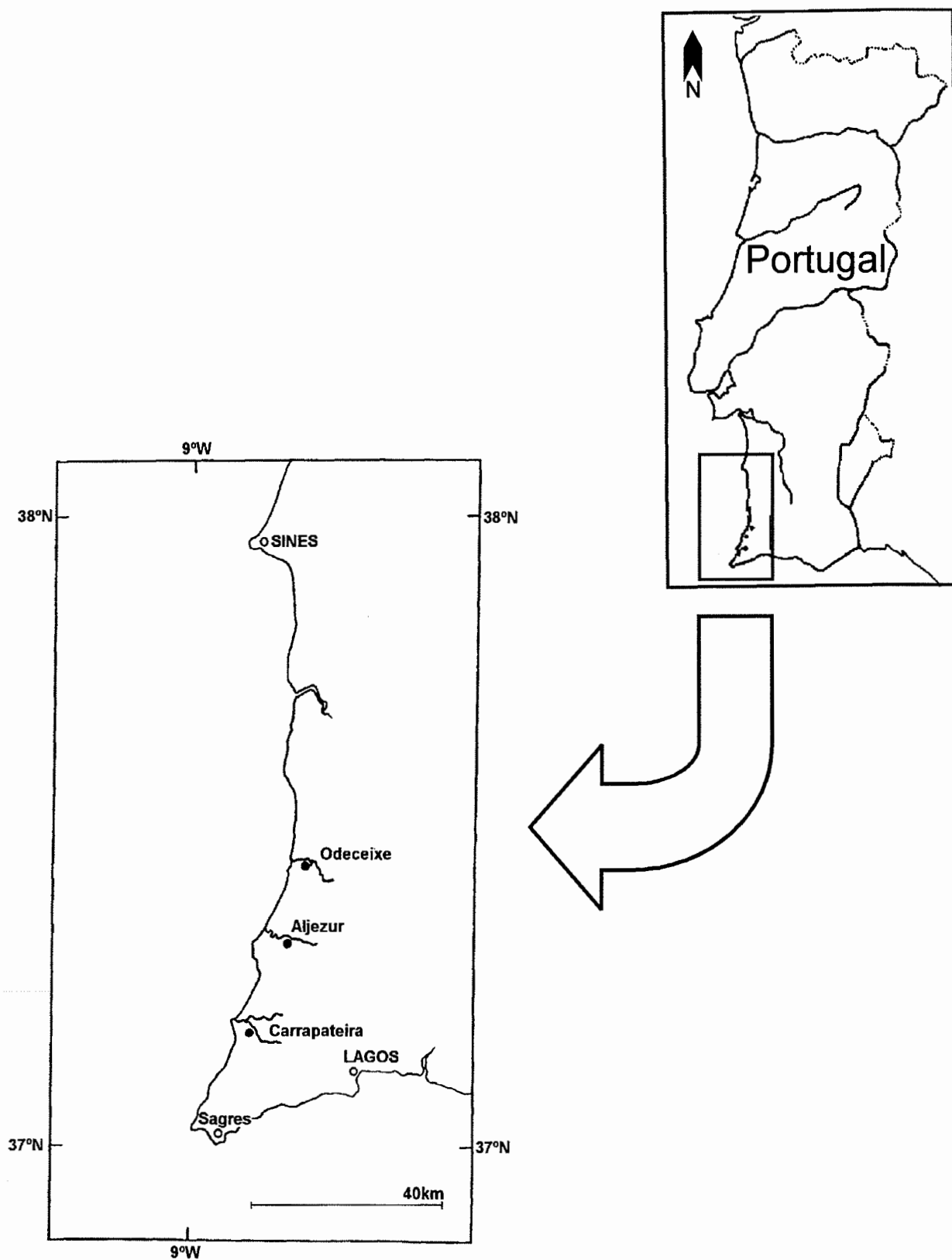


Figure 1 – Map showing the sampling stations on the south-western coast of Portugal.

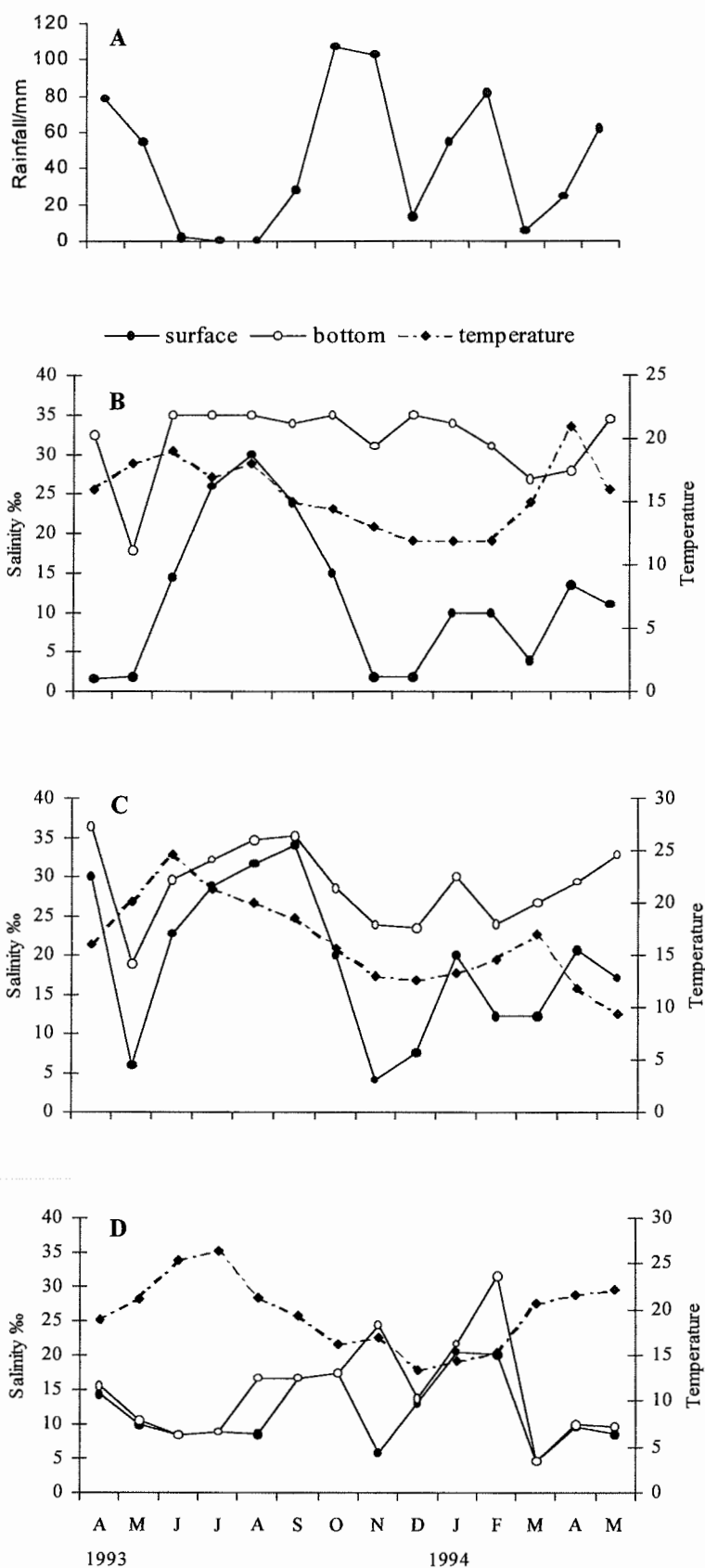


Figure 2 – A) Rainfall fluctuation on the South-western coast of Portugal; B) Monthly values of salinity (‰) and temperature (°C) in ODX; C) Monthly values of salinity and temperature in ALZ; D) Monthly values of salinity and temperature in CAR.

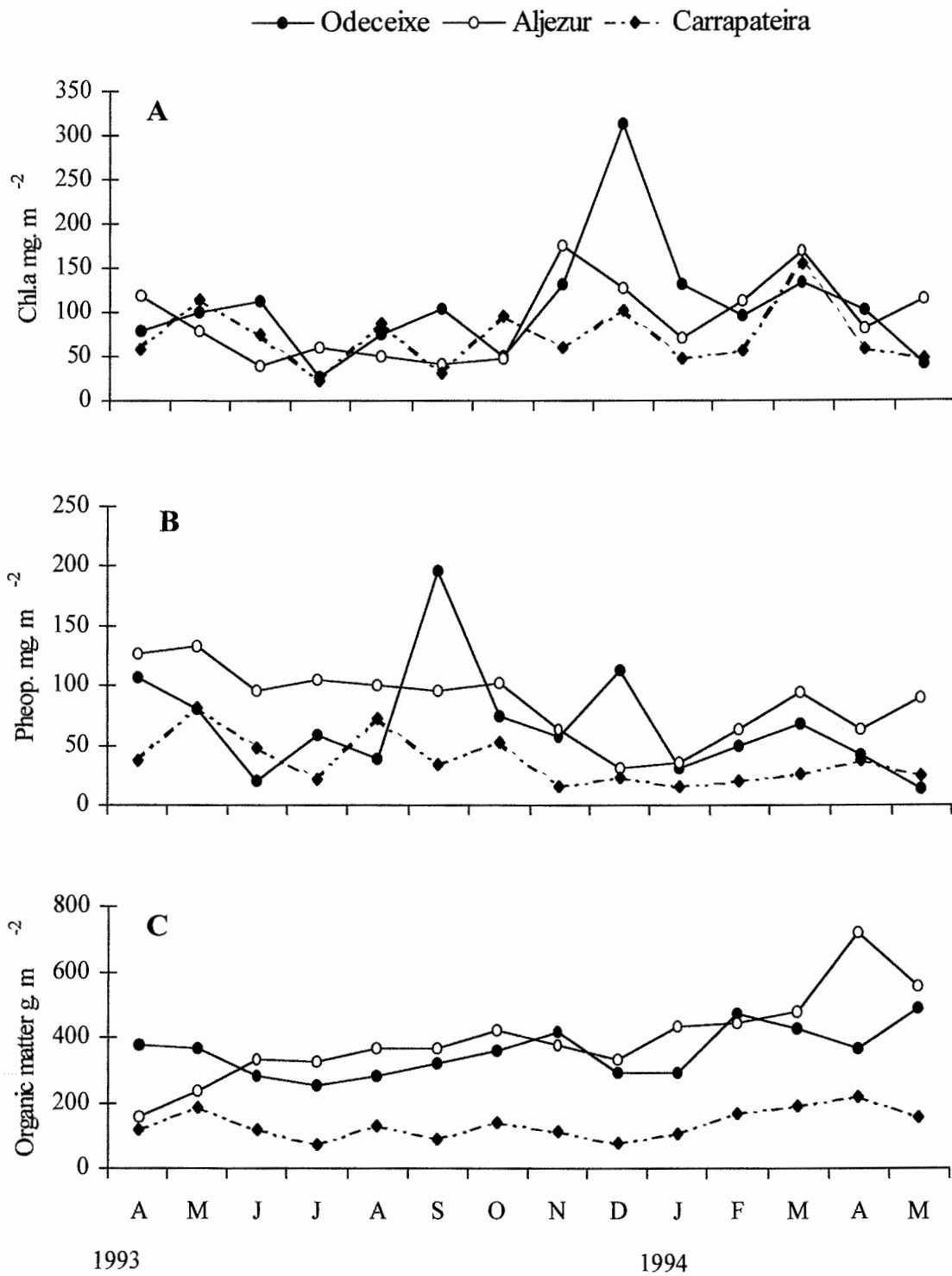


Figure 3 – A) Monthly chlorophyll a values (mg.m⁻²) in ODX, ALZ and CAR. B) Monthly phaeopigment values (mg.m⁻²) in ODX, ALZ and CAR. C) Organic matter content (g.m⁻²) in ODX, ALZ and CAR.

