Universidade de Aveiro Departamento de Biologia
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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia, realizada sob a orientação científica do Doutor Henrique Queiroga, Professor Auxiliar com Agregação do Departamento de Biologia da Universidade de Aveiro e do Doutor Peter Tiselius, Professor do Departamento de Ecologia Marinha – Faculdade de Ciências da Universidade de Gotemburgo (Suécia).

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To my mother, my brother and my lovely wife

“At some future period, not very distant as measured by centuries, the civilized races of man will almost certainly exterminate, and replace the savage races throughout the world.”

Charles Darwin (1809-82)

o júri

presidente

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palavras-chave

Canal de Mira (Ria de Aveiro); populações estuarinas de copépodes; *Acartia clausi*; *Acartia tonsa*; modelos crescimento dependentes da temperatura; forçamento ambiental; abundância; produção secundária.

resumo

Os copépodes são pequenos e frágeis crustáceos que constituem um dos grupos de organismos metazoários mais abundantes do mundo. Em ambientes marinhos e estuarinos, os copépodes assumem um papel de extrema relevância ao nível das cadeias tróficas, nomeadamente na transferência de matéria e energia de níveis tróficos inferiores (fitoplâncton) para níveis tróficos superiores (ex. larvas de peixe). A importância ecológica dos copépodes reflecte-se no elevado número de citações constantes no ISI Web of Knowledge (7716 citações entre 1969 e 2006) e no destaque que os mesmos continuam a possuir em estudos recentes de planctologia marinha e estuarina. Esta dissertação teve como objectivos principais (1) descrever variações espacio-temporais em termos de abundância e biomassa de populações estuarinas de copépodes da Ria de Aveiro (Portugal) e a sua relação com parâmetros hidrológicos (salinidade, temperatura, clorofila *a* e precipitação; (2) comparar as taxas de crescimento e desenvolvimento de populações alopátricas de copépodes; (3) definir modelos de crescimento dependentes da temperatura para as formas juvenis (nauplius e copepoditos) de *Acartia tonsa*; (4) avaliar o forçamento ambiental na distribuição e abundância de populações de *Acartia* e (5) calcular taxas de produção secundária potenciais para as populações de *Acartia*. Numa primeira fase, foi objecto de estudo a comunidade de copépodes estuarinos, para a qual foram descritos os padrões temporais de abundância e biomassa e obtidas estimativas de produção secundária. Os resultados obtidos neste estudo permitiram concluir que, entre outros aspectos, a abundância e biomassa da comunidade de copépodes da Ria de Aveiro se encontra significativamente correlacionada de modo positivo com a salinidade e com a temperatura da água. As estimativas das taxas de produção secundária derivadas da aplicação de modelos gerais de crescimento mostraram ser algo diferentes, sendo a estimativa dada pelo modelo de Hunthey & Lopez (1992) mais elevada do que a obtida pelo modelo de Hirst & Bunker (2003). O crescimento e desenvolvimento de espécies de *Acartia* foram estudados sob condições controladas em termos de alimento e temperatura, de forma a serem definidos modelos de crescimento dependentes da temperatura. A partir destes estudos concluiu-se que as populações alopátricas possuem diferentes respostas à temperatura. Além deste aspecto, também se observou que, pelo menos no caso da *A.tonsa*, as taxas de crescimento das formas juvenis (nauplius e copepoditos) estimadas *in situ* ou sob condições saturantes de alimento são similares.

resumo

O forçamento ambiental das populações de *Acartia* no Canal de Mira foi avaliado através de uma análise de componentes principais (ACP), que permitiu a análise simultânea das alterações espaciais e temporais das diferentes populações. Esta análise identificou três zonas distintas no estuário com base na abundância de *Acartia* spp.. Para cada zona, análises de correlação com diferentes desfasamentos temporais entre as variáveis ambientais e a abundância de copépodes, permitiram detectar a existência de forçamentos ambientais específicos, assim como um efeito positivo da biomassa fitoplanctónica na abundância do zooplâncton verificada meses mais tarde. Esta tese demonstrou igualmente a grande importância que as populações de *Acartia*, especialmente a mais abundante – *A. tonsa* – assumem na transferência de matéria e energia no ambiente planctónico da Ria de Aveiro (Portugal).

keywords

Canal de Mira (Ria de Aveiro); estuarine copepod populations; *Acartia clausi*; *Acartia tonsa*; temperature-dependent growth models; environmental forcing; abundance; secondary production.

abstract

Copepods are small fragile and tiny crustaceans that form one of the world's most abundant groups of metazoan organisms. In estuarine and marine environments copepods assume a key role in what trophic chains are concerned, namely in the transfer of matter and energy from lower trophic levels (phytoplankton) to higher trophic levels (ex. fish larvae). Copepods ecological importance is proven by the high number of quotations in ISI Web of Knowledge (7716 quotations between 1969 and 2006) and in the significance that they still have concerning current studies on estuarine and marine planktonic studies. The main goals of the present thesis were (1) to characterize and to describe the spatial-temporal patterns of abundance, biomass and production of the estuarine copepod community from Ria de Aveiro (Portugal) and its relationship with hydrological data (salinity, temperature, chlorophyll *a* and rainfall regime); (2) to compare growth and developmental rates of allopatric copepod populations; (3) to define temperature dependent growth models for nauplii and copepodites of *Acartia tonsa*; (4) to evaluate environmental forcing on the distribution and abundance of *Acartia* populations; and (5) to estimate potential secondary production rates of *Acartia* populations. In a first stage estuarine copepods community was studied, time patterns of abundance and biomass having been described and estimates of secondary production having been obtained. Results achieved by this study have led us to the conclusion that, among other aspects, abundance and biomass of the copepods community in Ria de Aveiro is positively correlated with water salinity and temperature. Estimates of secondary production rates deriving from the use of general growth models were different, the estimate obtained by Hunthey & Lopez (1992) model being higher than the one resulting from the Hirst & Bunker (2003) one. In order to define specific temperature-dependent copepod growth models, the growth and development of *Acartia* species were studied under controlled conditions of food and temperature. From those studies it was concluded that allopatric populations have different temperature responses. Additionally, it was also observed that, at least for *A.tonsa*, the growth rates of nauplii and copepodites at saturated food conditions and *in situ* conditions of food are similar.

abstract

Environmental forcing of *Acartia* populations in Canal de Mira was evaluated by means of 3-mode PCA, which analyses simultaneously spatial and temporal changes of multispecies assemblages. This analysis identified three distinct zones along the estuary based on *Acartia* spp. abundance. For each zone time-lagged correlations between environmental variables and copepods abundance indicated that different zones were forced by different combinations of variables and the existence of delayed effects of phytoplanktonic biomass on the abundance of *A.tonsa*. This thesis also demonstrated the high importance that *Acartia* populations, in particular the most abundant *A.tonsa*, assume on the transfer of matter and energy in the planktonic realm of Ria de Aveiro (Portugal).

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Chapter 1

General introduction

1.1 Copepods and their ecological significance

Minute aquatic crustaceans, copepods are among the most abundant metazoans organisms in the world (Mauchline 1998) and are the dominant mesozooplankton in the marine environment, comprising as much as 80% of its total biomass (Kiørboe 1998). The word 'copepod' has its root in two Greek words: 'kope' which means 'oar' and 'podos' which means 'foot', and refers to the animal's flat, laminar swimming pair of legs (Mauchline 1998).

Taxonomically, the copepods are presently classified in the Phylum Arthropoda, Subphylum Crustacea, Class Maxillopoda, Subclass (Copepoda) and divided into two Infraclasses and ten Orders (Table 1.1). These invertebrates occur in a vast number of aquatic environments being more abundant in marine environments. Their life style can be pelagic, benthopelagic, benthic, commensal or parasitic. Within the ten orders of copepods, the Calanoida includes the most abundant marine and estuarine species therefore assuming a high ecological importance in such ecosystems.

Table 1.1 Taxonomic classification of copepods including some ecological notes. After Huys and Boxshall (1991) and Humes (1994) and adapted from Mauchline (1998).

Subclass Copepoda Milne-Edwards, 1840	
Infraclass Progymnoplea Lang, 1948	
Order Platycopioida Fosshagen, 1985	Marine, benthopelagic species
Infraclass Neocopepoda Huys & Boxshall, 1991	
Superorder Gymnoplea Giesbrecht, 1882	
Order Calanoida Sars, 1903	Marine (75%) and fresh water primarily pelagic species
Superorder Podoplea Giesbrecht, 1882	
Order Misophrioida Gurney, 1933	Primarily benthopelagic and inhabitants of anchialine caves
Order Cyclopoida Burmeister, 1834	Marine and fresh water, and can be pelagic, commensal species
Order Gelyelloida Huys, 1988	Occur in karstic systems in France and Switzerland.
Order Mormonilloida Boxshall, 1979	Marine pelagic species
Order Harpaticoida Sars, 1903	Primarily marine (10% freshwater), most are benthic species
Order Poecilostomatoida Thorell, 1859	Marine commensal or parasitic species
Order Siphonostomatoida Thorell, 1859	Marine commensal or parasitic species
Order Monstrilloida Sars, 1903	Marine species, pelagic as adults but parasitic when young

The life cycle of calanoid copepods starts, like in all copepod species, with an egg that is produced by females and fertilized by males. The eggs can be carried by adult females or free spawned in the water column. Following egg hatching, calanoid copepods pass through 6 naupliar stages (NI to NVI) and five copepodite stages (CI to CV). The last instar is the adult stage, normally referred as CVI. The discrimination between males and females of calanoid copepod begins on the 4th copepodite stage (CIV).

The body of adult calanoid copepod is divided into three sections: the cephalosome, metasome and urosome (Fig. 1.1). In some species, the first segment of the metasome is fused with the cephalosome, and/or the fourth and fifth segments of the metasome are fused. Additionally, the metasome may appear, in some species, to have only three segments.

The urosome consists of the genital somite and posterior segments. The genital somite of females consists of fused segments differing from the males which are separated. The cephalosome and metasome together are known as the prosome. Its length, from the anterior end of the cephalosome to the posterior edge of the 5th segment of metasome is frequently used as a direct measure of body length (Mauchline 1998).

Copepods, like other crustaceans, have paired appendages (antennule, antenna, mandible, maxillule, maxilla and maxilliped) that are used in swimming, detecting and obtaining food, and mating (Fig. 1.1). The mouth comprises a labrum and a labium. The labrum is a muscular lobe which forms the anterior margin of the mouth and the paired lobes of the labium form the posterior and part of the lateral margins of the mouth (Mauchline 1998).

The first four metasome segments of females and males always have paired, biramous swimming legs that are similar in both sexes. Depending on the family, the fifth pair of legs can be similar or different from the first pair. The fifth pair of legs present in females can be greatly reduced in size and structure while those from males are normally enlarged in order to grasp the female during mating (Fig. 1.1).

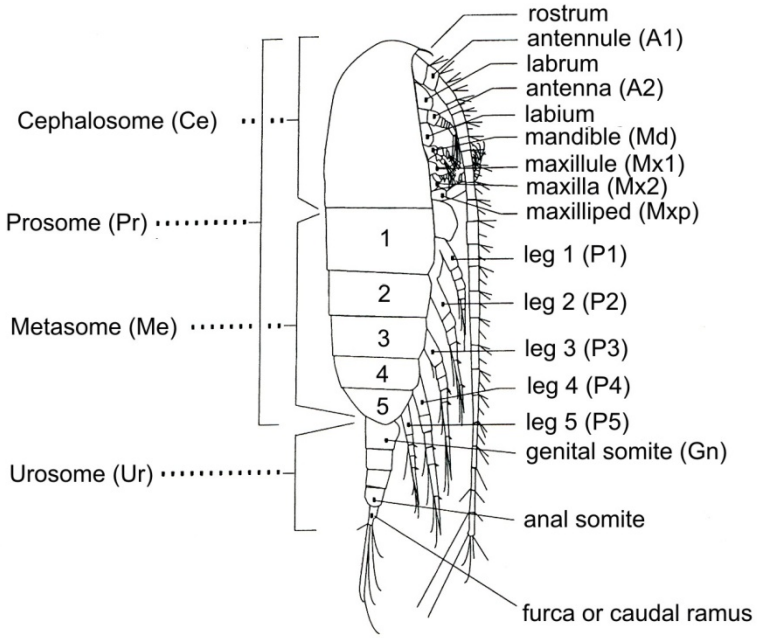

Figure 1.1 External morphology and appendages of a female calanoid copepod (adult). The metasome has five clearly defined segments, numbered 1-5. Legs 1-5 are the swimming legs. Adapted from Mauchline 1998.

The ecological importance of copepods is reflected by the high number of citations in *ISI Web of Knowledge* (7716 citations, from 1969 to 2006) and by the continuous interest manifested by marine biologist. Copepod are important grazers of phytoplankton and microzooplankton (Atkinson 1996) and play a key role in the trophic chain of marine and estuarine ecosystem by transferring matter and energy from low (phytoplankton) to high trophic levels (e.g. fish larvae).

The relevance of copepods is such that the reproductive and recruitment success of several pelagic fish and shellfish species of high economic value are extremely dependent on the dynamics of copepod populations (Conover et al. 1995, Beaugrand et al. 2003).