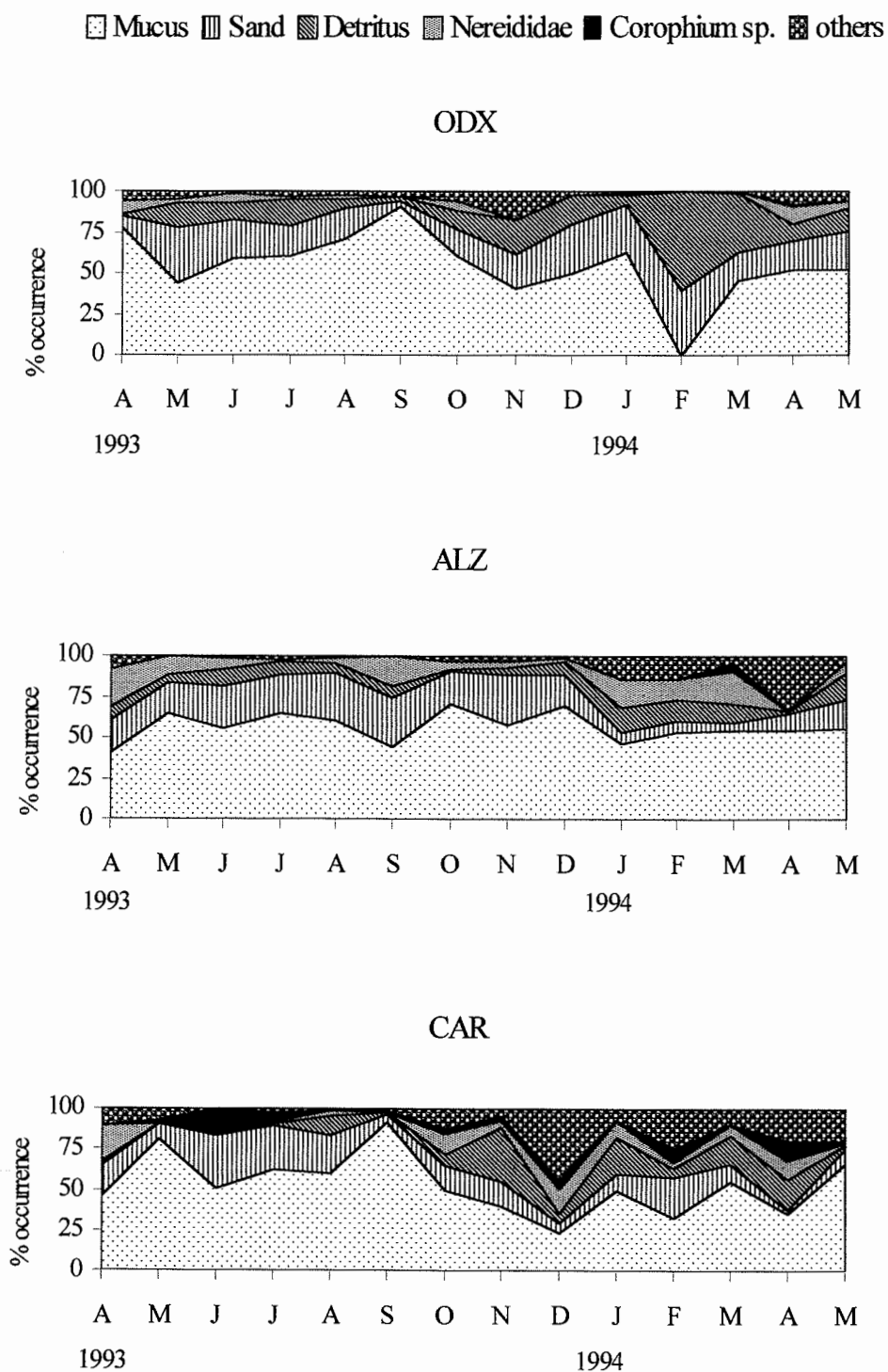


Figure 4 – Different food items ingested by *N. diversicolor* during sampling period in ODX, ALZ and CAR.

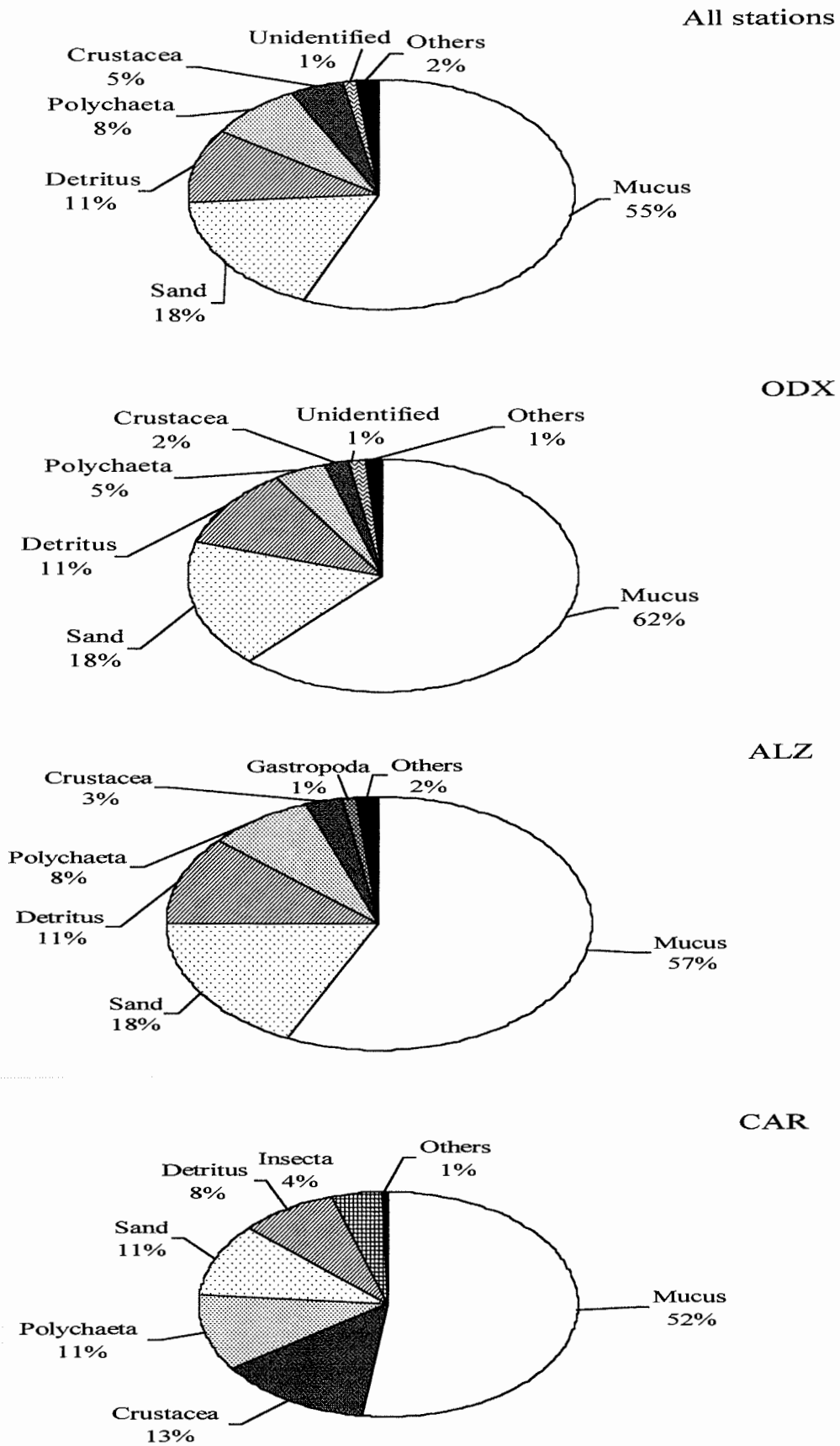


Figure 5 – Graphs representing the main gut contents of *N. diversicolor* (%) in the whole sampling stations and in each station separately.

Table 1 -
Gut content of *N. diversicolor* (%)
in all stations, with priority (**bold**) to
the main items.

Gut contents	%
mucus	56.33
sand	17.56
mud	0.08
unidentified	0.91
detritus	10.22
seaweed	0.05
<i>Rupia</i> sp.	0.41
Foraminifera	0.27
Gastropoda	0.01
Hydrobidae	0.71
Bivalvia	0.01
Annelida	0.12
Polychaeta	0.02
Nereididae	7.68
Spionidae	0.07
Capitellidae	0.03
Acari	0.01
Crustacea	0.44
Ostracoda	0.19
<i>Cyprideis</i> sp.	0.91
Copepoda	0.02
Cirripedia	0.01
Isopoda	0.18
<i>Cyathura carinata</i>	0.44
<i>Sphaeroma hookeri</i>	0.33
Anthuridae	0.08
Amphipoda	0.29
Corophium sp.	1.76
Gammarus	0.01
Insecta	0.87

Table 2 – Gut content of *N. diversicolor* (%) separated in (S1) small, (S2) medium and (S3) large size classes.

	S1		S2		S3		F2,904	p
	n	avg. ± se.	n	avg. ± se.	n	avg. ± se.		
Mucus	344	51.9 ± 1.90	542	58.8 ± 1.40	21	66.1 ± 7.05	5.26	0.0053
Sand		21.2 ± 1.52		15.5 ± 0.98		10.7 ± 4.78	6.37	0.0017
Detritus		8.9 ± 1.02		11.1 ± 0.80		10.4 ± 2.57	1.48	0.23
Nereididae		8.1 ± 1.33		7.4 ± 0.90		5.7 ± 3.71	0.22	0.79
<i>Corophium</i> sp.		2.7 ± 0.75		2.1 ± 0.33		0.7 ± 0.70	2.5	0.08

Table 3 – Male and female gut contents of *N. diversicolor* (%).

	♂		♀		t	p
	n	avg ± se.	n	avg ± se.		
Mucus	62	63.0 ± 4.3	159	58.5 ± 2.8	0.85	0.40
Sand		11.7 ± 2.6		12.6 ± 1.7	-0.28	0.78
Detritus		9.9 ± 2.4		11.1 ± 1.6	-0.40	0.69
Nereididae		5.9 ± 2.4		7.9 ± 1.9	-0.60	0.55
<i>Corophium</i> sp.		1.4 ± 1.3		2.5 ± 1.1	-0.61	0.54

Capítulo IV

Cultivo de *Nereis diversicolor* em condições laboratoriais

Artigo 5

REPRODUCTION AND GROWTH IN CAPTIVITY OF THE POLYCHAETE *Nereis diversicolor* O. F. MÜLLER, 1776 USING TWO DIFFERENT KINDS OF SEDIMENT: PRELIMINARY ESSAY.

Fidalgo e Costa, P.

Boletín del Instituto Español de Oceanografía, 15(1-4), 351-355 (1999).

Artigo 6

LABORATORY REARING OF *Nereis diversicolor* O.F. MÜLLER UNDER DIFFERENT CONDITIONS OF TEMPERATURE, SALINITY AND SEDIMENT TYPE.

Fidalgo e Costa, P., Passos, A.M. & Cancela da Fonseca, L.

(Artigo submetido à revista *Aquaculture*).

Artigo 7

GROWTH, SURVIVAL AND FATTY ACID PROFILE OF *Nereis diversicolor* O. F. MÜLLER, 1776 FED ON SIX DIFFERENT DIETS.

Fidalgo e Costa, P., Narciso, L. & Cancela da Fonseca, L.

Bulletin of Marine Science, 67(1): 337-343 (2000).

Reproduction and growth in captivity of the polychaete *Nereis diversicolor* O. F. Müller, 1776, using two different kinds of sediment: Preliminary assays

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ABSTRACT

The polychaetes used as bait for sport and commercial fishing are a natural resource exploited in a large number of estuaries. One of the most common species captured is the ragworm *Nereis diversicolor* O. F. Müller (1776). This species is also used in aquaculture, mainly because of its easy availability to local dealers, and plays an important role as a nutrient stimulating gonad maturation and spawning in hatcheries.

The high economic value of these worms, and the size of their catch in the main Portuguese estuaries, have led to their intense exploitation as commercial bait.

Accordingly, a small-scale reproduction study of this species was carried out under controlled conditions (temperature, salinity and luminosity) in two different sediments (sand and mud), following its development from the larval to the adult stages. Such elucidation of certain aspects of its life cycle is instrumental for the species's future culture in artificial systems. The present study represents the starting point of large-scale production that will reduce the anthropogenic disturbance to biological estuary communities. The main objective of this study was to obtain preliminary results that would enable us to understand the period of growth necessary to reach a commercially valuable size. Such growth was rapid in both types of sediment, taking only a few months.

Key words: *Nereis diversicolor*, reproduction, growth, sediments, culture.

RESUMEN

Reproducción y crecimiento en cautiverio del poliqueto *Nereis diversicolor* O. F. Müller, 1776 en dos tipos de sustrato: ensayos preliminares

Los gusanos de mar utilizados como cebo, tanto en la pesca profesional como en la deportiva, constituyen un recurso que es explotado en un gran número de estuarios. Uno de los gusanos más usados con esta finalidad es el nereido *Nereis diversicolor* O. F. Müller, 1776. Además de ser normalmente utilizada como cebo fresco, es una especie cada vez más empleada en acuicultura debido, por un lado, a la facilidad de su obtención en el mercado y, por otro, al importante papel que parece desempeñar en la maduración de las gónadas e inducción de las puestas en especies cultivadas.

El elevado valor económico asociado a la captura de estos nereidos en los principales estuarios portugueses ha provocado la intensificación de su extracción con fines comerciales.

En este ámbito se inició el estudio de la reproducción de esta especie a pequeña escala bajo condiciones controladas (temperatura, salinidad y luminosidad) en dos tipos de sustrato (arena y fango). Se estudió el desarrollo de la especie desde su fase larvaria hasta su estadio adulto, con la finalidad de conocer aquellos as-

pectos de su ciclo de vida que posibiliten su cultivo futuro en sistemas artificiales. Estos resultados constituyen el embrión para una posible producción a gran escala de este gusano, que posibilitaría, por otro lado, la reducción de la perturbación sobre las comunidades biológicas estuarinas. En este trabajo se presentan los resultados preliminares de este estudio. Los datos obtenidos permiten inferir que el crecimiento es muy rápido en ambos tipos de sedimento, alcanzándose en pocos meses las tallas comerciales.

Palabras clave: *Nereis diversicolor*, reproducción, crecimiento, substrato, cultivo.

INTRODUCTION

The ragworm *Nereis diversicolor* O. F. Müller (1776) is one of the species belonging to the Nereididae family that is used as commercial bait. There are several families (Arenicolidae, Eunicidae, Glyceridae, and Nephtyidae) exploited worldwide in sport and commercial fishing. The quest for these species is increasing rapidly, mostly due to their important role as a nutrient stimulating gonad maturation and spawning in hatchery-reared species, e.g. *Solea vulgaris* (Quensel, 1806), *Solea senegalensis* (Dinis, 1986), *Penaeus kerathurus* (Luis, 1989) and *Penaeus vannani* (Lytle, Lytle and Ogle, 1990).

These features, along with artificial fertilisation (Ozoh and Jones, 1990), apparently made these species the easiest ones to reproduce in captivity. In addition, the absence of a planktonic larval stage made the culture process cheaper.

The other main point of the culture and commercial use of this bait is to reduce the substrate harvesting disturbance, and the great biogeochemical and benthic community impact (Gambi *et al.*, 1994).

With the purpose of elucidating certain aspects of *N. diversicolor*'s life cycle, especially the sediment type suitable to its culture, a small-scale experiment was conducted under controlled conditions of temperature, salinity and light using two different kinds of sediments (sand and mud). In this assay, the development was followed from the larval to adult stages.

Experiments by Dean and Mazurkiewicz (1975) on polychaetes with benthic development, such as *N. diversicolor*, showed that some species may develop simply without sediment, while others prefer the same sediment used by adults. Some larvae stop growing and die when the type of sediment changes, showing an extreme sensitivity to this parameter. The elucidation of this and other questions regarding to culture of this species will make it possible, in the near future, to develop large-

scale artificial systems, like those already being developed with other species in the UK and the Netherlands (Olive, 1994). Large-scale culture provides not only an increasing market of live bait, but will also decrease the biological impact on estuarine communities, with lower environmental effects.

MATERIALS AND METHODS

Aquaria ($38 \times 45 \times 45 \text{ cm}^3$) with constant aeration were mounted, with a 15 cm sediment layer (sand and mud) from the estuary-lagoon systems of the southwest coast of Portugal (Aljezur and Carrapateira), locations where this species develops freely. Two different kinds of sediments were used (figure 1): medium sand with strong focus on classes 1 through 2 PHI (500-250 μm) proceeding from Carrapateira lagoon, and mud with high concentrations of silt and clay, but also mixed with some fine and very fine sand (3 and 4 PHI 125 and 63 μm) from the Aljezur estuary. The sediments of these aquaria were previously dried out in a heater (at 90 °C during 24 h) to eliminate other animals

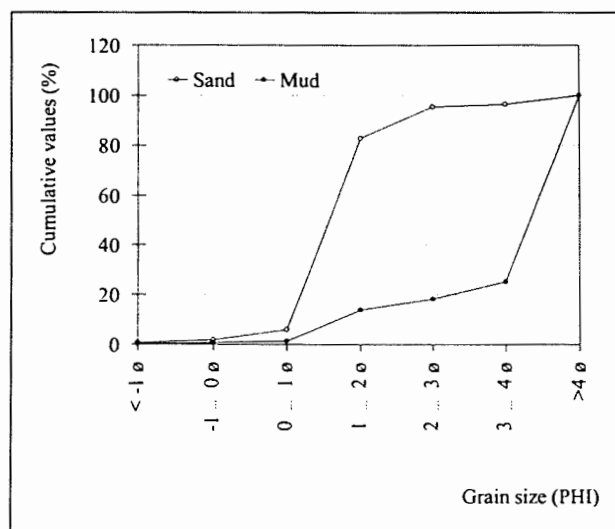


Figure 1. Grain-size distribution (cumulative curves) of the sediments (sand and mud)

(crustaceans, molluscs and annelids) and possible contamination that could interfere with the experiment. Due to the difficulty in establishing the sex of mature animals (Dales, 1950), 10 *N. diversicolor* adults (around 10 cm long) bought from a commercial dealer were transferred to each aquarium. Since it was impossible to establish gender, this number was used in order to guarantee a male/female presence in each aquarium.

During the experiment, water salinity was kept between 14 and 17 ‰, values considered ideal for this species's reproduction (Bartels-Hardege and Zeeck, 1990) and the temperature was 20 ± 2 °C (Duchon, 1971). To avoid the accumulation of catabolism products and consequent deterioration of the water quality, new water was added every week. The photoperiod was adjusted to a long photophase (16 h of daily light, LD 16:8) and a 100-lux intensity was supplied by a fluorescent light during the first month. Later on, a second lamp was installed that increased the luminosity to 175 lux.

Initially, each individual was fed every three days with commonly available commercial food used for tropical fish (Tetra Menu). This food type had been used successfully with this species (Garwood and Olive, 1981; Bartels-Hardege and Zeeck, 1990). After 22 days, they were fed daily, because some cannibalism was detected. To establish the beginning of the reproduction period, sediments sampled next to the gallery holes of the 'reproducers' were collected, since Bartels-Hardege and Zeeck (1990) stated that the eggs are fertilised there. Six individuals were collected 3 times a week from each aquarium and anaesthetised with MS-222, an anaesthetic commonly used on cold-blooded animals. Later they were measured (total length, from the antenna base to the anal cirrus insertion) using a binocular microscope and, when needed, a microscope. After measurements, the worms were stored in a plastic bag filled with nitrogen and lyophilised, being weighed afterwards to calculate biomass. The linear growth rate (mm/day) was calculated using the difference between the final average length and the initial one, divided by the total number of days of the experiment. The number of setae was recorded for each individual studied.

RESULTS

The first larval stages were not detected, which might be due to the fact that larvae stay inside the

gallery tubes protected by the females and attached to the mucus. Dispersion usually happens after the female's death (Bartels-Hardege and Zeeck, 1990), the larvae becoming more active when the tenth setae is fully developed (Clay, 1967). However, based on the number of setae of the first individuals found (8 parapodia and setae) the conclusion is that the hatching might have occurred about a month after the beginning of the experiment. The first eggs were observed in the samples taken after 4 weeks, so this was considered day zero, the beginning of the culture. The results (linear growth, weight growth and the increase in setae) are shown in figures 2 and 3; the linear growth rate obtained for both sand and mud are, respectively, 0.97 and 0.84 mm/day. Commercial size, according to observation at the local market, was obtained in 64 days (sand) and 62 days (mud). After 72 days, individuals were attained with a length of 7 cm (70 % of the initial reproducers' size).

DISCUSSION

The high rate of growth observed in this experiment (18 setae in 14 days, compared with Dales' (1950) result of individuals with 3 setae in 2 weeks) might be related to the fact that the culture temperature used was double the average one used previously. According to Neuhoﬀ (1979), only at temperatures close to 15 °C does *N. diversicolor* present a high growth rate. Olive *et al.* (1991), reporting on *Nereis virens* (Sars, 1835) culture, found that the growth rate is a complex function between the temperature and culture density.