Within the trophic marine chain, copepods also have a significant positive effect on the "microbial loop" by adding substances to the pool of dissolved organic matter (DOM) through excretion processes, leakage of faecal pellets and sloppy feeding. Half of the

carbon requirement of the bacterioplankton is fulfilled by the DOM originating from copepod's activity (Azam et al. 1983, Moller & Nielsen 2001).

Additionally, copepods are also very important in the upper ocean layer, exporting, redistributing and repackaging nutrients, as well as in the biogeochemistry of nitrogen (Hays et al. 1997) and carbon cycle (Banse 1995). The importance of copepods in the carbon cycle, and ultimately in climate change, is due to its active role in the biological pump of carbon into deep-ocean. The moulted exoskeletons and faecal pellets produced by copepods in upper ocean layers will tend to sink to the sea bottom where the carbon dioxide will be sequestered and, as a result, removed from the surface ocean layer and atmosphere.

Additionally, the diel vertical migrations of these organisms may also contribute to a net downward flux of carbon dioxide, because of the time spent during the day in deeper waters, by respiring and excreting surface-ingested particulate matter (Schnetzer & Steinberg, 2002). This mechanism constitutes a novel part of the biological pump of carbon (Janson et al. 2004).

1.2 Distribution of copepod populations in estuaries

Only a few species are able to develop in estuaries and their dynamics and distribution are forced by environmental parameter (Frometin & Ibannez 1994, Beaugrand et al. 2001, Licandro et al. 2001). The potential range of estuarine zooplanktonic populations is determined by its specific tolerance to salinity (Cervetto et al. 1999, Gaudy et al. 2000), its response to temperature variation (Leandro et al 2006a, b), food availability (Paffenhöfer & Stearns 1988), turbidity (Soetaert & Rijswijk 1993, David et al. 2005) and biotic relationships (Kimmel & Roman 2004).

Estuaries are strong advective environments as a direct consequence of the tidal currents (ebb and flood) and river flow, imposing an additional factor of stress for

calanoid copepods (Soetaert & Rijswijk 1993). Advective processes tend to increase the potential range of populations distributions (Soetaert & Rijswijk 1993) by the continuous transport of individuals to downstream of its normal habitat.

Copepods of the genus *Acartia* are retained in estuaries due to the combined effect of the swimming behaviour that allow regulation of its vertical distribution (Kouassi et al. 2001), the production of diapause eggs (Castro-Longoria 2001) in response to unfavourable environmental conditions and life cycle traits such as short generation times (Leandro et al. 2006a) and high reproductive potential. These life cycle strategies adopted by *Acartia* spp., allow them to be among the most abundant in the estuarine zooplanktonic communities and also some of the most widespread organisms (Day et al. 1989).

In estuaries located in the North Atlantic, the presence of 2 or 3 *Acartia* species, namely *Acartia tonsa*, *A.clausi* and *A.bifilosa*, is very frequent. However, *Acartia* species are commonly segregated in space (Castro-Longoria 2003, Lawrence et al. 2004, Marques et al. 2006). As previously referred, such spatial segregation is a result of physiological constrains (Gaudy et al. 2000, Calliari et al. 2006) or interspecific relationships such as predation (Tiselius et al. 1997) or competition (Tester & Turner 1991).

Additionally, the occurrence of *Acartia* species in a given estuary could also be limited in time as well. Such fact could be attributed to the differential hatching of dormant eggs from the sediment, a phenomenon well known for species of *Acartia* genus (Soetaert & Rijswijk 1993).

1.3 Secondary production and growth rates

Zooplanktonic secondary production is, by definition, the amount of new zooplankton biomass elaborated each day or year (Miller 2004). That biomass will be available to higher trophic levels and ultimately will support a great variety of marine resources.

The increase of the number of studies on European fish stocks at the beginning of the 20th century made it possible to conclude that marine zooplankton constitute the principal food source of most marine fishes (Huntley & Lopes 1992). This conclusion provided a driving economic force for studying the distribution and abundance of zooplankton and ultimately led to interest in quantifying their productivity (Huntley & Lopes 1992).

In planktonic ecology, secondary production is of special interest since it is a measure of energy flow through zooplanktonic populations and is an indicator of its physiological or nutritional state (Kimmerer 1987). Although there are a large number of studies involving zooplanktonic secondary production, no general and universal methodology to measure copepod production exists.

Over the course of the last century, a variety of methods to the estimate of marine zooplankton production have evolved. Within such methodologies we may find cohort analysis (Bougis 1974), cumulative growth (Omori & Ikeda 1976), turnover rates (Rigler & Downing 1984) and predictive models. Those models, derived from large datasets, relay upon the determination of more easily measured parameters such as temperature (Huntley & Lopez 1992), temperature and size-distributed biomass (Ikeda & Motoda 1978, Hirst & Lampitt 1998) and more recently temperature, body weight and chlorophyll *a* (Hirst & Bunker 2003).

Over recent years, the methodologies used more frequently in copepod production studies belong to the so-called growth rate approach. This approach is based on *in situ* determination of growth rate and copepod biomass (Poulet et al. 1995). The basic principle associated with this method is that growth rates are measured for individual stage/age classes and are multiplied by the biomass of that particular stage. The production rate of the entire population is then obtained by summing all stages. This approach is simple and does not require knowledge of mortality rates (Kimmerer 1987).

The simplest way to obtain growth rates estimates is from temperature-dependent growth models defined at food saturated conditions. Until recently, it was widely accepted that copepods are food limited *in situ*, thus resulting in an overestimation of production rates when derived from such temperature-dependent growth models. However, the results obtained by Hirst & Bunker (2003) contradict such fact as juvenile copepods in the field were shown to grow at rates close to maximum laboratory determined rates. Consequently, the growth rates given by the temperature-dependent growth models will give us realistic production rate values.

The egg production method is a simplification of the growth rate approach and is based on two key assumptions: first, that within a given species all stage-specific growth rates are equal and, second, that specific egg production is equal to the growth rates of adult females (Poulet et al. 1995). In this situation, production rate will be simply determined by multiplying the total copepod population biomass by the egg production rate.

However, growing evidences concerning differences between egg production rates and juvenile growth rates are contradicting one of the assumptions of egg production method. Under *in situ* conditions, adult female growth rates are frequently lower than the respective juvenile growth rates (Hirst & Bunker 2003). Additionally, *in situ* food conditions could be limiting for adult growth (decrease of egg production) but not necessarily for the juvenile stages. In this situation, juvenile growth rates will be underestimated and, consequently, so will the production rate.

The majority of secondary production studies based on copepod populations realized until now were performed in estuarine ecosystems of North America (Heinle 1966, Durbin et al. 1983) and Northern Europe (Escaravage & Soetaert 1995, Irigoien & Castel 1995).

References to estuaries of southern European are non-existent. Exception is the study performed by Vieira et al (2003a, b) and Pastorinho et al. (2003) in Mondego Estuary –

Portugal. Although, this study was based on cohort analysis applied on monthly data which is an inappropriate methodology since copepods exhibit high growth and short generation times (Kimmerer 1987, Escaravage & Soetaert 1995).

In spite of the difference in the absolute values of secondary production from one ecosystem to another (Table 1.2), the studies realized so far have demonstrated a strong seasonal variation, with appearance of secondary production peaks after the occurrence of primary production peaks (Kiorboe & Nielsen 1994, Irigoien & Castel 1995, Irigoien et al. 2000). However, comparisons of primary and secondary production values indicate that other carbon sources than phytoplankton need to exist in order to achieve such values of productivity (White & Roman 1992, Irigoien & Castel 1995).

For this particular ecosystem - Ria de Aveiro (Portugal) - recent data has shown that total respiration rates in the water column are greater than primary phytoplanktonic production (Queiroga et al 2001). Such results indicate that other organic carbon sources than phytoplankton must exist for the maintenance of secondary production, therefore reinforcing the importance of detritus in heterotrophic processes that occur in the water column. Additionally, this fact also supports the proposition that estuaries are heterotrophic systems and become more heterotrophic when nutrient inputs are higher (Heip 1995).

Table 1.2 Daily secondary production rates (mg C m^{-3} d^{-1}) and turnover rates (P/B, d^{-1}) obtained in different geographic regions and habitats. (a - annual average; b - assuming a carbon-dry weight conversion of 0.5; c - assuming 365 days per year; d - integrated for the water column mean depth).

Area / Habitat	Species	Production	P/B	Temporal scale	Method	Reference
Patuxent (USA) / Estuary	*Acartia tonsa*	28	0.52	short term (69 days)	Cohort analysis	Heinle 1966
Narragansett Bay (USA) / Estuary	*Acartia clausi*	2 - 4	0.25	Long term (8 month, weekly sampling)	Original-development time; recalculated using the growth rate approach	Durbin & Durbin 1981
Narragansett Bay (USA) / Estuary	*Acartia tonsa*	4 - 5	0.7	Long term (8 month, weekly sampling)	Original-development time; recalculated using the growth rate approach	Durbin & Durbin 1981
Skagerrak (Norway, Denmark) / Shelf waters	Copepoda	11.4	0.18	Short-term (10 days, August)	Growth rate approach (egg production and juvenile growth)	Peterson et al 1991
Kattegat (Denmark) / Shelf waters	Copepoda	1.18 [a,c,d]		Long term (1 year, fortnight sampling)	Growth rate approach (egg production)	Kiørboe & Nielsen 1994
Westerschelde (The Netherlands) / Estuary	*Acartia tonsa*	1.9 [b]	0.27	Long term (1 year, 10 days between sampling)	Growth rate approach (temperature dependent growth model)	Escaravage & Soetaert 1995
Westerschelde (The Netherlands) / Estuary	*Eurytemora affinis*	2.5 [b]	0.09	Long term (1 year, 10 days between sampling)	Growth rate approach (temperature dependent growth model)	Escaravage & Soetaert 1995
Solent (UK) / Shelf waters	Copepoda	0.09 [a,c]		Long term (14 month, monthly sampling)	Predictive model	Hirst et al 1999
Gironde (France) / Estuary	*Eurytemora affinis*	3	0.09			Castel & Feurtet 1989
Elbe (Germany) / Estuary	*Eurytemora affinis*	2.7	0.11	Long term (6 months, twice a week sampling)	Cohort analysis	Peitsch 1995
Gironde (France) / Estuary	*Acartia bifilosa*		0.08	Long term (6 months, monthly sampling)	Growth rate approach (instantaneous growth rate)	Irigoien &Castel 1995
Mondego (Portugal) / Estuary	*Acartia clausi*	0.17 [a,c]	0.07	Long term (1 year, monthly sampling)	Cohort analysis	Vieira et al 2003
Mondego (Portugal) / Estuary	*Acartia tonsa*	0.06 [a,c]	0.08	Long term (1 year, monthly sampling)	Cohort analysis	Pastorinho et al 2003
Mondego (Portugal) / Estuary	*Acartia bifilosa*	0.12 [a,c]	0.03	Long term (1 year, monthly sampling)	Cohort analysis	Vieira et al 2003

1.4 Planktonic studies in Ria de Aveiro (Portugal)

Given the high social, economical and ecological importance of Ria de Aveiro (Portugal), this estuarine ecosystem has been intensively studied during the last decades. Those studies focused on different scientific fields, such as sediment chemistry (e.g. Abreu et al. 2000), geology (e.g. Rocha et al. 2005), tidal propagation (Dias et al. 2000), hydrological characterization (e.g. Dias et al. 1999), water quality (Lopes et al. 2005) and estuarine biology. In biological studies, some of the research programs focused on planktonic ecology, namely bacterioplankton, phytoplankton and zooplankton communities.

Studies of bacterioplankton communities include analysis of patterns of ectoenzymatic and heterotrophic bacterial activities (Cunha et al. 2000), bacterial productivity (Almeida et al. 2001a, Almeida et al. 2001b), bacterial abundance (Cunha et al. 2000, Almeida et al. 2001a), fluxes of bacterioplankton between estuary and adjacent coastal area (Cunha et al. 2003) and relationships between bacterioplankton production with primary production and respiration (Almeida et al. 2005).

Some of the most relevant results indicated that bacterial abundance in Ria de Aveiro ranges from 0.2 to 15.3 × 10^9 cells l^{-1}, depending on the estuarine zone and tidal cycle, and confirmed the existence of 2 distinct bacterial communities (marine and brackish water). Based on the estimates obtained by Almeida et al (2005), planktonic respiration in Ria de Aveiro varies between 0.1 and 8.2 g C m^{-3} d^{-1}, primary production between 0.2 and 19.1 g C m^{-3} d^{-1} and bacterial secondary production between 2.7 and 7444 mg C m^{-3} d^{-1}. These colleagues also concluded that bacterioplankton growth is largely dependent on non-phytoplanktonic derived carbon sources.

More recently, novel biomolecular tools were applied to bacterioplankton studies in Ria de Aveiro (Portugal). Henriques et al. (2006) studied the dynamics of a free living bacterial community by means of 16S rDNA PCR-denaturing gradient gel electrophoresis (DGGE), and concluded that the specific composition shifts within the bacterial community of Ria de Aveiro (Portugal) occur between the brackish and

freshwater sections. Additionally, those authors also identified the dominant bacterial groups of the brackish and freshwater sections.

Within phytoplanktonic communities, the studies yet performed are limited to marine and estuarine diatomological communities. Resende et al. (2005) described the abundance cycles and spatio-temporal distribution patterns of the most abundant taxa along a gradient of salinity (Canal de Mira – Ria de Aveiro). These authors clearly identified the existence of two different communities, one typical of marine environments and located downstream and the other situated further upstream and dominated by freshwater forms. Additionally, optima and tolerance values for salinity and temperature were also defined for the main diatom species.

Phytoplankton laboratorial experiments revealed that some marine planktonic diatom species are able to produce and exudate exopolysaccharides (EPS). The EPS production was shown to be related to nutrient depletion (N and P) in the culture medium and growth phase, and mainly occurred when the cultures reached the stationary growth phase (Leandro et al. 2003). The exopolysaccharides produced by the three species, essentially polymers of uronic acids, were very similar in their composition of neutral sugars (Leandro et al. 2003).

Studies of zooplanktonic organisms mainly focused on decapod larvae. At the ecological level, some studies were devoted to the vertical migrations and selective tidal-stream transport in larvae of the crab *Carcinus maenas* (Queiroga 1997, 1998). Those studies demonstrated the presence of active vertical migration synchronised with the tidal cycle in first and last stage larvae of the species, and demonstrated the importance of the shift in vertical position during the tidal cycle to the efficiency of upstream and downstream transports.

Additionally, some important works on decapod larvae ecology addressed the importance of wind speed and direction and of tidal currents on recruitment processes of estuarine crab species, also using *Carcinus maenas* as a biological model. (Almeida & Queiroga 2003, Queiroga 2003, Queiroga et al. 2006, Marta-Almeida et al. 2006). The

main conclusions of these studies were that onshore transport of megalopae is inversely related with the intensity of upwelling favourable winds, and that hydrological variables associated with spring flood tides enhance inout into estuaries.

According to Pereira et al. 2000, decapod larvae in Ria de Aveiro (Portugal) display three types of net larval flux along a lunar month. Type 1 includes first zoeas that are consistently exported to sea. Type 2 comprises late zoeas, megalopae and juveniles that are consistently imported into the estuary and Type 3 includes first zoeas that are imported and exported. In species of this type import periods appeared to alternate with export periods according to lunar phase. The results obtained by Pereira et al. 2000 showed that imports and exports of larvae depended not only on abundance but also on larval vertical distribution time-patterns.

The most significant and comprehensive study about the ecology of zooplankton in the Ria de Aveiro was performed by Morgado (1997). In his study, specific composition of zooplanktonic communities (125, 335 and 500 μm), monthly occurrence and spatial distribution of the most important species were related to hydrological parameters. This study also described dynamic aspects of the zooplankton communities related to the major environmental cycles by the evaluation of longitudinal transport of organisms at high and low tide (at spring and neap situation) as well as tidal, nychtemeral and lunar dynamics.

The abundance of the zooplankton fluctuated widely throughout the year, with three main peaks, in Spring, Summer and Autumn. The comparative analysis of the three taxocenosis (125, 335 and 500 μm) indicated that the contribution of the smaller species and developmental stages is the most important to the annual zooplankton production of Ria de Aveiro. This study also investigated the faunistic composition and showed that the holoplankton of Ria de Aveiro is mainly composed of Copepoda, Siphonophora, Chaetognatha and Appendiculata. Meroplankton was constituted by Hydromedusae, polychaeta larvae, Mollusca larvae and eggs, Cirripedia nauplii, Decapoda larvae, Isopoda, Mysidacea and Pisces larvae and eggs.

The Copepoda were the most abundant and frequent organisms in this community. The most abundant species in this group in terms of abundance were *Acartia tonsa* (29%), *Euterpina acutifrons* (20%), *Oithona nana* (15%) and *Acartia clausi* (8%). Other copepod species encountered included *Paracalanus parvus*, *Pseudocalanus elongatus*, *Clausocalanus* spp., *Temora longicornis*, *Calanipedia aquaedulcis*, *Oithona similis*, *Cyclopina gracilis*, *Oncae media* and *Tachidius discipes*.

1.5 General objectives

The aim of this thesis was to better understand the importance of copepod populations on the carbon flux in a southern European estuary (Ria de Aveiro, Portugal) and particularly *Acartia* spp populations. To fulfil such purpose, *in situ* studies - field campaigns encompassing a period of 22 consecutive months, were combined with laboratory methodologies - temperature-dependent copepod growth models.

Specifically, the objective of this thesis was (1) to characterize and describe the spatial-temporal patterns of abundance, biomass and production of the estuarine copepod community from Ria de Aveiro (Portugal) and its relationship with hydrological data (salinity, temperature, chlorophyll *a*, suspended matter and rainfall regime), (2) to compare growth and developmental rates of allopatric copepod populations, (3) to define temperature dependent growth models for nauplii and copepodites of *Acartia tonsa*, (4) to evaluate environmental forcing on the distribution and abundance of *Acartia* populations and (5) to estimate potential secondary production rates of *Acartia* populations.

1.6 List of manuscripts

Excluding chapter 1 (General introduction) and chapter 7 (Conclusion remarks), each chapter of the present thesis corresponds to a manuscript that was already published or submitted on an international peer-reviewed journal.

1.6.1 Published manuscripts

1. Leandro SM, Morgado F, Pereira F, Queiroga H (2007) Temporal changes of abundance, biomass and production of copepod community in a shallow temperate estuary (Ria de Aveiro, Portugal). Estuarine Coastal and Shelf Science 74: 215-222 (1.733)

2. Leandro SM, Queiroga H, Rodriguez L, Tiselius P (2006). Temperature dependent development and somatic growth in two allopatric populations of *Acartia clausi* (Copepoda: Calanoida). Marine Ecology Progress Series 322: 189-197 (2.286)

3. Leandro SM, Tiselius P, Queiroga H (2006) Growth and development of nauplii and copepodites of the estuarine copepod *Acartia tonsa* from southern Europe (Ria de Aveiro, Portugal) under saturating food conditions. Marine Biology 150: 121-129 (1.756)

1.6.2 Submitted manuscripts

1. Leandro SM, Tiselius P, Queiroga H (submitted). Spatial and temporal scales of environmental forcing of *Acartia* populations (Copepoda: Calanoida) in the Canal de Mira (Ria de Aveiro, Portugal). Limnology & Oceanography (3.287)

2. Leandro SM, Queiroga H, Tiselius P (submitted) Biomass and production of juvenile stages of Acartia (Copepoda: Calanoida) populations from a Southern

European estuary (Canal de Mira – Ria de Aveiro). Journal of Plankton Research (1.617)

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Chapter 2

Abundance, biomass and production of an estuarine copepod community

Section 2.1

Temporal changes of abundance, biomass and production of copepod community in a shallow temperate estuary (Ria de Aveiro, Portugal)

2.1.1 Abstract

The present study reports on temporal changes of abundance, biomass and secondary production of the copepod community of Ria de Aveiro (Portugal). Zooplankton sampling and hydrological measurements (salinity, temperature, chlorophyll *a* and nutrients concentrations) were conducted at four occasions (June 2000, September 2000, December 2000 and March 2001), at 6 sampling stations and during ebb and flood. The contribution of copepods (from nauplius to adult) to the total abundance and biomass of the zooplankton community of Ria de Aveiro (Portugal) was equal to 63.6% and 62.0%, respectively (annual average). The estimate of nauplius abundance given by two zooplankton nets with different meshes was significantly different ($P < 0.001$) with the 64 μm net collecting 13.9 times more than the 125 μm one. No significant differences were found for copepodites and adults. The abundance of all development stages (except adults) was positively correlated ($P < 0.05$) with salinity and temperature. The seasonal patterns of abundance and biomass were similar to those found in other temperate coastal waters. Mean daily secondary production rate (mean ± SE) estimated by the Huntley and Lopez growth model (Huntley & Lopez, 1992) was 22% higher than the value given by the application of the Hirst and Bunker model (Hirst & Bunker, 2003): 3.71 ± 0.540 and 2.90 ± 0.422 mg C m^{-3} d^{-1}, respectively.

Key words: Estuarine copepod community; abundance; biomass; secondary production rate; Ria de Aveiro (Portugal)

2.1.2 Introduction

Copepods are frequently considered the most important link between phytoplankton and higher trophic levels (Mauchline 1998, Uye et al. 2000). These organisms comprise as much as 80% of the total mesozooplankton biomass (Kiørboe 1997) and are the most significant component of marine and estuarine environments as herbivores and as prey for higher levels (e.g. the larvae of fish). Absolute values of biomass corresponding to estuarine copepod communities have been estimated during the last decades. In Atlantic waters, those estimates were made mainly on estuaries located on North America (e.g. Durbin & Durbin 1981, Park & Marshall 2000), or North Europe (e.g. Escaravage & Soetaert 1995, Peitsch 1995), and few or none relate to southern European estuaries.

Estuaries are amongst the most productive natural ecosystems in the world (Schelske & Odum 1962, Baban 1997, Wilson 2002), supporting a great variety of marine resources with economic potential (e.g. fish, crustaceans and molluscs). These high levels of productivity are due to the existence of three different types of primary producers (marsh grass, benthic algae and phytoplankton), to water movements resulting from tidal action, to the existence of abundant nutrients supplies (run-off and rivers) and to the rapid regeneration and conservation of nutrients (Schelske & Odum 1962).

The abundance, biomass and distribution of marine zooplanktonic organisms are indirectly affected by the physical conditions and depend on the food conditions (quality and quantity) that occur in a given ecosystem. In the water column, the productivity of each trophic level will depend on the amount of phytoplankton production and also on the transfer efficiency of matter and energy between each level (Uye & Shimazu 1997).

In Ria de Aveiro (Portugal), a temperate shallow coastal lagoon, the mesozooplankon has been previously studied in terms of spatial distribution of abundance and diversity at seasonal and tidal scales (Morgado, pers. comm). The abundance of the

zooplankton showed important variations during the year, with three main peaks, in Spring, in Summer and in Autumn. The Copepoda were the most abundant and frequent organisms in the community. The most abundant species in this group in terms of concentration were *Acartia tonsa* (29%), *Euterpina acutifrons* (20%), *Oithona nana* (15%) and *Acartia clausi* (8%).

The objectives of the present study consisted in describing temporal patterns of abundance and biomass of the copepod estuarine community of Ria de Aveiro (Portugal) lagoon and obtaining an estimate of secondary production. Several estimates of production of estuarine copepods are available in literature, (e.g. Heinle 1966, Heinle 1969, Durbin & Durbin 1981, Miller 1983, Castel & Feurtet 1989, Escaravage & Soetaert 1995, Escribano & Mclaren 1999, Hirst et al. 1999, Ara 2001), but there seems to be a lack of reliable estimates for southern European estuaries.

2.1.3 Materials and Methods

2.1.3.1 Study site

Ria de Aveiro (40º 38' N, 8º 44'W) is a shallow coastal lagoon situated on the north west coast of Portugal (Fig. 2.1), separated from the sea by a sand bar. Its mouth is artificially maintained and the mean depth is about 1 m, except in the navigation channels where it ranges from 7 to 20 m. The Ria de Aveiro has an irregular and complex geometry, where four main channels can be identified: Canal de Ílhavo, Canal de Mira, Canal de Ovar and Canal do Espinheiro. With maximum length and width of 45 and 10 km, the lagoon covers an area of 83 km^2 at spring high tides and of 66 km^2 at spring low tides.

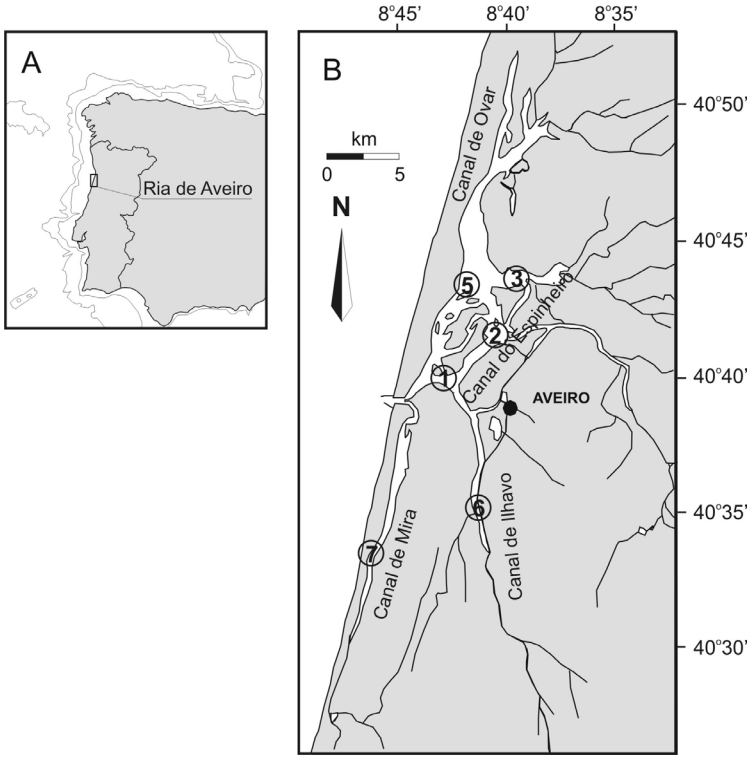

Figure 2.1 Map of Ria de Aveiro (Portugal) with the localization of the sampling stations used on this study.