Specimens showed a steady development, measured in terms of setigerous increase (lower standard deviation) in both sediments, as opposed to the one obtained with the linear or weight growth, where the deviation values are higher (figures 2 and 3). This reflects a morphological difference between individuals of the same age. This could indicate a problem for the commercial culture of *N. diversicolor*, which could be overcome by selecting the reproduction stock among those individuals with higher growth. The observed linear growth is very similar using both sediments, although the linear growth rate was higher in sand. There was also a clear gradual increase of total length, and consequently, of the biomass. Its standard deviation also increases, which explains what was mentioned pre-

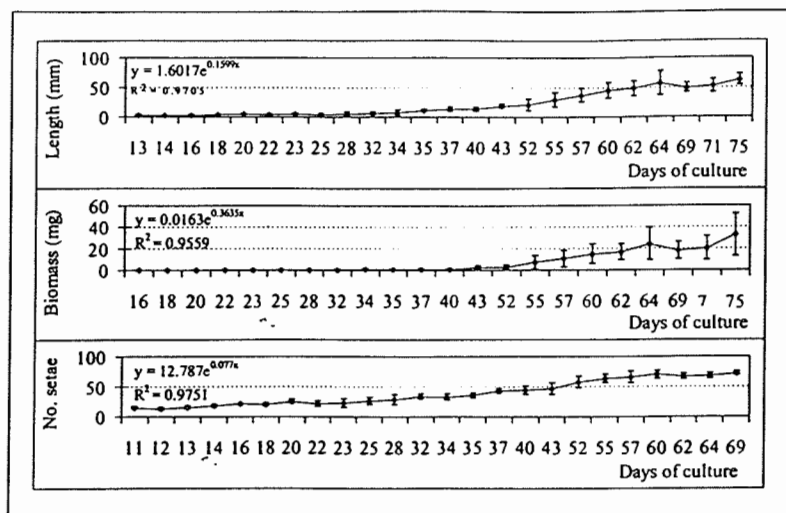


Figure 2. Growth of *N. diversicolor* using sand under controlled conditions. Temporal variation of average values (\pm standard deviation) of total length, biomass and setae number (the regressions adapted to the results are indicated)

viously about differences at an individual level. Bridges *et al.* (1996), working with *Nereis (Neanthes) arenaceodentata* (Moore, 1903), noted these differences with the start of competition for space and food, and also when the number of worms per square metre is still low, reflecting a positive influence on growth. When the density is high, growth declines. This increase (linear and weight growth) of some individuals becomes more and more evident over time, since the larger worms use their size for both food and territory control. When comparing the biomass graph with the one of setae numbers, in the last four days of culture, it is possible to infer that there is a strong weight difference among individuals, which cannot be verified if we compare it with the setae numbers, which are almost the same (figures 2 and 3).

It is noteworthy that there were 10 *N. diversicolor* in each aquarium, due to the lack of gender determination. Since it was impossible to previously determine the total number of males and females, consequently the number females that spawned was also unknowable.

Analysing the biological parameters (total length, biomass and number of setae) obtained using both substrates, and taking into account that the observation, handling and sampling of worms were easier in the aquaria using sand as substrate I suggest its use in future experiments.

Although preliminary, these results, combined with the high commercial value of this species, explain the importance that this resource may have in aquaculture, to both commercial trade (fishermen) and aquaculture professionals.

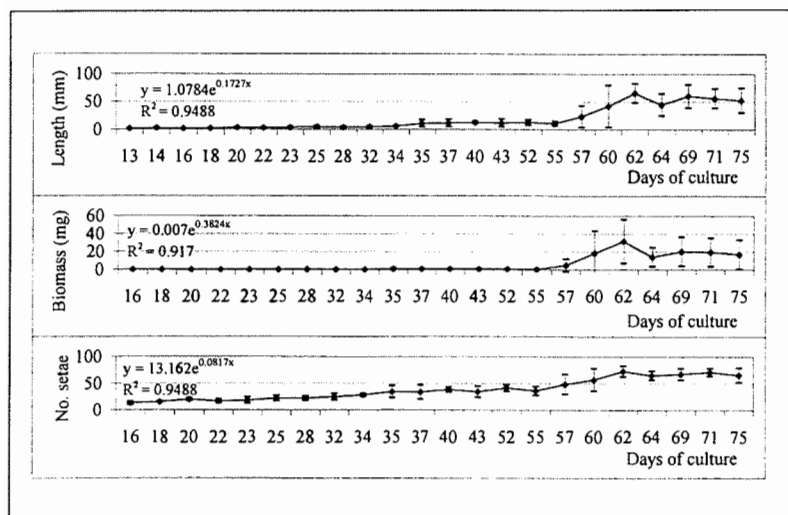


Figure 3. Growth of *N. diversicolor* using mud under controlled conditions. Temporal variation of average values (\pm standard deviation) of total length, biomass and setae number (the regressions adapted to the results are indicated)

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LABORATORY REARING OF *Nereis diversicolor* O.F. MÜLLER UNDER DIFFERENT CONDITIONS OF TEMPERATURE, SALINITY AND SEDIMENT TYPE

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Abstract

Commercial importance of polychaetes (Annelida) is increasing due to their use as bait for sport and professional fishing, and also as a nutritional resource used for fish (Soleidae) and crustacean (Penaeidae) aquaculture. In Portugal, the price of *Nereis diversicolor* reaches values higher than those of the edible finfish (eg. *Sparus aurata*), for what the culture of this worm may be profitable in a near future. Reproduction and growth in captivity of *N. diversicolor* was attempted successfully. Development from larvae to adult has been performed to the fourth generation. In order to optimise this culture, preliminary rearing experiments were undertaken during 60 days. The main goal was to establish the best rearing conditions of temperature, salinity and sediment type to enhance its growth and survival. Firstly, experiments on medium and fine sand, using 15 and 25 ‰ for salinity and 15, 20, 25°C for temperature, were carried out in the laboratory. Kruskal-Wallis tests showed no significant differences in growth and survival between trials using different type. Then, medium sand was chosen as the best sediment due to easy observation and handling of the individuals by reducing the water turbidity and improving water quality. Two extreme salinity conditions, 5 and 35 ‰, were tested under initial temperatures. The results demonstrated that 15‰ and 25° C were the best conditions for both growth ($6.31 \pm 0.33 \%d^{-1}$) and survival (100%). All the experiments have been carried out 30 days after hatching. Each salinity and temperature conditions were combined, and three replicates with six individuals were used per treatment. Worms were fed on commercial Tetramim® flakes, and the photoperiod adjusted to LD 16:8 with an average light intensity of 175 Lux. At the end of the experiments all worms were counted and the coelomic content analysed as to determine the development of oocytes.

Keywords: *Nereis diversicolor*; Rearing; Salinity; Sediment type; Temperature

Introduction

Commercial interest for annelids is becoming more significant due to their use as fresh bait in sport and professional fishing. Its use in nutrition in aquaculture of crustacean (Penaeidae) and fish (Soleidae) is also increasing due to its important role in the development of the gonads. In Portugal, the price of *Nereis diversicolor* O.F. Müller reaches values higher than those of the edible finfish (eg. *Sparus aurata*) (Diniz, 1986, Croz, et al., 1988, Luís, 1989, Lytle, et al., 1990).

There are several species used currently as fresh bait, caught manually in many coastal environments throughout Europe. Baitdigging is done without any environmental consideration, causing severe disturbance in these areas. One of these species frequently found in estuaries and lagoons is the polychaete *N. diversicolor* (Jensen, 1988, Ozoh and Jones, 1990, Ozoh and Jones, 1990, Arias and Drake, 1995). This species is known because it tolerates a large range of salinities, temperature, and dissolved oxygen (Mettam, 1979, 1981, Neuhoff, 1979, Bartels-Hardege and Zeeck, 1990, Gillet, 1990) and it is found living and breeding in different sediment type (Clay, 1967, Wolff, 1973, Kristensen, 1988, Grant, 1992, Fidalgo e Costa and Cancela da Fonseca, 1998).

The aquaculture of Polychaeta in Europe began in the Netherlands and in the United Kingdom and it has been developing in the last few years. However the demand is still higher than the offer (Olive, 1994). Its implementation in different countries aims, besides profit, to prevent the intense dilapidation of the natural stocks. This culture can be of the intensive type, like it has been done in the United Kingdom with *Nereis virens*, or a semi-intensive culture using residual waters from an aquaculture which are rich in pseudo-faeces and in food residues. In contrast, the integrated aquaculture system, which has predominantly been adopted in Asia and Oceania for other species, produces reasonable quantity of biomass with reduced environmental impacts (Johannes, 1978, Ruddle and Zhong, 1988, Folke et al., 1998).

N. diversicolor has some traits that ease its growth, such as a totally benthonic life-cycle, an easy artificial fertilisation and lecithotrophic larvae (Dales, 1950, Wolff,

1973, Bartels-Hardege and Zeeck, 1990, Ozoh and Jones, 1990). This last trait results on the delaying of the beginning of feeding, which allows the larvae to be fed early on with the same food given to adults (Reish, 1974). The main goal was to establish the best rearing conditions of temperature, salinity and sediment type to enhance its growth and survival.

Materials and Methods

Experimental design

After previous experiments in reproduction and growth in captivity done in aquariums (Fidalgo e Costa, 1999), development from larvae to adult has been performed to the fourth generation. Aiming to clear and improve some aspects related to that culture, an experimental development was initiated and lasted for a total 60 days with specimens of *N. diversicolor* born in captivity. All the experiments have been carried out 30 days after hatching, with animals weighting in average $32.7 \pm SD 19$ mg of fresh weight. This fact was a rather unusual procedure in this species, since larger size individuals captured in nature are more frequently used. The only food item provided since the larvae began feeding was the commercial Tetramim[®], used successfully in polychaete cultures (Reish, 1974, Garwood and Olive, 1981, Guérrin 1987, Bartels-Hardege and Zeeck, 1990, Fidalgo e Costa et al., 1999). The photoperiod was maintained to a long photophase (16 hours of light daily, LD 16:8), and an intensity of 175 lux provided by a fluorescent lamp. Feeding became *ad libitum* according to Guérrin (1987). Two litre plastic cups were used as culture containers with 6 cm of medium sand (500–250 μm) and fine sand mixed with some silt (250–63 μm). Three replicates with six individuals were used per treatment, corresponding to a density of about 390 ind. m^{-2} , similar to the average density found in nature, more specifically in the Carrapateira estuary, Southwest coast of Portugal (Fidalgo e Costa et al., 1998). The containers were filled with salt water previously filtered with 50, 10, 5, 1 e 1 μm nets and kept constantly aerated. Salinity was then corrected in order to obtain values of 15 and 25‰, and the tested temperatures were respectively 15, 20 e 25°C. These salinity values, and in particular, the temperature chosen to this experiment are close to the average values obtained in nature where *N. diversicolor* grows and breeds (Fidalgo e Costa et al., 1998). Afterwards, and after establishing that medium sand was the best type of sediment to use, two extreme salinity

conditions were tested (5 e 35‰) under initial design (density, replicate numbers and temperatures). All individuals used were weighted at the beginning and at the end of the experiment, and specific growth rates (μ , d^{-1}) were calculated according to Jørgensen (1990) and Riisgård et al. (1996) using the equation:

$$\mu = \ln (W_t/W_0)t^{-1}$$

where W_0 and W_t correspond to body mass (fresh weight) at day 0 and at day t respectively. To enable the comparison of the results with other authors, the specific growth rates were converted to percentages ($\%d^{-1}$). At the end of the experiment all individuals were and counted to establish survival rates. To determine the type of sediment in which *N. diversicolor* had better growth and survival rates, data values were log-transformed and Kruskal-Wallis tests was used. The Dunn test was applied afterwards to determine what are the best temperatures and salinities. The program used to the statistical analyses of the data was Statistics 5.0 for Windows.