Vouga and Antuã rivers, which have average flows of 29 and 2 $m^3 s^{-1}$, accordingly (Dias et al. 2000), account for most of the freshwater discharge into the lagoon. The total mean river discharge during a tidal cycle is about 1.8 x 10^6 m^3. The tidal prism at the mouth in a spring tide with a tidal range of 2.48 m is about to 70 x 10^6 m^3 (Vicente 1985). Therefore, circulation in the lagoon is dominated by tides, except during periods of peak discharge which are coincident with heavy rainfall (Barrosa 1985, Vicente 1985, Dias 2000).

Tides are semidiurnal with an average range of 2.1 m and ranges of ca. 1 and 3 m in extreme neap and spring conditions. In each of the main channels the tidal prism value relative to the value at the mouth is of about 38% for Canal de S. Jacinto, 26% Canal do Espinheiro, 10% Canal de Mira and 8% Canal de Ílhavo (Dias et al. 2000). The residence time estimated for the lagoon is lower than 2 days in the central area close to the mouth, revealing a strong marine influence in this area. Residence times higher than 1 week are found at upper reaches of the main channels (Dias 2000).

2.1.3.2 Sampling

The sampling strategy used in the present study considered the need to account for the spatial complexity of the lagoon, the seasonality of the salinity and temperature regimes, the tidal circulation, and logistic reasons. Four seasonal field sampling campaigns were performed in June, September and December 2000 and in March 2001. Sampling was carried out at six stations (St.1, St.2, St.3, St.5, St.6 and St.7) distributed throughout of the lagoon (Fig. 2.1). The mean water depth at each station was equal to 5.8, 2.7, 3.4, 5.1, 3.3 and 1.7 m, respectively.

At each station, sampling took place during ebb and during flood of the same day. In order to standardise sampling in relation to the tidal and tide amplitude cycle, zooplankton sampling and hydrological measurements always took place after the new moon, during average amplitude tides (2.0 m), 2 h ($\pm$ 30 min), after flood and ebb slack times at each station. Slack times for each station were estimated from the average tide of March 20th, 2000. The sampling programme included a total of 48 sampling occasions (4 campaigns x 6 stations x 2 tidal phases).

2.1.3.3 Hydrological parameters

Temperature and salinity were measured *in situ* with an AANDERAA STD-Sensor Model No 3230. Mean values for the water column were determined by averaging the values obtained in vertical profiles (1-meter steep readings). In order to determine nutrient concentrations (ammonium, nitrates/nitrites, phosphates and silicates) and chlorophyll *a* content, water samples were taken with a van Dorn bottle.

The nutrient concentrations (μM) were determined with an auto-analyser following the classical methods described by Strickland & Parsons (1972), after water filtration through 0.45 μm acetate filters. Three replicates of 500 ml of seawater were filtered through GF/C filters and the chlorophyll *a* concentration (mg m^{-3}) was determined fluorimetrically as described by Yentsch & Menzel (1963).

2.1.3.4 Copepod abundance and biomass

Zooplankton samples were collected at mid-water by towing two coupled zooplankton nets during less than 1 min, in order to prevent net clogging. The nets had mesh sizes of 64 and 125 μm and had diameters of 29.5 and 25.4 cm, respectively. A KAHLSICO Digital Flow Meter was fitted on the 64 μm net. Total volume filtrated by the two nets was assumed to be the same and varied from 0.4 to 8.8 m^3. Excluding nauplii data, this assumption showed to be acceptable since no significant differences were verified between the estimates of copepodite and adult forms obtained from the two nets (see results).

Immediately after the collection, the samples were preserved in buffered formaldehyde solution at a final concentration of 4%. In the laboratory, samples were diluted to a known volume and aliquots were taken to get a minimum of 200 individuals per stage (nauplius, copepodite and adult). Sorting and counting were made on a stereomicroscope Olympus SZH10. Abundances were expressed in individuals m^{-3}. The sorted organisms where washed with distilled water and transferred to small aluminium caps.

After drying at 60ºC for 24 hours, the weight was taken on a CAHN C-32 electrobalance (± 0.1 μg). Dry weights were corrected for weight lost during preservation according to Omori and Ikeda (1984) by a factor of 1.3 (corresponding to a loss of 30%) and then converted to carbon weight (μg C) assuming this to be 40% of the dry weight (Omori & Ikeda 1984, Båmstedt 1986). The total biomass of nauplii, copepodites and adults for the different sampling campaigns, was obtained by the product of its abundance and respective mean individual carbon weight, expressed in μg C m^{-3}.

2.1.3.5 Secondary production rate

Daily secondary production rate was estimated by the product of copepods biomass and the growth rate:

$$P = B \times g \qquad \text{(Eq. 1)}$$

where *P* is the daily secondary production (mg C m^{-3} d^{-1}), *B* is the copepods biomass (mg C m^{-3}) and *g* is growth rate (d^{-1}). Estimates of copepod growth rate were obtained from two different general growth models:

Huntley & Lopez (1992): $g = 0.045\ e^{0.111T}$

Hirst & Bunker (2003): $\log_{10} g = 0.0186\ T - 0.288 \log_{10} BW + 0.417 \log_{10} Ca - 1.209$

where *g* is the growth rate (d^{-1}), *T* is the temperature (ºC), *BW* is body weight (µg C ind^{-1}) and Ca is the chlorophyll *a* concentration (µg Chl *a* l^{-1}). The equation in Hirst and Bunker's model used in the present study describes the growth rate of juvenile and adult copepods, whether they are broadcasters or sac-spawners.

2.1.3.6 Statistical analyses

The effect of mesh size (64 and 125 µm), season (Winter, Spring, Summer and Autumn) and tidal phase (Ebb and Flood) on the abundance of nauplii, copepodites and adults of the copepod community was analysed with a 3-way orthogonal ANOVA. Data were log-transformed before analysis, which homogenised the variances according to a Cochran's test (Sokal & Rohlf, 1987).

Spearman rank correlations were computed in order to investigate the relationships between environmental variables (salinity, temperature and chlorophyll *a*) and abundances, and between chlorophyll *a* and nutrient concentrations (ammonium, nitrites/nitrates, phosphates and silicates). For each variable under study, a mean

value and standard error was calculated based on all the data obtained in the respective sampling campaign (n = 12, corresponding to 6 stations and 2 tidal phases). All statistical analyses were done using Statistica version 6.0 (StatSoft Inc. 2001).

2.1.4 Results

2.1.4.1 Hydrological parameters

Salinity and water temperature showed a markedly seasonal variation (Fig. 2.2). Salinity ranged from 33.3 in September 2000 to 7.4 in March 2001. Water temperature varied from 21.2ºC in June 2000 to 13.2ºC in December 2000. Precipitation data for the study period was obtained from the meteorological station located in the University of Aveiro. The highest value of chlorophyll *a* was found in June 2000 (5.88 ± 0.98 mg m^{-3}), and the lowest in December 2000 (0.94 ± 0.12 mg m^{-3}).

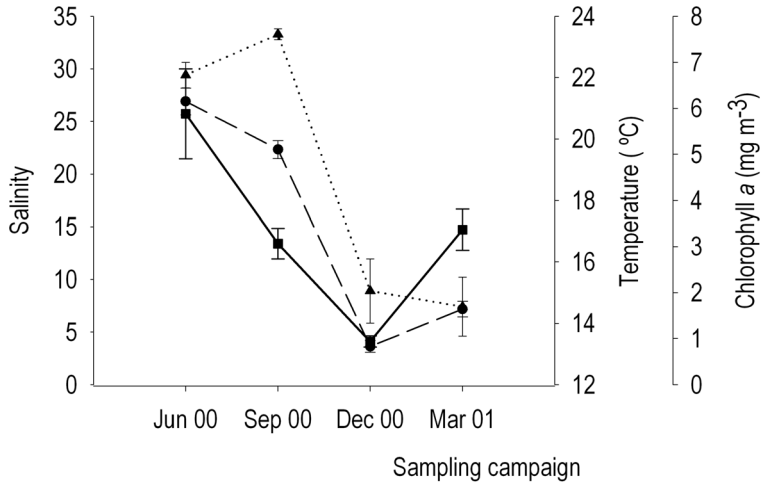

Figure 2.2 Hydrological parameters determined at each sampling campaign. [(▲) Salinity, (●) Temperature and (■) Chlorophyll *a*]. Symbols and bars denote means and standard errors, respectively.

Figure 2.3 shows the seasonal variation in the nutrient concentration. Ammonium (NH_4) and nitrates/nitrites (NO_3 + NO_2) concentrations were highest in December 2000 (8.03 and 135.71 μM) and lowest in June 2000 (1.13 and 12.68 μM). A different

pattern of variation was found for phosphates (PO_4) and silicates (SiO_2). Phosphates showed a maximum of 3.36 μM in September 2001 and a minimum of 0.83 μM in March 2001. For silicates, the concentration ranged from 5.89 μM in September 2000 to 78.92 μM in December 2000.

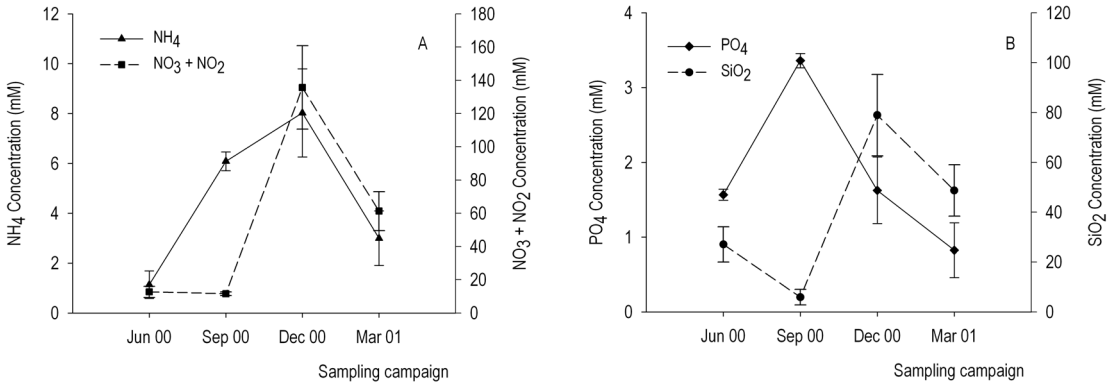

Figure 2.3 Concentration (μM) of ammonium (NH_4), nitrates/nitrites (NO_3 + NO_2) (A) and phosphates (PO_4), silicates (SiO_2) (B) at each sampling campaign. Symbols and bars denote means and standard errors, respectively.

Spearman´s rank correlation indicated a significant negative association between the concentration of NH_4 and chlorophyll *a* (r = - 0.46, d.f. = 48, $P < 0.05$) and between the concentration of NO_3^- + NO_2^- and chlorophyll *a* (r = - 0.42, d.f. = 48, $P < 0.05$). No significant associations were found between the concentrations of phosphates or silicates and chlorophyll *a*.

2.1.4.2 Abundance

Although this paper aims to study the copepod community in the Ria de Aveiro, the abundance and biomass of other zooplanktonic groups were also estimated. The contribution of copepods (including nauplii, copepodites and adults) to the total zooplankton abundance was equal to 63.6% (annual average).

Smaller contributions were provided by strictly carnivorous organisms (including siphonophores, chaetognaths, medusae and nematods) with 10.2%, gastropod larvae

(10.4%), ciliates (10.4%), cirripedes (1.9%), appendicularians (1.7%), cladocerans (1.0%), ostracods (0.7%) and mysidaceans (< 0.1%). The total zooplanktonic biomass was also dominated by the copepods (62.0%) followed by the carnivores (12.3%), gastropod larvae (7.1%), ciliates (6.0%), ostracods (4.5%), cladocerans (2.9%), appendicularians (2.9%), cirripedes (2.2%) and mysidaceans (< 0.1%).

A three-way orthogonal ANOVA (Table 2.1) showed the existence of a highly significant effect of the season on the concentration of nauplii and copepodites ($P < 0.001$), and a significant effect on the concentration of adults ($P < 0.05$) indicating a seasonal effect on the abundance of the different development classes of the copepod community.

The effect of tidal phase was generally not significant, except for a significant interaction of Season x Tide detected for the nauplii ($P < 0.01$). Highly significant differences ($P < 0.001$) were found between the estimates of the concentrations of nauplii obtained with the two mesh sizes, the 64 μm net producing much higher estimates (18551 ± 3148 and 1331 ± 198 ind. m^{-3}, for the 64 and 125 μm nets, respectively). Differences between estimates of concentration obtained for copepodites and adults with the two mesh sizes were not significant (Table 2.1).