Oocytes measurement

The individuals that revealed a green colour, typical aspect of an advanced maturation stage (Dales, 1950, Clay, 1967), had their coelomic content observed using a magnifying glass in order to determine their sexual maturation stage. For each female, a number of 36 oocytes were measured; following this proceeding a representative sample of different oocytes size is secured, while techniques similar to those used by other authors are maintained (Mettam, 1979, Olive and Garwood, 1981).

Results

The type of sediment tested, medium and fine sand, do not cause a differentiated effect on the growth of *N. diversicolor* (Fig. 1) Using the Kruskal-Wallis test, the values obtained do not reveal significant differences ($H(1, n=190)$, $P=0.236$). For this reason it was decided to test two new salinities (5 and 35‰), considered extreme when compared with the values registered in the previously studied estuaries (Fidalgo e Costa et al., 1998). Medium sand was chosen for this test, because it presents practical advantages over the fine sand when used in culture. Medium sand

does not disturb water, making it easier to observe the worms and also decreases the risk of anoxia and consequently loss of water quality.

The results obtained in medium sand reveal significant differences in the growth of *N. diversicolor* between the tested temperatures (Fig. 2) (Kruskal-Wallis: $H(2, n=190) = 7.63, P < 0.05$ test). However, in specific terms, this differences were significant between 15 and 25°C (Dunn: $Q = -2.733, P < 0.05$ test). Survival rate of 100% was obtained with 15°C temperature in all salinities tested, and the higher daily growth rate was found in 15‰ at 25°C, and with a value of $6.31 \pm 0.33 \%d^{-1}$. Concerning weight growth, there was an average increase between 32.7 mg wet weight and 440.4 mg wet weight in the whole experiment, although on 15‰ and 25°C, growth average was much higher, between 13.1 mg wet weight and 541 mg wet weight.

Regarding salinity (Fig. 3), the growth differences were also significant (Kruskal-Wallis: $H(3, n=190) = 10.986, P < 0.025$ test) in salinities of 15 and 35‰ (Dunn: $Q = 3.256, P < 0.01$ test).

By observing sexual maturation through oocytes diameter, we can say that all females reared in the highest temperature (25°C) were almost at the final stage of maturation with oocyte diameters of $198 \pm 37.8 \mu m$ in all salinities and sediments. Also, the individuals reared in 20°C showed a high oocytes development with an average diameter of $202 \pm 37.8 \mu m$. In the lowest temperature tested (15°C) the individuals reared in 5‰ e 15‰ were not matured. However, when reared at 25‰ and 35‰ *N. diversicolor* showed oocytes in development with an average diameter of $123 \pm 28 \mu m$.

Discussion

With this study we were able to conclude that *N. diversicolor* has a high growth rate in the sediment, salinities and temperatures is which it was tested. This high growth rate becomes more obvious in the first months of development, as seen in this experiment, but also throughout the first year of life, as seen in wild samples of this species (Fidalgo e Costa et al., 1998). After the first year of life, *N. diversicolor* concentrates in sexual maturation, reducing growth rates (Heip and Herman, 1979,

Kristensen, 1984). The fact that there are no significant differences in the growth of *N. diversicolor* between the type of sediment tested, confirms that this factor, although extremely important to species of benthonic development (Dean and Mazurkiewics, 1975), does not have a clear influence in the growth of this species. It is known that in wild state *N. diversicolor* grows and reproduces in different types of sediment without any preference (Kristensen, 1988, Miron and Kristensen, 1993, Fidalgo e Costa, 1994, Fidalgo e Costa and Cancela da Fonseca, 1998, Fidalgo e Costa, 1999). On the other hand, the same tests, reveal a preferential adaptation of this species to brackish environments with mean salinities (Neuhoff, 1979; Gillet, 1990; Miron and Kristensen, 1993a, 1993b), showing better survival rates in 15‰ salinities. The highest growth rate of *N. diversicolor* is obtained at 25°C .

The growth rates obtained in this study ($6.31 \pm 0.33 \%d^{-1}$) can be compared to the ones obtained by Esnault et al. (1990) with larger individuals fed with Tetramin® ($4.0-6.0 \%d^{-1}$), Riisgård et al. (1996) which supplied *Rhodomonas* sp. as food ($4.9 \pm 0.8 \%d^{-1}$) and by Nielsen et al. (1995) that used shrimp ($7 \%d^{-1}$). These growth rates reflect the controlled conditions to which these organisms are submitted and the importance of food in the growth and development of this species. The differences in growth rates become more obvious when animals reared in captivity are compared with those growing in wild (even within individuals with the same age). This fact can be observed in the wild populations in the Southwest coast of Portugal where the average growth rates found were of $1.13 \%d^{-1}$, even for individuals with less than a year (Fidalgo e Costa et al., 1998). Considering that breeding in this species was continuous throughout the year, with new individuals becoming part of the population every month, the growth rates were obviously underestimated. Similar values were obtained to *N. diversicolor* by Chambers and Milne (1975) with $1.5 \%d^{-1}$ to the Ythan estuary in Scotland. Riisgård et al. (1996) found a growth rate of $2.5 \pm 0.8 \%d^{-1}$ in a coast bay in Denmark. The best growth rate was obtained by Vedel and Riisgård (1993), with $3.9 \%d^{-1}$ individuals captured in a eutrophicated fjord, also in Denmark. These values were similar to the ones found by Heip and Herman (1979), with a rate of $4.2 \%d^{-1}$, obtained in a population in a small brackish water lagoon without any connection to the sea.

Based on this study and on the one done previously (Fidalgo e Costa, 1999), we concluded that sand is the best option for the culture of *N. diversicolor* because it is

easier to observe and to collect the reared organisms. On the other hand, it functioning as biological filter, and also decreases the risks of the anoxia associated to the thinner sediment and with higher percentages of organic matter (Nichols, 1970, Cancela da Fonseca, 1989). Regarding temperature, 25°C seems to be the best choice, combined to a salinity of 15‰. This combination revealed the best results.

The variety in the oocytes size distribution in individuals within a population serves as an indicator: the smaller the difference between the oocytes size, the closest is the spawning season (Olive and Garwood, 1981, Fischer, 1984). Although at the end of the experiments all individuals were about three month old, all revealed an advanced maturation stage, specially the individuals reared at 25°C and 20°C, which do not reveal differences in oocytes size. Precocious maturation seems to be related to food offer, because, according to Grémare et al. (1988) in a study done on polychaetes from another species, increasing the food offer results in a premature maturation of females and in an increasing of the number of eggs. Guérin (1987) in a study done on worms fed with Tetramin[®] as well, obtained a rapid growth, a precocious maturation and a lower lifetime. According to this author, Tetramin[®] has the nutritional requisites targeted to the larvae stages, interfering with growth speed control. Overfeeding, besides providing a fast growth of individuals in culture, favours a fast and precocious maturation. This singular growth and ripening can be used in future studies to obtain larvae intensively. However, it will be necessary a detailed study of the success of these gametes and the impact on gametogenesis promoted by extremely accelerated maturation. On the other hand, it is yet to solve a fundamental aspect in a future culture of *N. diversicolor*, the morphological differences between individuals with the same age. This fact is frequently observed in animals born in laboratory (Fidalgo e Costa, 1999), and it is extremely important to continuously select the parenting among the larger sized animals in an attempt to minimise the negative impact in the culture and commerce of this species.

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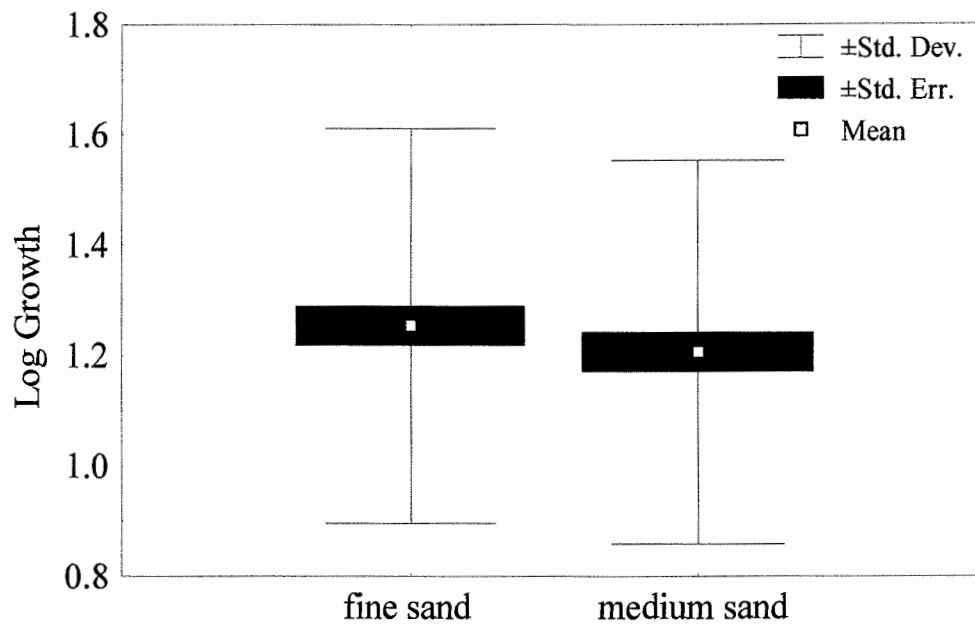


Fig. 1 – *Nereis diversicolor* growth on fine and medium sand.

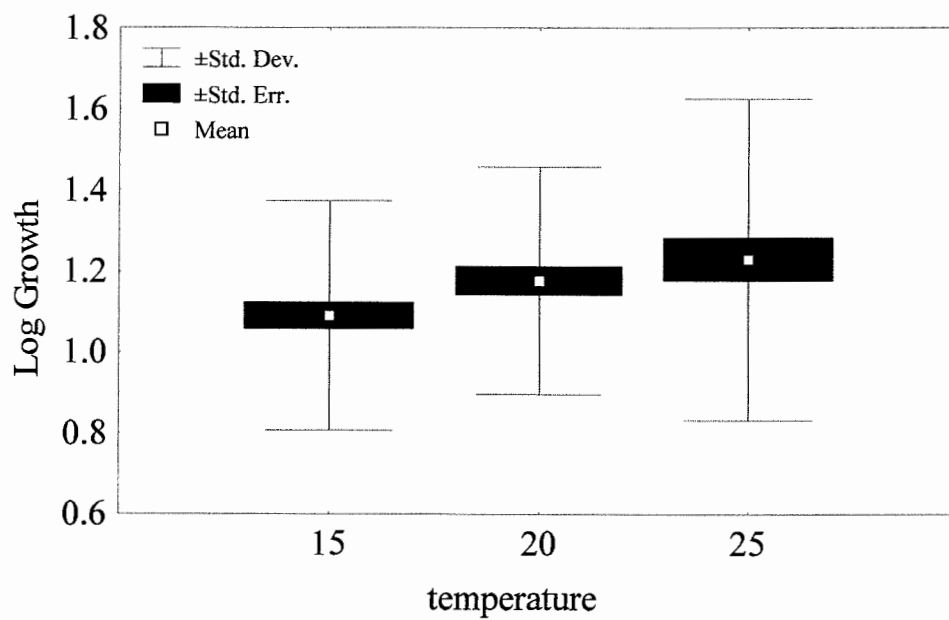


Fig. 2 –*Nereis diversicolor* growth under different temperatures (°C).