Table 2.1 3-way orthogonal ANOVA of the effects season (Winter, Spring, Summer and Autumn), tidal phase (ebb and flood) and mesh size (64 μm, 125 μm) on the abundance of nauplii, copepodites and adults. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

	Nauplii			Copepodites			Adults		
	d.f.	MS	F	d.f.	MS	F	d.f.	MS	F
Season	3	2.988	12.096***	3	11.766	27.141***	3	1.028	3.756*
Tide	1	0.001	0.004	1	1.165	2.687	1	0.054	0.199
Net	1	33.278	134.723***	1	0.001	0.002	1	0.411	1.503
Season x Tide	3	1.020	4.128**	3	0.534	1.231	3	0.709	2.589
Season x Net	3	0.559	2.264	3	0.367	0.846	3	0.128	0.467
Tide x Net	1	0.009	0.038	1	0.293	0.677	1	0.058	0.211
Season x Tide x Net	3	0.108	0.436	3	0.190	0.439	3	0.133	0.487
Error	75	0.247		75	0.434		75	0.274	
TOTAL	90			90			90		

Based on these results, the abundance of nauplii was defined only from the estimate given by the 64 μm net. For copepodites and adults, the abundance was obtained by averaging the estimates given by the two nets. The highest abundances of the copepod community (from nauplius to adults) were noted in September ($65.8 \times 10^3 \pm 13.4 \times 10^3$ ind. m^{-3}) and June ($20.4 \times 10^3 \pm 5.12 \times 10^3$ ind. m^{-3}), in contrast to December and March where the mean abundances were equal to 11.4×10^3 ($\pm 2.61 \times 10^3$) ind. m^{-3} and 10.9×10^3 ($\pm 3.17 \times 10^3$) ind. m^{-3}, respectively (Fig. 2.4).

Spearman rank correlations between abundance and hydrological parameters showed that the abundances of all stages were positively correlated with salinity and with temperature ($P < 0.05$), except for the adults that were correlated only with salinity. Significant correlations with chlorophyll *a* were not found.

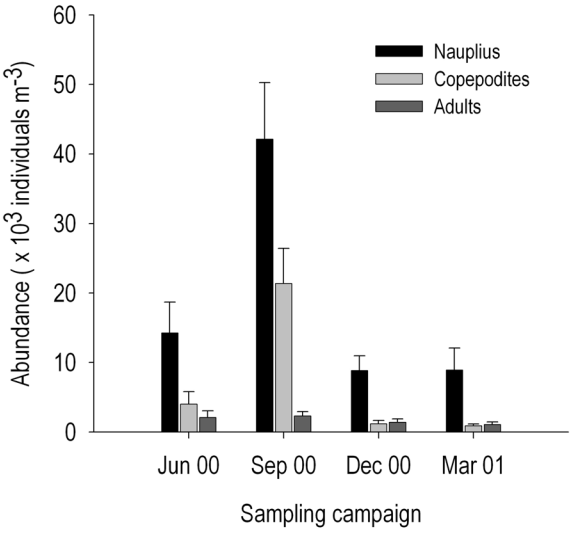

Figure 2.4 Abundance (individuals m^{-3}) of the copepoda community (nauplius, copepodites and adults) in the Ria de Aveiro (Portugal). Data are means with standard errors indicated by error bars.

2.1.4.3 Biomass and secondary production

The variation between sampling dates of the biomass, in terms of carbon content, of the copepod community of the Ria de Aveiro is shown in Figure 2.5. The highest values were found in September 2000 (12.40 ± 2.31 mg C m^{-3}) and June 2000 (10.94 ± 3.34 mg C m^{-3}), and the lowest were found in December 2000 (4.63 ± 1.03 mg C m^{-3}) and March 2001 (5.41 ± 1.85 mg C m^{-3}).

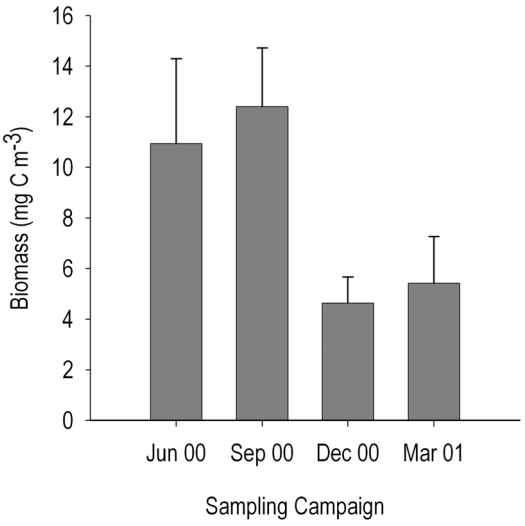

Figure 2.5 Temporal changes of biomass (mg C m^{-3}) of the copepoda community (from nauplius to adults) of Ria de Aveiro (Portugal). Symbols and error bars indicate means and standard errors, respectively.

As expected from the models used to calculate secondary production, the temporal change of production followed the pattern of concentration and biomass, with highest values during summer and lowest in winter and early spring. Daily secondary production rates (minimum ± SE – maximum ± SE) ranged from 1.25 ± 0.284 – 7.06 ± 1.106 mg C m^{-3} d^{-1} (Huntley & Lopez model) and 0.96 ± 0.236 – 5.65 ± 0.894 mg C m^{-3} d^{-1} (Hirst & Bunker model) (Fig. 2.6).

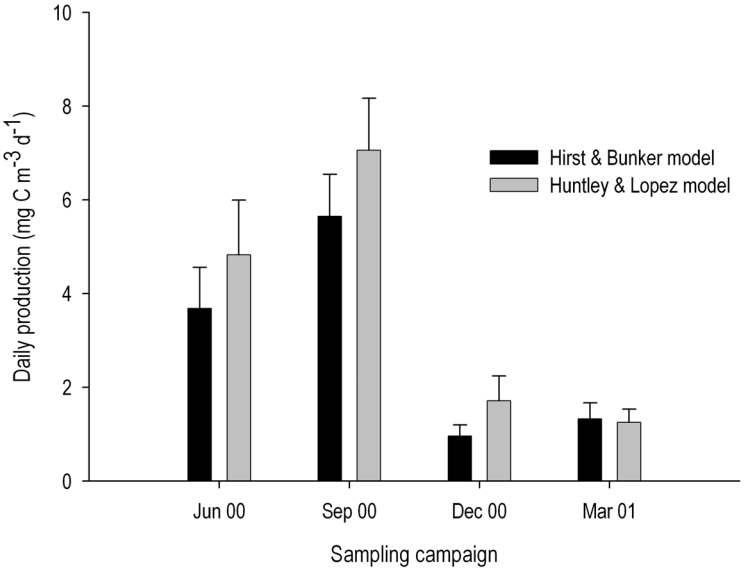

Figure 2.6 Temporal changes of daily secondary production rate (mg C m^{-3} d^{-1}) of the copepoda community of Ria de Aveiro (Portugal). Production rates were estimated from the general growth models of Huntley & Lopez (1992) and Hirst & Bunker (2003). Data are means with standard errors indicated by error bars.

By the application of the Huntley & Lopez model, the mean daily secondary production rate was 22% higher than the one obtained from the Hirst & Bunker model (3.71 ± 0.540 and 2.90 ± 0.422 mg C m^{-3} d^{-1}, respectively).

2.1.5 Discussion

2.1.5.1 Hydrological characteristics and precipitation regime

The seasonal pattern of salinity variation in Ria de Aveiro determined from the present study is strongly related to precipitation, which is in agreement with previous studies that have shown that this lagoon behaves as a seasonal poikilohaline estuary (Moreira et al. 1993, Dias et al. 1999). An unusual peak of precipitation that occurred in December 2000 contributed to a large input of freshwater to the lagoon. Such input was reflected not only on the low salinity values measured in that period, but also on the low values of chlorophyll a concentration and copepod abundance (see below).

Periods of lower phytoplankton biomass (winter and spring) were paralleled by an increase in the N:P ratio, which is consistent with the hypothesis of a lower nitrogen consumption and higher availability during periods of short days and low primary production. The significant negative correlation found between nitrogen and chlorophyll *a* concentration supports this interpretation.

2.1.5.2 Copepod abundance and biomass

The most abundant zooplanktonic group found during this study was the Copepoda (all development stages). Their relative contribution to the total zooplanktonic abundance and biomass of the Ria de Aveiro was 63.6 and 62%, respectively. The decrease of copepod abundance in December was more pronounced during ebb, where significant values of the interaction Season x Tide was obtained for the nauplii. In this study, the biomass of copepods was converted to carbon content for easy comparison with secondary production data already estimated in other regions.

Due to the lack of a relationship between the dry weight and the carbon content for the different development stages and for the different species of copepods that comprise the estuarine community of Ria de Aveiro, it was assumed that 40% of the dry weight of all development stages and species corresponds to carbon. Such an

assumption may contain a potential source of error based on the evidence that the carbon content differs between species and, within a given species, temporal variability occurs due to food availability, reproductive status or development stage (Mauchline 1998).

2.1.5.3 Copepod production

There are a few alternative methodologies available to estimate the copepod secondary production. In addition to the values for biomass, all of them require also the determination of growth rates. Growth rates of copepods can be determined from preserved samples, demographic information, direct measurements of somatic growth or reproductive rates, or taken from models based on physiological rates (Huntley & Boyd 1994), temperature (Huntley & Lopez 1992), or temperature and body weight (Hirst & Sheader 1997, Hirst & Lampitt 1998), and temperature, body weight and chlorophyll *a* concentration (Hirst & Bunker 2003).

Due to the lack of more detailed information, we decided to use the growth models defined by Huntley & Lopez (1992) and Hirst & Bunker (2003) in order to estimate the secondary production rate achieved by the copepod community of the Ria de Aveiro. Comparing the results obtained from the two models, it is clear that the application of the Huntley and Lopez's model often shows higher values than the Hirst and Bunker's model. The Huntley and Lopez's model depends only on temperature and do not assume that copepod growth may be food limited during some periods of the year (Burkill & Kendall 1982, Kimmerer & McKinnon 1987, Peterson et al. 1991).

This aspect is taken into account in the Hirst and Bunker model with the inclusion of a food descriptor (chlorophyll *a*). In addition, the Huntley and Lopez's model tends to overestimate growth rates (Kleppel et al. 1996). As pointed out by Hirst & Sheader (1997), such overestimation is justified by the fact that growth rates are obtained from field generation times which may be biased since cohort's may grow asynchronously, and slow growing individuals may have higher mortality rates (Lopez 1991). They may

be also influenced when peaks in abundance are used to follow growth, as these peaks are made up of optimally growing individuals (Carlotti & Nival 1991).

Although the production rates were different depending on the method applied, they were similar to the rates obtained for copepod communities of other temperate coastal waters. For example, the copepod production in the Westerschelde (The Netherlands) was estimated as about 4 mg C m^{-3} d^{-1} (Escaravage & Soetaert 1995), that of the Kattegat (Denmark) as 1.18 mg C m^{-3} d^{-1} (Kiorboe & Nielsen 1994) and a value of 6.85 mg C m^{-3} d^{-1} was obtained for the Inland Sea of Japan (Uye & Liang 1998). Marked differences are found when the production at the Ria de Aveiro is compared to that of the Mondego estuary which lies 50 km to the south (Vieira et al. 2002, Pastorinho et al. 2003, Vieira et al. 2003). The production of the dominant copepod populations (*Acartia* spp.) of the Mondego was estimated as 0.36 mg C m^{-3} d^{-1}, which is about 10 times lower than the estimate for the Ria de Aveiro. This difference is most certainly due to the use of cohort analysis for the calculations made for the Mondego estuary, which is an inappropriate methodology when copepods exhibit high growth rates and short generations times (Kimmerer 1987, Escaravage & Soetaert 1995).

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Chapter 3

Growth and development of copepod allopatric populations

Section 3.1

Temperature dependent development and somatic growth in two allopatric populations of *Acartia clausi* (Copepoda: Calanoida)

3.1.1 Abstract

This study compares the effect of temperature on the post-embryonic development time and weight-specific growth rate in two populations of *Acartia clausi* originated from different biogeographic areas (Northern and Southern Europe). Development was followed from nauplius I to adult at 3 temperatures (10, 15 and 18ºC) at saturating food conditions. The relationship between development time and temperature was established by fitting a Belehradek´s function. The north population had a shorter generation time at all temperatures. At 10ºC, the development time was estimated to 33.9 and 36.4 days decreasing to 16.3 and 17.4 days at 18ºC for the north and south population, respectively. Prosome length decreased with temperature and the southern population had longer individuals at all temperatures. ANCOVA revealed a significant ($P < 0.001$) positive effect of temperature on the growth rates and nauplii grew faster than copepodites (except at 18ºC - south population and 20ºC – north population). Significant differences between populations were noted during larval growth, with nauplii from the north grew faster at high temperatures (18ºC). The results indicate that the two *A. clausi* allopatric populations which are subjected to different temperature regimes have different temperature responses, in particular at high temperatures.