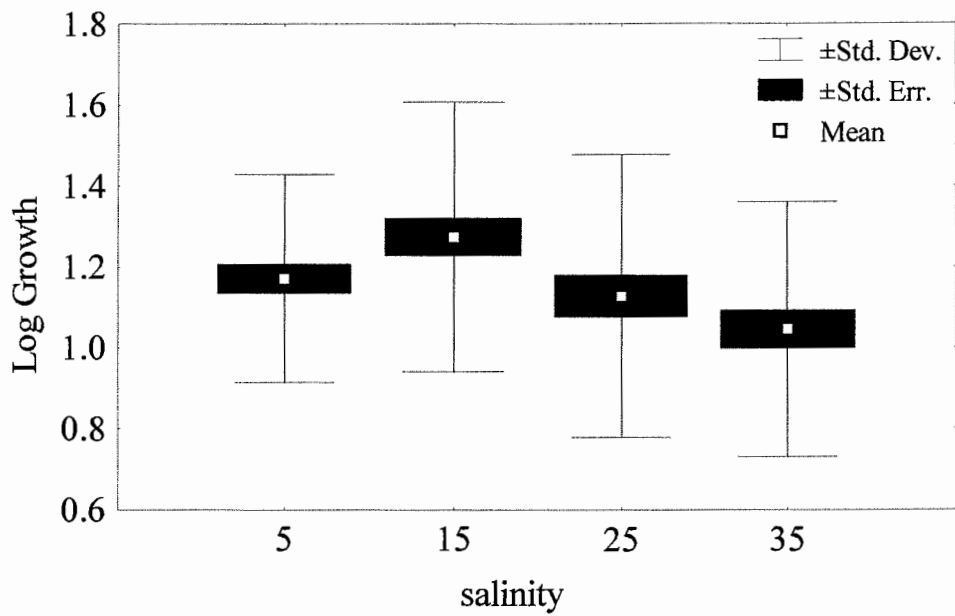


Fig. 3 – *Nereis diversicolor* growth under different salinities.

GROWTH, SURVIVAL AND FATTY ACID PROFILE OF
NEREIS DIVERSICOLOR (O. F. MÜLLER, 1776)
FED ON SIX DIFFERENT DIETS

P. Fidalgo e Costa, L. Narciso and L. Cancela da Fonseca

ABSTRACT

Cultured *Nereis diversicolor* are used as food for flat fish and shrimp on aquaculture farms. In order to improve cultures of *N. diversicolor* we determined the growth and survival rates of juvenile worms fed with six different diets for 60 d in the laboratory. Experiments were carried out with 1 mo old worms and fed every other day ad libitum. The six diets were: decapsulated *Artemia* cysts, sea bream dry food, Lansy (diet for late post larval stage of shrimp), Tetramin (fish dry food), extruded soy and pollen. The photoperiod was adjusted to 16L:8D with an average light intensity of 175 Lux. Temperature and salinity were maintained at $20 \pm 2^\circ\text{C}$ and 15‰, respectively. Three replicates were setup for each treatment with six individuals per replicate. Worms fed Lansy had the highest daily growth rate (13.94 mg d^{-1}) and 100% survival rate. Each type of food was analyzed for organic matter, caloric and total lipid content. The organic matter content of sediments was also analyzed. No direct relationship between growth and dietary type was noted. The fatty acid content of the worms was related to the specific diet. Higher levels of the total lipids, DHA (docosahexaenoic acid) and EPA (eicosapentanoic acid) were more closely related to the 1 mo old juvenile worms. However, when diets have a high DHA/EPA ratio, there does not appear to be any relationship to juveniles which seems to indicate that *N. diversicolor* does not have a high DHA requirement. In any event we believe that higher HUFA (high unsaturated fatty acid) levels (mainly EPA and DHA) result in higher survival rates and better growth rates.

Polychaetes are gaining in importance commercially because they are used as bait for sport and professional fishing and as a food source in aquaculture (Gambi et al., 1994; Olive, 1994). Commercial harvesting of polychaetes causes disturbance to the benthic community and the ecosystem. If harvested polychaetes are shipped to areas where the species is not native, there is the risk of introducing an allochthonous species to that region (Gambi et al., 1994). Commercial rearing of polychaetes offers a solution which is both ecologically and environmentally sound to reduce potential environmental problems of harvesting specimens from the field.

The ragworm *Nereis diversicolor* (O. F. Müller, 1776) is typically an inhabitant of estuarine mud flats in Europe where it is one of the commonest of all shore polychaetes (Chambers and Milne, 1975; Heip and Herman, 1979; Mettam, 1979; Olive and Garwood, 1981; Fidalgo e Costa, 1994; Fidalgo e Costa and Cancela da Fonseca, 1995). This species is nearly restricted to the littoral zone where it lives in a U-shaped burrow in the sediment. Researchers have reported that *N. diversicolor* utilizes a variety of feeding strategies: as a herbivore feeding on diatoms and other micro-organisms (Harley, 1956 in Evens, 1971); a predator or scavenger (Olafsson and Parsson, 1986; Heip and Herman, 1979); omnivore (Neuhoff, 1979) and facultative filter-feeder (Riisgård, 1991; Riisgård et al., 1992; Vedel and Riisgård, 1993; Riisgård et al., 1996). It has recently been discovered that if the phytoplankton concentration is sufficiently high, this species shifts from predatory/surface deposit-feeding to suspension-feeding (Nielsen et al., 1995). Therefore, *N. diversicolor*

can utilize different types of food, both in the field and under laboratory conditions (Neuhoff, 1979; Garwood and Olive, 1981; Guérrin, 1987; Bartels-Hardege and Zeeck, 1990; Esnault et al., 1990; Vedel and Riisgård, 1993; Nielsen et al., 1995; Riisgård et al., 1996; Vedel, 1998).

The fatty acid content of *N. diversicolor* seems to play an important role in stimulating gonad development and spawning in the hatchery-reared common sole *Solea vulgaris* (Flüchter and Tromsdorf, 1974), Senegalese sole *Solea senegalensis* (Dinis, 1986) and penaeid shrimp *Penaeus kerathurus* (Luis and Ponte, 1993; Luis, 1989). The purpose of this experiment was to determine growth and survival rates of *N. diversicolor* fed with six different diets in the laboratory and to determine which food produces the best growth rates as related to the fatty acid composition. These results will be of importance in supporting flat fish and shrimp broods in aquaculture farms.

MATERIALS AND METHODS

One-month old laboratory reared worms were fed one of six different types of food every other day ad libitum for 60 d following the procedure of Guérrin (1987). The diets were: decapsulated *Artemia* sp. cysts, sea bream dry food (SBDF), Lansy (diet for late post larval stage of shrimp), Tetramin (fish dry food), soy and pollen. There were three replicates, with six worms per replicate, for each diet. Worms were placed in 2-L containers. The containers were aerated and the water changed weekly to maintain good water quality. Containers were examined daily and dead animals were removed and the number recorded. Natural sand (250–500 μm) was dried in a heater at 90°C for 24 h to kill any organisms present. The sand was placed in the container to a depth of 5 cm. The photoperiod was adjusted to 16L:8D with an average light intensity of 175 Lux. Temperature and salinity were maintained at $20 \pm 2^\circ\text{C}$ and 15‰, respectively.

At the end of the experiment, all worms were weighed individually (fresh weight) and the growth was expressed in two ways: (1) by the daily growth rate (mg d^{-1}), obtained by the difference between the final and the initial weight average, divided by the number (60) of days and (2) the specific growth rate (μd^{-1}) according to Jørgensen's formula which is: $\mu = \ln(W_t - W_0) t^{-1}$ where W_0 and W_t are the average biomass of the polychaetes on day 0 and day t, respectively, divided by the number of days (60). This measure of growth has been used with *N. diversicolor* by Jørgensen (1990), Riisgård (1991), Riisgård et al. (1992, 1996), Vedel and Riisgård (1993) and Nielsen et al. (1995).

CHEMICAL ANALYSIS.—Organic matter content of the sediments and diets were measured in triplicate at the end of the experiment by loss on ignition for 24 h at 450°C (Cancela da Fonseca, 1989). A 1425 Semimicro Oxygen Bomb Calorimeter was used to measure the caloric content of diets.

Fatty acid compositions were determined by capillary gas chromatography (GC). The lipid extracts were saponified for 40 min under nitrogen at 100°C with 0.5M KOH in methanol. The resultant fatty acids were recovered and their methyl esters prepared by reaction with a 14% $\text{BF}_3/\text{CH}_3\text{OH}$ solution for 8 min at 100°C under nitrogen (Metcalf and Schmitz, 1961). The fatty acid methyl esters, after solvent evaporation, were recovered in 2 ml of isoctane. They were analyzed by GC (Varian 3300) using a split 12:1 and separated on a 30 m WCOT SP1000 fused silica 0.32 mm i.d. capillary column, operated for 7 min at 180°C then programmed at 4°C min^{-1} to 200°C with 1 ml min^{-1} helium carrier gas. Injector port and Flame Ionization Detector (FID) were kept at 250°C. Column performance was monitored by routine injection with a secondary standard of fatty acid methyl esters prepared from cod-liver oil.

RESULTS

SURVIVAL AND GROWTH EXPERIMENTS.—The greatest growth rates were obtained when worms were reared when the salinity was 15‰ (Nielsen et al., 1995). Survival of *N. diversicolor* was 100% when fed Lansy, sea bream and soy, 94.4% with pollen, 89% with Tetramin and 78% with *Artemia* cysts. Growth rate as mg d⁻¹ was greatest when fed Lansy (13.9 mg d⁻¹) followed by SBDF (9.4), Tetramin (9.4) and soy (8.8). Low growth rates were measured on diets of pollen (6.9) or *Artemia* cysts (6.2). The calculated daily growth rate was greatest on diets of Lansy and Tetramin ($\mu = 0.068$ d⁻¹) followed by SBDF (0.061), soy (0.058), *Artemia* cysts (0.054) and pollen (0.053) (Table 1). These results showed that *N. diversicolor* was able to survive with a single food source.

Table 1. Growth rates and survival of *Nereis diversicolor* fed on six different diets.

Diet	Growth rate (mg/day)	Specific growth rate (d ⁻¹)	Survival (%)
Lansy	13.9	0.068	100
SBDF	9.4	0.061	100
Tetramin	9.4	0.068	88.8
soy	8.8	0.058	100
pollen	6.9	0.053	94.4
<i>Artemia</i>	6.2	0.054	77.7

ORGANIC MATTER, CALORIC CONTENT AND TOTAL LIPIDS.—The organic matter content was higher in worms when fed prepared diets than from sediments except for soy (Table 2). This indicates that nearly 50% of the organic matter present in the diets was used by the worms and the remainder either secreted as mucus with in tube construction excreted in feces. *Artemia* cysts had the highest caloric value and pollen the lowest.

The fatty acid composition of the prepared diets and the worms fed on different diets is given in Table 3. The majority of the fatty acids in *N. diversicolor* in all tests were C 16:0, C 18:1n-9, C 18:1n-7, C 18:2n-6 and C 20:5n-3. Lesser amounts were C 16:1n-7, C 18:0, C 18:3n-3, C 20:2n-6 and C 20:4n-6. The fatty acid composition in the worms was unsaturated. The fatty acid composition of the rag worms reflects its diet. *N. diversicolor* is apparently able to biosynthesize de novo some fatty acids such as C 20:5n-3 (EPA) and C 22:6n-3 (DHA). Animals fed on diets low in EPA and DHA (soy and pollen) always had higher amounts of DHA than the food. However, when worms were fed diets rich in DHA (sea bream or Lansy), the DHA profiles were lower than the food. This is indicated in the DHA/EPA ratios which were always lower than 1 even when the diet had a higher ratio (Table 3).

Table 2. Caloric and organic matter content of the diets and organic matter content found in the sediment.

		<i>Artemia</i>	SBDF	Lansy	Tetra	Soy	Pollen
Calories (cal/g)	average	5,422	5,180	4,938	4,836	4,636	4,581
	st. dev.	121.1	94.0	144.9	118.3	53.8	168.4
% O.M. (Diets)	average	0.940	0.890	0.900	0.900	0.100	1.000
	st. dev.	0.005	0.000	0.000	0.020	0.005	0.000
% O.M. (Sediment)	average	0.360	0.500	0.470	0.430	0.370	0.630
	st. dev.	0.050	0.100	0.050	0.050	0.050	0.050

Table 3. Fatty acid profile of the diets (P) and the ragworms *Nereis diversicolor* (W) fed on the six tested diets.

FAME ($\mu\text{g mg}^{-1}$ D.W.)	Artemia		SBDF		Lansy		Tetramin		Soy		Pollen	
	P	W	P	W	P	W	P	W	P	W	P	W
EPA	6.4	5.4	6.4	7.8	10.7	9.1	2.6	3.1	0.0	4.2	0.0	3.8
DHA	0.1	0.8	8.4	4.9	14.4	6.7	3.1	1.6	0.0	0.7	0.0	1.0
Saturates	17.4	10.5	24.9	16.3	19.5	13.5	23.2	9.2	2.0	10.7	5.6	9.6
Monounsaturates	39.0	16.9	44.4	28.3	23.7	17.9	28.5	11.7	1.2	10.9	3.5	8.4
Diunsaturates	5.2	4.2	7.7	7.2	7.4	5.5	19.7	8.6	3.9	7.9	6.7	6.3
Polyunsaturates	18.5	21.8	21.5	20.1	34.0	22.4	12.2	9.9	0.9	13.7	6.2	15.6
Unsaturates	63.1	42.9	73.5	55.5	65.1	45.8	60.4	30.3	6.1	32.5	16.5	30.3
Branched	3.4	1.7	0.9	0.8	0.8	0.8	0.5	0.6	0.1	1.2	0.1	0.7
HUFA	11.5	14.1	17.4	20.2	29.8	23.1	9.1	11.4	0.3	13.4	1.4	13.9
n-3	10.7	19.5	20.3	18.0	32.0	20.8	10.9	7.5	0.8	9.3	6.1	11.1
n-6	9.6	6.3	7.9	9.0	8.2	6.9	20.8	10.9	4.0	12.2	6.8	10.1
n-3/n-6 ratio	1.1	3.1	2.6	2.0	3.9	3.0	0.5	0.7	0.2	0.8	0.9	1.1
DHA/EPA	0.0	0.1	1.3	0.6	1.4	0.7	1.2	0.5	0.1	0.2	0.0	0.3
%Total lipids	16.4	18.2	11.5	13.6	12.7	12.5	20.7	7.9	1.6	9.4	7.5	20.0
FAME ($\mu\text{g mg}^{-1}$ dw)	86.5	55.1	99.4	72.7	85.3	60.1	84.2	40.1	8.1	44.4	19.3	40.6

EPA: eicosapentanoic acid, DHA: docosahexaenoic acid, HUFA: high unsaturated fatty acids, FAME: fatty acid methyl esters, dw: dry weight, SBDF: sea bream dry food.

The n-3/n-6 ratio and the total fatty acid methol esters (FAME) depended upon the diet. Depending upon the source of the food, vegetable, non-vegetable, marine or non-marine, the n-3/n-6 ratio was more than one for foods of marine origin (*Artemia* cysts, SBDF and Lansy) and less than one for foods of non-marine origin (Tetramin, soy and pollen) (Table 3).

DISCUSSION

The fatty acid profile of the diets played an important role in the survival and growth rates of *N. diversicolor* in laboratory experiments. These worms attained greater growth rates compared to those grown in the field (Chambers and Milne, 1975; Heip and Herman, 1979; Kristensen, 1984). The DHA/EPA ratios were always less than one (Table 3). This may be due to the omnivorous and opportunistic feeding behavior of *N. diversicolor*. Typically, carnivorous marine animals have higher DHA/EPA ratios. It is worthy of note that the worm retains nearly all the EPA from their diet. However, if the amount of EPA is low in the diet, they can biosynthesize it de novo. The process is different with DHA; if the amount is high in the diet, the worm can metabolize about 50%. If, however, the amount of DHA is low or absent in the diet, the worm will synthesize it de novo.

In conclusion, marine diets rich in EPA and DHA allow a higher incorporation of HUFA which results in higher survival rates and better growth in cultures of *N. diversicolor*.

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Capítulo V
Considerações Finais e Conclusões

Os ambientes estuarino-lagunares são caracterizados por variações extremas dos parâmetros ambientais causados por uma dinâmica cíclica sazonal e diária de massas de água (McLusky, 1981, Day, 1981, Day *et al.*, 1987, Bearman, 1989, Nybakken, 1996). As entradas de água doce por escorrência continental, bastante acentuadas durante o inverno formando enxurradas, bem como a dinâmica cíclica propiciada pela acção das marés, são as principais forças-motrizes que influenciam os factores abióticos, transporte de colonizadores de diferentes *pools* de espécies e por consequência as biocenoses destes sistemas litorais. Embora a acção das marés durante o presente trabalho, tenha sido praticamente imperceptível na estação da Carrapateira devido às características lagunares, a entrada de água doce no sistema deu-se por escorrência a partir da bacia hidrográfica. O ritmo determinado pelas massas de água, ora transportando sedimentos de origem marinha para dentro dos estuários, ora arrastando sedimentos finos, ao longo destes pequenos estuários, altera claramente a morfologia e a granulometria do leito destes sistemas. O conteúdo em matéria orgânica nos sedimentos acaba por sofrer influência destas alterações principalmente no que se refere às dimensões das partículas do substrato, devido ao facto de existir uma relação inversa entre o tamanho dos grãos e a concentração de matéria orgânica (Barnes, 1974). A proveniência desta matéria orgânica, maioritariamente de origem vegetal, está relacionada com a degradação de macrófitas, geralmente frequentes nestes sistemas costeiros (Wolff, 1987, Cancela da Fonseca, 1989, Sprung, 1994). Mas actividades como a pecuária, desenvolvidas à volta das bacias hidrográficas destes sistemas, contribuem igualmente com um aporte orgânico associado principalmente ao período chuvoso. Esta matéria orgânica após decomposição, se incorpora aos sedimentos através da adsorção a partículas minerais, principalmente siltes e argilas, tornando-se assim, disponível para as cadeias alimentares (Bordovskiy, 1965, McLusky, 1981, Day *et al.*, 1987). O aumento de matéria orgânica dissolvida, bem como a entrada de nutrientes lixiviados dos terrenos circundantes ocorrido principalmente durante o inverno, são os responsáveis pelo aumento dos valores de clorofila *a* nesta estação do ano. A existência de um ciclo sazonal para o microfitobentos com um pico de biomassa durante o verão é restrito a sistemas localizados em latitudes mais elevadas (Colijn & Dijkema, 1981, Sundbäck *et al.*, 2000), e ausentes em regiões com um fotoperíodo mais alargado (Varela & Penas, 1985, Brotas *et al.*, 1995). A pequena escala dos ecossistemas estudados, faz com que estes sejam um caso particular dentro das zonas costeiras temperadas. Os

aumentos de biomassa microfitobêntica estão dependentes, não só do alargamento do fotoperíodo, mas principalmente da entrada de nutrientes como azoto e fósforo provenientes de actividades como a agricultura, transportados pelas chuvas para dentro destes sistemas.

É possível constatar através da amplitude dos parâmetros ambientais da água e do sedimento, que *Nereis diversicolor* está sujeito a tensões fisiológicas que influenciam de forma determinante a sua distribuição e abundância bem como, e mais significativamente, o próprio ciclo de vida nos três sistemas estudados. Em Odeceixe e Aljezur a gametogénese não começa no primeiro ano de vida, e o grupo reprodutor é constituído por indivíduos que apresentam uma longevidade compreendida entre os 18 e os 24 meses. Como resultado os indivíduos obtidos nestas estações apresentam as maiores dimensões. Na Carrapateira a gametogénese evolui de uma forma mais rápida, embora com um padrão distinto do das demais estações e, como tal, a longevidade é reduzida para valores entre 18 e 12 meses (Fidalgo e Costa *et al.*, 1998). Devido ao amadurecimento precoce dos indivíduos nesta estação, o tamanho que atingem é inferior ao dos das demais estações. A razão sexual foi sempre favorável às fêmeas em todas as estações, parecendo ser este predomínio de fêmeas a regra à escala mundial (Dales, 1950, Olive & Garwood, 1981, Mettam, 1981, Mettam *et al.*, 1982, Pinto, 1997). Esta pluralidade na biologia desta espécie pode ser observada em diferentes populações ao longo da sua área de distribuição (Chambers & Milne, 1975, Heip & Herman, 1979, Mettam, 1979, Neuhoff, 1979, Olive & Garwood, 1981, Essink *et al.*, 1985, Möller, 1985, Mettam, 1979, Mettam *et al.*, 1982, Kristensen, 1984, Gillet, 1993, Zubillaga & Salinas, 1997, Nithart, 1998, Abrantes *et al.*, 1999).