Key words: Temperature; Development time; Weight-specific growth rate; *Acartia clausi*; Ria de Aveiro (Portugal); Gullmarsfjord (Sweden)

3.1.2 Introduction

Temperature is an important environmental factor for ectotherm organisms like copepods, and strongly affect vital physiological rates like respiration (e.g. Roddie et al. 1984, Gaudy et al. 2000) and excretion (e.g. Gaudy et al. 2000). Along with food conditions, temperature can also modify the life history traits of copepods through its influence on mortality rates (e.g. Hirst & Kiørboe 2002), egg production (e.g. Halsband-Lenk et al. 2002) and growth and development rates (Campbell et al. 2001, Hirst & Kiørboe 2002).

Calanoid copepods often dominate the zooplankton in terms of abundance and biomass, and some species occur over wide biogeographic regions (Mauchline 1998). The neritic copepod *Acartia clausi* Giesbrecht, has been recorded in different areas of the Atlantic Ocean like the North Sea (Tiselius et al. 1991), Irish Sea (Castellani & Lucas 2003), English Channel (Lindley et al. 1997), Cantabrian coast (Quevedo et al. 1999) and NW coast of Portugal (Morgado et al. 2003) as well as in other areas including the Mediterranean Sea (Gaudy et al. 2000), Black Sea (Gubanova et al. 2002), Gulf of Guinea (Pagano et al. 2003) and Pacific Ocean (Gomez-Gutierrez et al. 1999).

The populations are subjected to local conditions, creating site-specific adaptations and eventually genetic differences. Reduced gene flow between populations will over time create different genotypes. The degree of genetic exchange between populations depends on the capacity that a given sub-population has to remain and keep isolated in a given area. *Acartia* spp. species are common in extremely advective regions like coastal areas and estuaries, but can establish persistent populations. *Acartia clausi* is able to maintain isolated populations even at a relatively short spatial scales, in contrast to other species such *Calanus* spp (Bucklin et al. 2000).

The retention of *Acartia* spp. populations in a given region could be attributed to the combined effect of the swimming behaviour that allow regulation of vertical distribution (Kouassi et al. 2001), the production of diapause eggs (Castro-Longoria 2001) in response

to unfavourable environmental conditions and life history traits such as short generation times and high reproductive potential. The evidence that genetic differences occur among allopatric populations of *Acartia clausi* implies that population-specific responses to environmental conditions could exist.

The present study aimed at comparing the effect of temperature on the development and growth rate of two populations of *Acartia clausi* originating from north (58º15'N, 11º27'E) and south Europe (40º38'N, 8º44'W). The south population (Ria de Aveiro, Portugal) experiences a temperature range of 11-20ºC and show abundance peaks in July and October when water temperature varies from 17-20ºC (Leandro, unpublished). The north population (Gullmarsfjord, Sweden) lives where the water temperature fluctuates between -1-20ºC, and is most abundant from June to August (14-20ºC, Eriksson 1973). Our null hypothesis was that development time and growth rates of the two populations were not different when reared at the same temperature. To test this, development was followed from nauplius I to adults at 3 temperatures (10, 15 and 18°C) and at saturating food conditions.

3.1.3 Materials and Methods

3.1.3.1 Parental cultures

In order to avoid confounding effects of field temperature on the development and growth, all copepods for the parental cultures were collected at nearly the same water temperature (16ºC), but at different seasons for the north and south population. Accordingly, *Acartia clausi* was collected in the Gullmarsfjord, Sweden (58º 15' N, 11º 27' E, north population) in summer of 2002 and in Ria de Aveiro, Portugal (40º 38' N, 08º43' W, south population) in spring 2003. We did not replicate the sampling of parental animals, but studied the temperature response within each population, represented by a single sub-sample. Although genetic differences can occur over short distances (Bucklin et al. 2000), the sampling areas are highly dynamic and the populations continuously

reproducing and we assumed that sampling several thousand females on one occasion would give a good representation of the population in the area.

The copepods were caught with horizontal tows at 2 m using a 200 µm mesh net fitted with a 10 l plastic bag as a cod end. After collection, the samples were diluted in surface water and brought to the laboratory within 1 h. In the laboratory, ca. 2000 females of *A. clausi* from each location were sorted out using a binocular microscope and transferred to 20 l buckets. The animals were fed with a mixture of the diatom *Thalassiosira weissflogii* (equivalent spherical diameter, ESD = 14.5 µm) and the cryptophyte *Rhodomonas* sp. (ESD = 6.7 µm). The micro algae were grown in f/2 medium (Guillard, 1962) at 20°C under a 12:12 light:dark cycle. Both algal strains were provided by the marine algal culture centre at Göteborg University (GUMACC).

Eggs from the field caught females were harvested daily, rinsed in filtered sea water and stored in 10 ml plastic tubes at 4°C in the dark. Parental stock cultures for the north and south populations were subsequently started with the eggs collected from the corresponding field culture and placed in 40 l carboys containing filtered seawater (< 0.2 µm, salinity 32 PSU) and maintained at 18ºC. The cultures were fed ad libitum of *Rhodomonas* sp. during naupliar development and a mixture of *Rhodomonas* sp. and *T. weissflogii* after the appearance of copepodites.

3.1.3.2 Growth experiments

Growth experiments started after the parental cultures were acclimated for 1 generation (Table 3.1). For each treatment/replicate ≈ 10 000 eggs were harvested from the corresponding stock and transferred to polyethylene buckets containing 10 l of filtered sea water (< 0.2 µm, salinity 32 PSU) with gentle aeration.

The eggs were collected from the bottom of the “parental culture” by sieving through successive nylon screens with 200, 90 and 75 µm mesh in order to exclude adults, copepodites, nauplii and faecal pellets. The fraction collected on the 75 µm sieve was

then transferred to a small Petri dish and concentrated to the centre by slowly rotating movements and further cleaned from early nauplius and detritus.

For both populations, each treatment/replicate was setup at the same time. The growth experiments were performed in duplicate at 10, 15, 18°C with each population. In addition, one experiment at 20°C with the northern population and two experiments at 22°C with the southern population were performed. All experiments were carried out in a temperature controlled room, under a 12L:12D light cycle.

Food levels were always ≥ 1000 µg C l^{-1}, which is 3 times higher than the optimal food concentration previously defined for *Acartia clausi* (Klein Breteler & Schogt 1994). The food offered was *Rhodomonas* sp. during the naupilar growth (NI to NVI) and a mixture of *Rhodomonas* sp. and *Thalassiosira weissflogii* (copepodite stage I-VI).

Food concentration was checked daily by an electronic particle counter (Elzone 180XY). Algal biovolume (µm^3) was converted to carbon using the relationship defined by Strathmann (1967). The food concentration was adjusted daily by adding fresh culture and always kept ≥ 1000 µg C l^{-1}.

Table 3.1 Experiments performed. Date of collection of the field populations, ambient temperature (°C), end of acclimation period and dates for each experiment. Eggs for experiments stored at 4°C in the dark from end of acclimation to start of experiments.

	North	South
Ambient temperature, °C	16.8	15.6
Date of collection	19 July 2002	24 Feb 2003
End of acclimation	20 Aug 2002	25 March 2003
Start of Experiments		
10°C	06 Sep 2002	29 March 2003
15°C	27 Aug 2002	31 March 2003
18°C	06 Sep 2002	29 March 2003
20°C	27 Aug 2002	-
22°C	-	31 March 2003

3.1.3.3 Stage durations

The development was followed from nauplius I to the adult stage. Time 0 was defined as the time when eggs were harvested (eggs 0-24 h old) and incubation started. Each experiment was sampled daily by mixing the bucket and sieving 100-300 ml of water to yield at least 30 individuals for stage determination and length measurements. Total volume of the bucket was kept constant by adding filtered sea water.

The duration of each development stage (stage duration, SD) was calculated as the difference of the median development time (MDT) of two successive stages. The MDT of a certain stage was defined as the time when 50% of the organisms in a culture had passed that stage (Landry 1975b, 1983) which in turn was estimated from stage-

frequency data converted to stage proportion. The cumulative proportion of each stage over time was plotted against time and a gamma distribution function fitted (Klein Breteler et al. 1994).

The relationship between temperature and median development time (from egg to adult) of each population was estimated using the Belehradek's function:

$$D = a\,(T + c)^{-2.05}$$

where D is median development time (days), T is temperature (°C) and a and c are fitted coefficients (McLaren 1995). The coefficients were estimated by non-linear regression using the software STATISTICA 6.0.

3.1.3.4 Body length and weight measurements

Total body length of nauplii (µm) and prosome length of copepodites (µm) were measured with an inverted microscope (100 × magnification) with a calibrated eyepiece micrometer. Prosome lengths up to stage CIII were compared by 2-way ANOVA (population and temperature as factors) and when sexes were discerned (CIV-adults) by 3-way ANOVA (sex, population and temperature as factors).

The weight of nauplii (NI to NVI) was estimated from a length-weight regression (Hay et al. 1991). For each copepodite stage at each temperature and population, 10-20 individuals were rinsed briefly with distilled water and placed in pre-weighed small aluminium caps. After 24 hours at 60ºC, the dry weight was measured on a microbalance (Mettler Toledo, sensitivity 1 µg).

3.1.3.5 Weight-specific growth rates

The body weight of each developmental stage (natural logarithm transformed) was plotted against cumulative time for each temperature and population, separating nauplii and copepodites. Each data set was then fitted to a linear regression where the slope represents the growth rate (g, d^{-1}).

The effect of population (north and south), growth phase (nauplii and copepodites) and temperature on growth rate was analyzed through an ANCOVA, where the natural logarithm of dry weight (ln DW) was the dependent variable, development time (days) the covariate and population, growth phase and temperature the independent factors. The relationship between g and temperature was described by a non-linear regression assuming that individuals grow exponentially (Huntley & Lopez 1992, Mauchline 1998).

3.1.4 Results

3.1.4.1 Stage durations

Stage duration decreased significantly with temperature for both populations (Fig. 3.1, Table 3.2). Nearly isochronal growth with similar stage durations through the late naupliar stages (NIII – NVI) and early copepodite stages (CI – CIII) was noted for both populations (Table 3.2). The first naupliar stage (NI) was always shorter and the NII longer than other naupliar stages. Stage duration tended to increase with age.

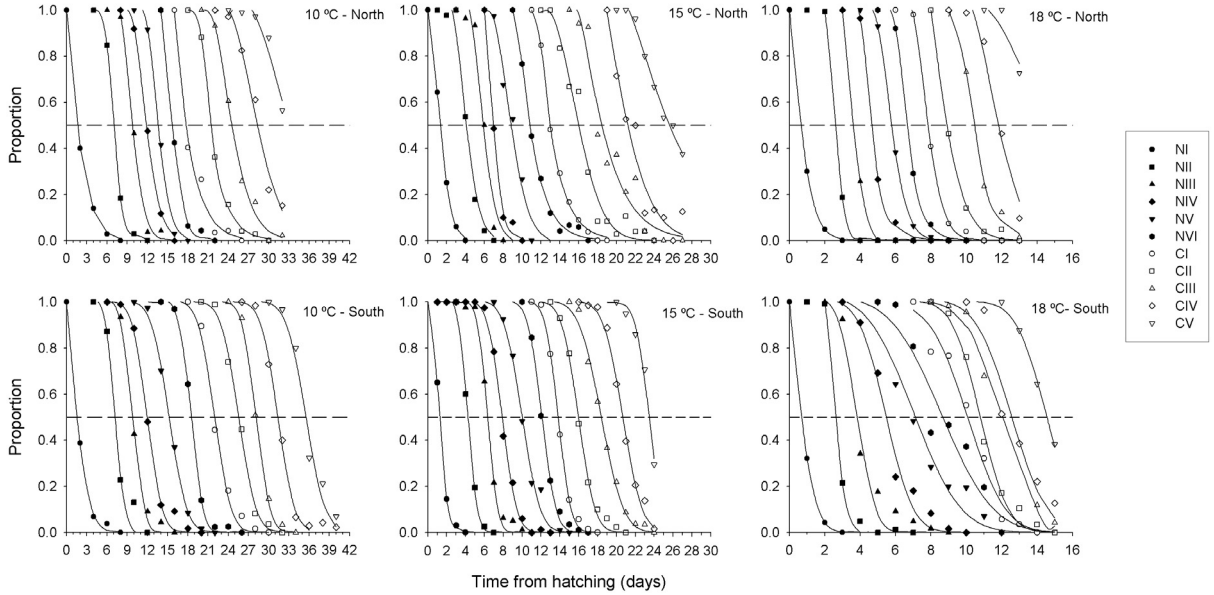

Figure 3.1 *Acartia clausi*. Post-embryonic development time for the two populations (North: top panels, South: bottom panels) reared at 10, 15 and 18ºC. For each development stage (nauplii, filled symbols; copepodites, open symbols), a Gamma distribution was fitted to the cumulative proportions plotted against time. The intersection between the dashed line (= 50%) and the fitted Gamma function defines the mean development time (MDT) for the particular stage.

Table 3.2 *Acartia clausi*. Stage duration (days) of north (N) and south population (S) estimated at 5 temperatures and under saturating food conditions (≥ 1000 µg C l^{-1}). Mean value (± standard error), n = 2; where no SE is given, n = 1. Experiments at 20 and 22ºC were not performed on both populations.