A população adulta de *N. diversicolor* mantém-se praticamente constante ao longo do ano, com a presença de indivíduos em diferentes etapas do ciclo gametogénico em todas as estações. Durante o verão, ocorre um decréscimo significativo no efectivo populacional em Odeceixe e Aljezur, atingindo as mais baixas densidades no mês de Setembro. Na estação da Carrapateira, onde as densidades obtidas se mantém mais estáveis ao longo do ano, o declínio no número de indivíduos em Setembro não se verifica. O decréscimo de efectivos na população adulta nesta época do ano pode ser devido ao facto desta espécie ser monotélica, morrendo após a emissão dos gâmetas por ruptura do corpo (e.g. Dales, 1950, Olive & Clark,

1977, Olive & Garwood, 1981, Mettam *et al.*, 1982, Fischer, 1984, Humpheys, 1985, Möller, 1985). Woodin (1974) demonstrou que a mortalidade após a emissão dos gâmetas e o sucesso do assentamento larvar explicam em muito as variações nas densidades dos adultos em populações de poliquetas intertidais. Outra hipótese, apesar de *N. diversicolor* apresentar um ciclo de vida completamente bentónico, é a possibilidade de migração para um outro local do estuário utilizando por exemplo as correntes de maré (e.g. Mettam, 1979, 1981, Caron *et al.*, 1993, Sardá com. pess.). Nos cultivos por nós realizados em condições laboratoriais, foi frequente observarmos *N. diversicolor* a fazer incursões prolongadas fora das galerias. O recrutamento desta espécie é contínuo ao longo do ano em todas as estações prospectadas, apresentando um acentuado pico no fim do verão, em Setembro, e um segundo de proporções mais reduzidas durante a primavera, em Abril / Maio. Em zonas temperadas, com invernos menos intensos, os períodos de reprodução de *N. diversicolor* estendem-se por um período mais alargado. Em geral, podemos afirmar, que em latitudes elevadas esta espécie apresenta um único recrutamento entre a primavera e o início do verão (Mettam, 1979, 1981, Olive & Garwood, 1981, Mettam *et al.*, 1982, Nithart, 1998). À medida que descemos de latitude, *N. diversicolor* passa a reproduzir-se de forma contínua ao longo do ano, ainda que com diferentes intensidades, segundo as épocas do ano (Sola & Ibañez, 1990, Gillet, 1990, Garcia-Arberas & Rallo, 1993, Dueso & Sardá, 1994, Arias & Drake, 1995).

Através das observações dos indivíduos selvagens, bem como dos nascidos em laboratório, foi possível concluir que a fase de maior crescimento é obtida nos primeiros meses de desenvolvimento até cerca dos 12 meses de vida. A partir do segundo ano de idade, começa haver uma redução substancial no crescimento somático devido a um investimento na reprodução, nomeadamente na produção de gâmetas (Mettam, 1979, Olive, 1983, Fischer, 1984). As taxas de crescimento linear foram calculadas por coorte durante o seu período de existência, a partir da conversão do valor de L_3 (prostómio, peristómio e 1º setígero) em comprimento total. As taxas médias de crescimento linear mensal por estação (Odeceixe – 0.2 cm, Aljezur – 0.33 cm, Carrapateira - 0.27 cm) revelam um crescimento inferior aos obtidos por Abrantes *et al.* (1999), com 0.4 e 0.5 cm/mês, para o Canal de Mira, na Ria de Aveiro. A população estudada por Lopes da Silva (1993), no estuário do Mira apresenta um crescimento igual ao obtido por nós em Odeceixe. O crescimento de *N. diversicolor* no primeiro ano de vida varia entre locais prospectados, sendo menor

em Odeceixe (0.17 cm/mês), e aumentando progressivamente em Aljezur (0.26 cm/mês) e Carrapateira (0.29 cm/mês). A variabilidade ambiental entre as estações é a responsável por estas diferenças, nomeadamente a temperatura que aumenta significativamente entre Odeceixe e a Carrapateira. Esse crescimento acelerado reflecte-se nas taxas de renovação da biomassa (P/B) encontradas, que foram respectivamente para Odeceixe, Aljezur e Carrapateira de 2.0, 3.1 e 4.0. As duas primeiras estações apresentam um P/B semelhante ao obtido para populações situadas em latitudes superiores à nossa (Gillet, 1990, Chambers & Milne, 1975, Heip & Herman, 1979, Nithart, 1998). O quociente P/B obtido para Carrapateira é ligeiramente inferior ao encontrado por Abrantes *et al.* (1999) para o Norte de Portugal, bem como para Marrocos (Gillet, 1986, 1993). O parâmetro P/B é dependente de aspectos como ciclo de vida, longevidade, biomassa do indivíduo maduro e biomassa em cada classe etária. Diferenças bióticas e abióticas das áreas em estudo e até mesmo erros de precisão no cálculo da biomassa e produção por parte dos investigadores, podem estar relacionados com as diferenças encontradas em estudos distintos (Chambers & Milne, 1975, Brey, 1990, Sprung, 1993). O elevado P/B estabelecido para a população da Carrapateira está relacionado com a elevada renovação da biomassa, proporcionada por um ciclo de vida mais curto, bem como a presença de um grande número de juvenis que constituem o contributo mais importante para esta relação. Möller (1985), para a costa ocidental Sueca, obteve valor similar para uma população também constituída maioritariamente por juvenis. As diferentes populações de *N. diversicolor* ao longo da sua área de distribuição têm normalmente dinâmicas distintas, por apresentarem diferentes respostas evolutivas às pressões selectivas (Levins, 1975). Estas diferenças influem nas épocas de reprodução, densidades e por conseguinte nas respectivas biomassas. No entanto as variações de densidade e biomassa para a Península Ibérica, demonstram um padrão comum, tal como observado em variados locais situados mais a Norte (Sardá *et al.*, em preparação). Existe uma dinâmica completamente dissemelhante para a Península Ibérica, onde as curvas de densidade e biomassa são opostas, apresentando respectivamente os valores máximos no verão e no inverno (Garcia-Arberas & Rallo, 1993, Dueso & Sardá, 1994, Arias & Drake, 1995, Fidalgo e Costa *et al.*, 1998). No Norte da Europa, biomassa e densidades apresentam uma evolução conjunta, com o pico no início do verão (Chambers & Milne, 1975, Heip & Herman, 1979, Kristensen, 1984, Gillet, 1990, Nithart, 1998). Logo pós o período onde se atinge as maiores densidades,

ocorre uma ligeira mas progressiva diminuição do efectivo e respectivas biomassas, facto este que não se observa nas populações de *N. diversicolor* da Península Ibérica. Aqui, logo em seguida aos valores de densidade mais elevados, ocorre uma queda abrupta deste parâmetro, acompanhado de um aumento proporcional dos valores de biomassa. Um crescimento mais acelerado, traduzido por um incremento da biomassa de *N. diversicolor* nestas latitudes é portanto confirmado, embora ocorra uma maior mortalidade nos meses subsequentes ao recrutamento (Sardá *et al.*, em preparação).

As produções anuais de *N. diversicolor* estimadas para a costa sudoeste de Portugal, apresentam valores intermédios aos diferentes sistemas estudados nas costas Atlânticas (Kristensen, 1984, Chambers & Milne, 1975, Humphreys, 1985, Sprung, 1994, Arias & Drake, 1995, Nithart, 1998, Abrantes *et al.*, 1999). No entanto a estação da Carrapateira, apesar de ser globalmente o local menos produtivo com valores semelhantes aos encontrados para a costa norte-africana (Gillet, 1993), apresenta para as classes mais novas uma produção cinco vezes superior à da estação de Odeceixe, e três vezes superior à de Aljezur.

Do ponto de vista trófico, *N. diversicolor* pode ser considerado uma espécie oportunista e generalista, adaptando as suas estratégias alimentares às variações no espaço e no tempo dos factores abióticos (e.g. Goerke, 1971, Reise, 1979, Fauchald & Jumars, 1979, Esselink & Zwarts, 1989, Esnault *et al.*, 1990, Riisgård, 1991, Masson *et al.*, 1995, Smith *et al.*, 1996, Lucas & Betru, 1997). As relações inter-específicas também assumem extrema importância na opção da melhor estratégia a adoptar, bem como com a maior ou menor disponibilidade e qualidade do alimento (Dinis, 1986, Zwarts & Esselink, 1989). Em Odeceixe e Aljezur esta espécie adopta hábitos mais filtradores e detritívoros. Por outro lado, na estação da Carrapateira a baixa presença de predadores faz com que *N. diversicolor* promova incursões às áreas circundantes às suas galerias, adoptando um comportamento maioritariamente carnívoro e canibal (cf. Capítulo III, Artigo 4).

N. diversicolor apresenta-se em condições laboratoriais tão eclético como no estado selvagem, alimentando-se das mais variadas dietas como por exemplo: Lansy (alimento para pós-larvas de camarões), Tetramim (ração para peixes ornamentais), *Artemia* sp., soja (desidratada em grãos), pólen (em grãos) e ração

comercial para douradas; atingindo elevadas taxas de crescimento e de sobrevivência. Acima de tudo, esta espécie é capaz de crescer e se desenvolver apenas com uma única dieta, o que favorece sobremaneira o seu cultivo (Fidalgo e Costa, *et al.*, 2000).

Nas análises de ácidos gordos realizadas aos indivíduos alimentados com as dietas anteriormente referidas, é interessante salientar que todo ácido eicosapentanoico (EPA) existente nas dietas é retido por *N. diversicolor*. No caso das quantidades de EPA nas dietas serem baixas é possível a bio-síntese *de novo* por parte de *N. diversicolor*. Por outro lado, se a quantidade de ácido docosahexaenoico (DHA) for alta na dieta, apenas 50% é metabolizado, mas se a quantidade de DHA for baixa ou ausente *N. diversicolor* é capaz de sintetizar *de novo*. Em conclusão, dietas ricas em EPA e DHA permitem uma alta incorporação de HUFA (ácidos-gordos insaturados de cadeia longa) por parte de *N. diversicolor*, permitindo a esta espécie atingir em condições de cultivo um bom crescimento e uma alta sobrevivência (Fidalgo e Costa *et al.*, 2000).

Embora a natureza do substrato seja um dos parâmetros ambientais mais importantes na distribuição da fauna bentónica (Nichols, 1970, Gray, 1981, Turner *et al.*, 1995), *N. diversicolor* tem a capacidade de se enterrar e viver numa grande variedade de substratos (Clay, 1967). Em condições laboratoriais esta espécie não apresenta diferenças significativas no crescimento e sobrevivência em relação aos tipos de sedimentos testados (Fidalgo e Costa, 1999). Por esse motivo concluímos que a opção mais acertada para o cultivo desta espécie é a areia, pois é o sedimento onde se faz com maior facilidade a visualização e recolha dos exemplares. Por outro lado, reduz-se com essa escolha os riscos dos fenómenos de anóxia tão associados aos sedimentos mais finos e com maior percentagem de matéria orgânica (Cancela da Fonseca, 1989).

Em relação à temperatura, 25°C aliada a uma salinidade de 15‰ resulta numa combinação na qual se obtém uma sobrevivência de 100%. O crescimento de *N. diversicolor* em cativeiro e em pequena escala é extremamente elevado, atingindo em 72 dias aproximadamente 70% do tamanho dos indivíduos encontrados à venda no mercado (Fidalgo e Costa, 1999), embora alguns destes em adiantado estado de maturação. Esta característica poderá ser aproveitada para a obtenção intensiva de

larvas, no entanto será necessário um estudo pormenorizado acerca da viabilidade destes oócitos e a certificação de que a maturação extremamente acelerada dos progenitores não tenha sido prejudicial à oogénese.

A curto prazo importa melhorar a técnica de cultivo desta espécie, nomeadamente aspectos relativos à reprodução em cativeiro, ultrapassar igualmente problemas referentes às diferenças de tamanho entre indivíduos da mesma idade. A médio prazo, e se tratando de um cultivo de maiores dimensões, a optimização do método de recolha dos indivíduos adultos será de igual importância. Testar a introdução ocasional de selvagens seleccionados de diferentes populações, quer para manter a variabilidade genética, quer para introduzir características genéticas desejáveis, deverá ser um procedimento a seguir.

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