	Temp	NI	NII	NIII	NIV	NV	NVI	CI	CII	CIII	CIV	CV
N	10	2.0 ± 0.39	5.9 ± 0.71	2.6 ± 0.40	2.2 ± 0.12	2.2 ± 0.50	2.4 ± 0.15	2.3 ± 0.39	3.5 ± 0.13	3.3	3.8	4.7
N	15	1.5 ± 0.20	3.2 ± 0.35	2.0 ± 0.31	1.5 ± 0.40	1.9 ±0.03	2.3 ± 0.37	2.2	3	2.4	2.8	4.5
N	18	0.7 ± 0.04	1.9 ± 0.01	1.1 ± 0.17	0.9 ± 0.16	1.2 ± 0.02	0.8 ± 0.05	1.2 ± 0.02	1.1 ± 0.05	1.4 ± 0.06	1.5 ± 0.01	2.8 ± 0.17
N	20	1.4	2.6	1	0.7	0.7	1	1	0.9	1.6	0.7	1.5
S	10	1.4 ± 0.07	5.4 ± 0.05	2.9 ± 0.15	1.9 ± 0.25	3.4 ± 0.16	3.4 ± 0.04	3.3 ± 0.08	3.9 ± 0.25	2.9 ± 0.25	3.4 ± 0.18	4.4 ± 0.19
S	15	1.2 ± 0.00	3.0 ± 0.01	2.1 ± 0.02	1.5 ± 0.00	2.1 ± 0.00	2.1 ± 0.00	1.7 ±0.01	2.2 ± 0.02	2.4 ± 0.01	2.3 ± 0.02	2.9 ± 0.01
S	18	0.7 ± 0.03	2.1 ± 0.24	1.4 ± 0.23	1.6 ± 0.08	1.4 ± 0.06	1.9 ± 0.17	1.5 ± 0.09	1.0 ± 0.21	1.3 ± 0.19	1.5 ± 1.07	1.4 ± 0.82
S	22	0.4 ± 0.16	2.1 ± 0.28	1.3 ± 0.28	1.0 ± 0.08	1.2 ± 0.03	1.0 ± 0.04	1.4 ± 0.11	1.0 ± 0.02	1.2 ± 0.05	1.1 ± 0.02	1.9 ± 0.03

3.1.4.2 Development times

The estimated development times equalled 33.9, 20.8 and 16.3 days for the north population and 36.4, 22.3 and 17.4 days for the south population at 10, 15 and 18ºC, respectively. For each population, the relationship between temperature and generation time was fitted to the Belehradek's function (Fig. 3.2).

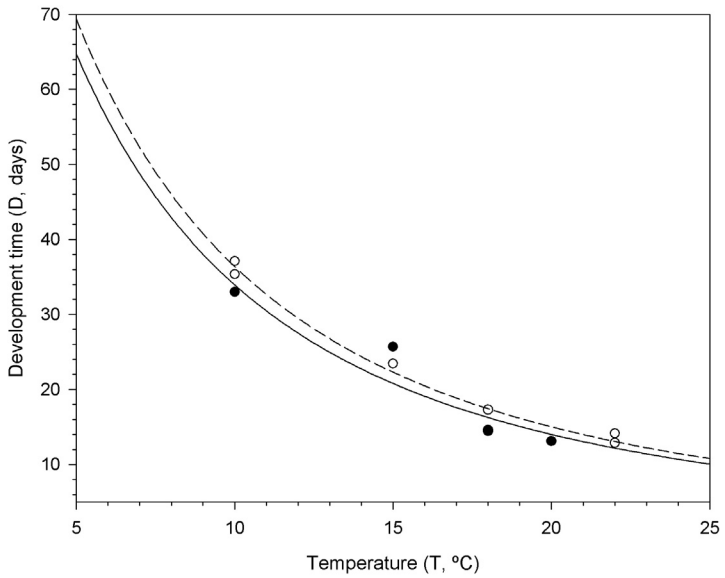

Figure 3.2 *Acartia clausi*. Relationship between temperature (T, ºC) and development time (D, days) for the North (filled symbols) and South (open symbols) population. The estimated parameters of the Belehradek´s function were: $D = 13490.3\ (T + 8.53)^{-2.05}$ ($r^2 = 0.897$, $P < 0.01$; solid line) for the north population and $D = 14410.4\ (T + 8.50)^{-2.05}$ ($r^2 = 0.978$, $P < 0.001$; dashed line) for the south.

3.1.4.3 Prosome length

The prosome length of all copepodites was longer in the south population than in the north and the prosome lengths decreased with temperature (Fig. 3.3). Both effects were significant (2-way ANOVA, $P < 0.001$). The effect of sex (CIV – CVI) was also highly significant (3-way ANOVA, $P < 0.001$), with females always being longer than males. The length-ratio of successive stages was nearly constant in both populations, 1.22 (south)

and 1.20 (north). A length-weight regression for all copepodite stages and adults (both populations pooled) was estimated:

$$\text{Log DW} = 2.064 \text{ Log PL} - 5.080 \qquad (r^2 = 0.846, P < 0.001)$$

where DW is dry weight (µg) and PL prosome length (µm)

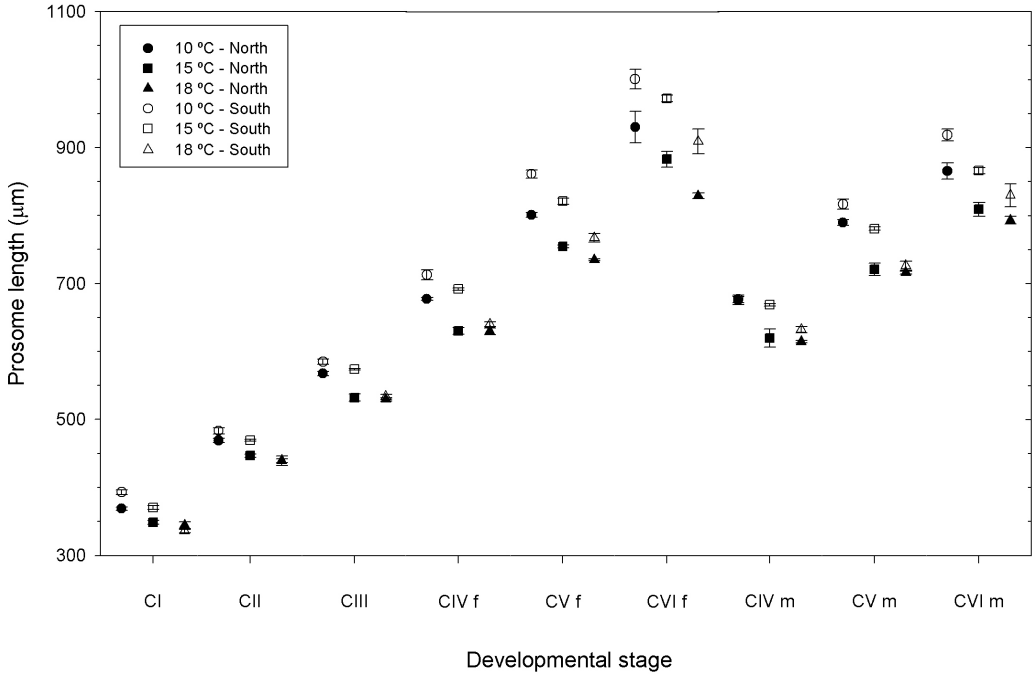

Figure 3.3 *Acartia clausi*. Mean prosome lengths (µm) of copepodites (CI-CVI, males and females separated from stage CIV) from the two populations. Error bars represent standard error.

3.1.4.4 Weight-specific growth rates

The weight-specific growth rate (g, d^{-1}) was estimated separately for each temperature and population (Fig. 3.4) from the slopes of the linear regression of ln DW on cumulative development time. The parameter estimates of the regressions are given in Table 3.3.

An ANCOVA testing for the homogeneity of slopes among the regression lines (Table 3.4) indicated that for both populations and within a given temperature, nauplii grew significantly faster than copepodites (ANCOVA homogeneity of slopes, stage × covariate). Exceptions to this rule were noted at 18ºC for the south population and at 20ºC for the north. Growth rates of nauplii and copepodites of both populations also increased significantly with temperature (ANCOVA homogeneity of slopes, temperature × covariate).

Table 3.3 *Acartia clausi*. Parameter estimates for linear regressions in Fig. 3.4 and respective significance (*P*). The regressions are of the form ln DW = a + b × D, where the slope (b) represents the weight-specific growth rate (d^{-1}), DW is the dry weight (µg) and D the development time (days) (N – nauplii, C – copepodites).

Temperature (ºC)	Population	Stage	a	b	r^2	*P*
10	North	N	-3.197	0.192	0.958	< 0.001
		C	-1.616	0.116	0.949	< 0.001
	South	N	-3.093	0.177	0.979	< 0.001
		C	-1.744	0.111	0.994	< 0.01
15	North	N	-3.105	0.272	0.984	< 0.001
		C	-1.106	0.124	0.971	< 0.01
	South	N	-3.061	0.265	0.988	< 0.001
		C	-1.658	0.164	0.986	< 0.001
18	North	N	-3.133	0.468	0.981	< 0.001
		C	-1.143	0.220	0.935	< 0.01
	South	N	-2.902	0.330	0.989	< 0.001
		C	-2.741	0.301	0.970	< 0.01
20	North	N	-3.304	0.421	0.891	< 0.01
		C	-2.107	0.308	0.977	< 0.01
22	South	N	-3.009	0.423	0.973	< 0.001
		C	-2.024	0.301	0.965	< 0.01

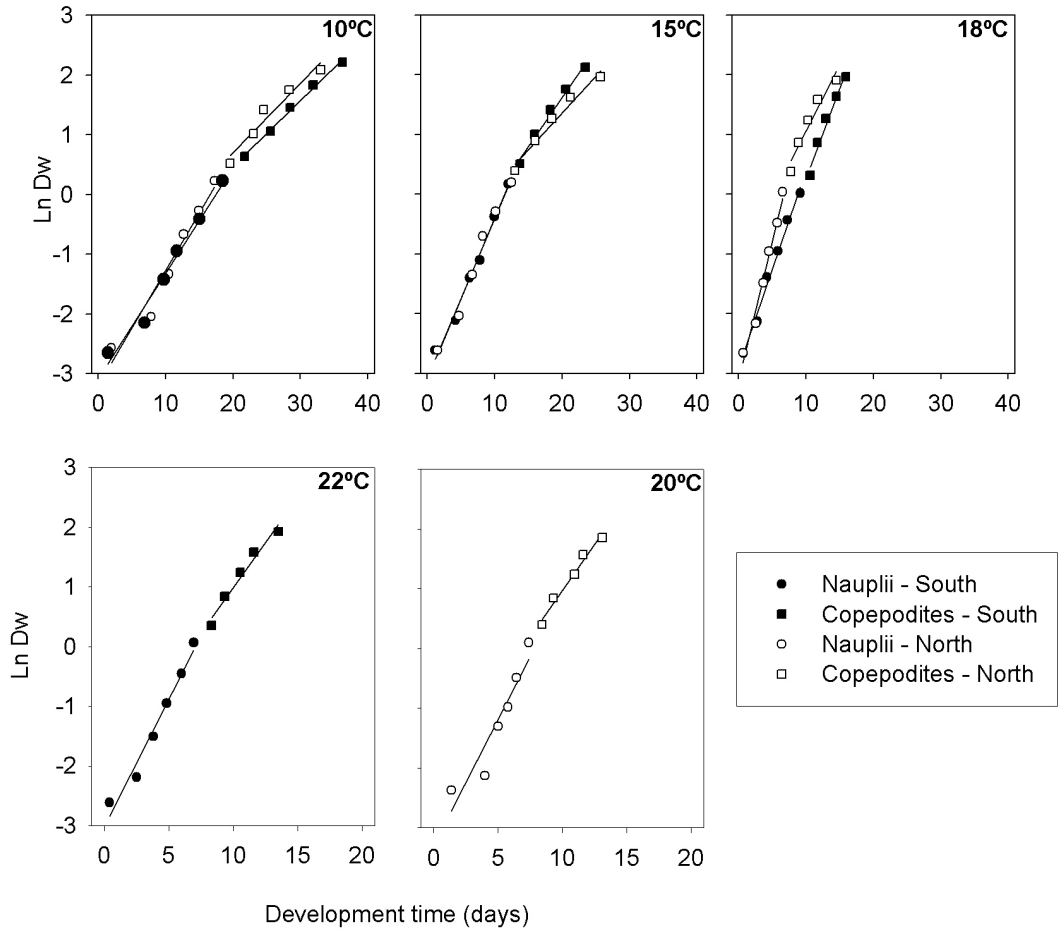

Figure 3.4 *Acartia clausi*. Linear regressions of ln-transformed body weight (µg) on cumulative development time (days) for each temperature and population (North - filled symbols, South-open symbols). Nauplii and copepodites are separated and parameter estimates for each regression are given in Table 3.3.

The effect of population was evaluated separately for nauplii and copepodites (within temperature and stage, effect of population). Nauplii from the north population grew faster than the ones from the south, but only at the highest temperature (18°C). No significant differences between the growth rates of copepodites from the north and south population were found.

Table 3.4 *Acartia clausi*. Results from ANCOVA comparing *g* (weight-specific growth rate), the slopes in Figure 3.4 and Table 3.3. The tests are for homogeneity of slopes within growth phase (effect of temperature), within a given temperature (effect of growth phase) and within temperature and stage (effect of population). Growth phase is for nauplii or copepodites.

	df	*F*	*P*	Pairwise comparisons
Within Growth phase, effect of temperature				
North Population				
Nauplii	3, 16	13.2	< 0.001	20ºC = 18ºC > 15ºC > 10ºC
Copepodites	3, 12	10.8	< 0.001	20ºC > 18ºC > 15ºC = 10ºC
South Population				
Nauplii	3, 16	29.3	< 0.001	22ºC > 18ºC > 15ºC > 10ºC
Copepodites	3, 12	28.1	< 0.001	22ºC = 18ºC > 15ºC > 10ºC
Within Temperature, effect of growth phase				
North Population				
10ºC	1, 7	8.1	0.025	Nauplii > Copepodites
15ºC	1, 7	47	< 0.001	Nauplii > Copepodites
18ºC	1, 7	27.9	< 0.001	Nauplii > Copepodites
20ºC	1, 7	1.5	0.264	Nauplii = Copepodites
South Population				
10ºC	1, 7	17.6	0.004	Nauplii > Copepodites
15ºC	1, 7	27.5	< 0.001	Nauplii > Copepodites
18ºC	1, 7	0.7	0.419	Nauplii = Copepodites
22ºC	1, 7	5.6	0.049	Nauplii > Copepodites
Within Temperature and stage, effect of population				
Nauplii				
10ºC	1, 8	0.4	0.545	North = South
15ºC	1, 8	0.1	0.786	North = South
18ºC	1, 8	15.1	0.005	North > South
Copepodites				
10ºC	1, 6	0.1	0.79	North = South
15ºC	1, 6	5.8	0.052	North = South
18ºC	1, 6	3	0.133	North = South

The weight-specific growth rate (*g*) for both nauplii and copepodites of *Acartia clausi* populations could be compared using the Huntley & Lopez (1992) equation:

$$g = a\, e^{(bT)}$$

where T is temperature (°C) and a and b are fitted constants.

In Figure 3.5 is represented the respective temperature-dependent growth model adjusted for nauplii and copepodites from each population. The north population increased growth by 8-13% for each degree increase in temperature, whereas the south population only increased growth by 7-8% per degree.

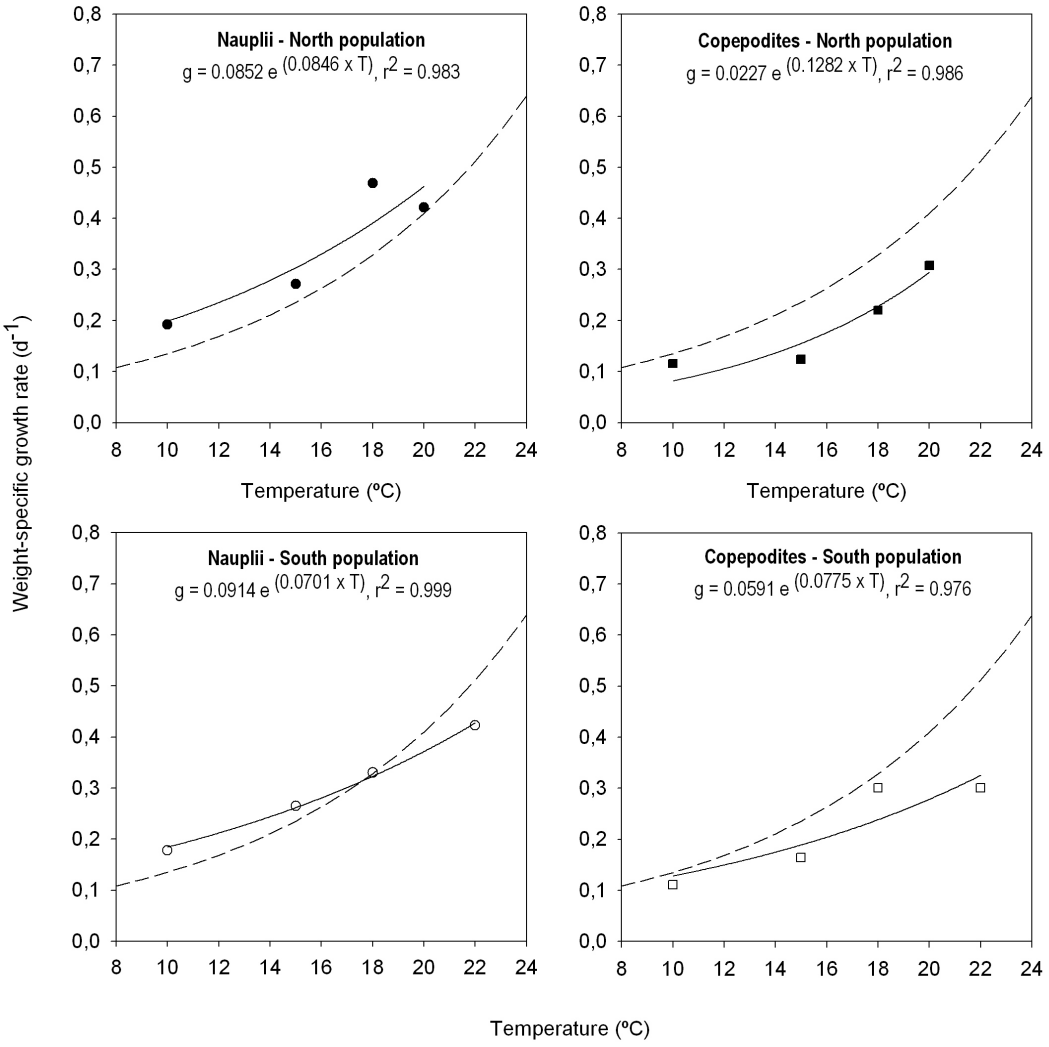

Figure 3.5 *Acartia clausi*. Non-linear regression of the weight-specific growth rate (*g*, d^{-1}) on temperature (T, ºC) for nauplii and copepodites of both populations. The relationship proposed by Huntley and Lopez (1992) is indicated by the dashed line.

3.1.5 Discussion

The aim of this study was to evaluate the importance of temperature on the development and growth rate of two allopatric populations. In the experimental design we therefore kept several other critical parameters constant such as salinity, food and light:dark cycle.

Although the two populations can (and probably do) encounter different food conditions in the field, this has been found to affect fecundity of adult females more than somatic growth of other stages (Hirst & Bunker 2003). We can not exclude that other factors may be more important or that synergistic effects do not exist, but we will only discuss the factor manipulated in the study, temperature.

3.1.5.1 Stage durations

Isochronal development (Miller et al. 1977, Landry 1983) states that for a given species, the time spent in each developmental stage is the same for all stages (Peterson 2001). We observed a nearly isochronal development of stages NIII to CIII. A common feature at all temperatures was the relatively short duration of the first naupliar stage. This stage does not feed, but uses the lipid reserves to fuel the growth.

In contrast, two stages showed a longer development, the NII (first feeding stage) and CV, the stage just prior to adult. During NII the animal develops a digestive tract and a foraging behavior and similar prolonged duration has been found in both *Acartia clausi* (Landry 1975) and *Acartia tonsa* (Berggreen et al. 1988). The increased stage durations of CV corresponds to the transition to adult males and females during which the animal undergoes external morphological differentiation and sexual maturation (Landry 1983, Peterson 2001).

We did not estimate the development time for males and females separately, but it is known that males develop faster than females (Landry 1983). Thus, a significant proportion of stage duration of CIV and CV owe to the development of the females.

Deviations from the isochronal pattern of development are not limited to *Acartia* spp., but have been noted in other species such as *Paracalanus parvus* (Landry 1983), *Pseudocalanus elongatus* (Klein Breteler et al. 1995) and *Calanus finmarchicus* (Campbell 2001).

3.1.5.2 Development times vs. temperature

The two allopatric populations of *Acartia clausi* were exposed to different temperature regimes in the field, with the north population living at lower temperatures, but also experiencing a wider range of temperatures, -1-20°C vs. 11-20°C in the south. The north population has a shorter generation time even at higher temperatures than they normally experience in the field.

A strong response to changes in temperature can be selected for if the temperature range experienced by the population is wide. The selection of fast growing animals in the colder environment could have resulted in different genotypes with specific temperature responses. During juvenile development and growth, the cold acclimated organisms had a stronger response to temperature than warm acclimated ones. A similar response to high temperatures has been reported for egg development in *A. clausi* (Landry 1975 a,b).

With decreasing temperature, the south population showed a gradual delay in development compared to the north and the largest difference (2.5 days) occurred at 10°C. This delay could be due to depressed metabolic rates at the lower limit of its thermal range, indicating that the optimum temperature of each population is different. The lower limit of the south population would occur at higher temperatures than for the north population.

3.1.5.3 Body size

Phenotypic plasticity (Atkinson 1994) was exhibited by individuals of both populations when reared at different temperatures. The ontogenetic response to temperature was an increased body length with the decreasing temperature. Increase in size and slow development at low temperatures is common in several marine copepods species.

A corresponding shortening of the life cycle at high temperatures would allow a population to take advantage of favourable environmental conditions, by growing faster and achieve maturity earlier (Atkinson 1994). In addition, a physiological explanation for this pattern is that in the majority of ectotherm species, an increased temperature would increase rates of growth and differentiation and thereby reduce the size at a given stage due to the imbalance between anabolic and catabolic reactions (Ray 1960).

This negative correlation of prosome length with temperature has been found in several copepod species both in the field (e.g. Hirst et al. 1999) and in laboratory studies (e.g. Campbell et al. 2001). In addition to phenotypic plasticity, our study also indicated a latitudinal variation in the prosome length of *Acartia clausi*. The copepodites and adult females from the south population were always significantly larger than the north at a given temperature. This conclusion contradicts the general rule about the influence of temperature on the prosome length of copepods. However, the fact that at a given temperature, the south population had slower development rates than the north population could justify such latitudinal variation.

3.1.5.4 Growth rates

At high temperatures, the north population grew faster than the south and this was due to a faster naupliar development. Growth rates estimated from the general equation of Huntley & Lopez (1992) were always higher than those observed for copepodites (Fig. 3.5). At 22ºC, the Huntley & Lopez model overestimates growth of copepodites of the south population by 36%. The Huntley & Lopez model is based on estimates for many

species with different tolerances inhabiting regions with variable temperature ranges. As we show here, two populations of the same species may respond differently when subjected to the same temperature depending on genetic differences or different temperature history of the individuals. *Acartia clausi* from the south population may be at the thermal limit for growth at 22°C, whereas the Huntley & Lopez model should fit species at their thermal optimum, since it is based on field measurements. Populations exposed to extremes of their natural environmental conditions (like our experimental treatments) will not fit the model well. Conversely, population responses to extreme conditions in the field will not be well predicted by the model. We suggest the use of specific growth models instead of general models if estimates of potential growth rates are sought.

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Chapter 4

Growth and development of *Acartia tonsa*

Section 4.1

Growth and development of nauplii and copepodites of the estuarine copepod *Acartia tonsa* from southern Europe (Ria de Aveiro, Portugal) under saturating food conditions

4.1.1 Abstract

A temperature-dependent growth model is presented for nauplii and copepodites of the estuarine calanoid copepod *Acartia tonsa* from southern Europe (Portugal). Development was followed from egg to adult in the laboratory at 4 temperatures (10, 15, 18 and 22ºC) and under saturating food conditions (> 1000 μg C l^{-1}). Development times versus incubation temperature were fitted to a Belehradek´s function, showing that development times decreased with increasing incubation temperature: at 10ºC, *A. tonsa* need 40.3 days to reach adult stage, decreasing to 8.9 days when reared at 22ºC. ANCOVA (homogeneity of slopes) showed that temperature ($P < 0.001$) and growth phase ($P < 0.01$) had a significant effect on the growth rate. Over the range of temperatures tested in this study, highest weight-specific growth-rates were found during naupliar development (NI to NVI) and varied from 0.185 d^{-1} (10ºC) to 0.880 d^{-1} (22ºC) with a Q_{10} equal to 3.66. During copepodite growth (CI to CV), the weight-specific growth-rates ranged from 0.125 d^{-1} (10ºC) to 0.488 d^{-1} (22ºC) with a Q_{10} equal to 3.12. The weight-specific growth rates (g) followed temperature (T) by a linear relationship and described as Ln g = -2.962 + 0.130 T ($r^2 = 0.99$, $P < 0.001$) for naupliar stages and Ln g = -3.134 + 0.114 T ($r^2 = 0.97$, $P < 0.001$) for copepodite stages. By comparing *in situ* growth rates (juvenile growth and fecundity) for *A.tonsa* taken from the literature with the temperature-dependent growth model defined here we suggest that the adult females of *A. tonsa* are more frequently food limited than juveniles.

Key words: Postembryonic development times; Somatic growth; Weight-specific growth rates; *Acartia tonsa*; Temperature-dependent growth model; Ria de Aveiro (Portugal)

4.1.2 Introduction

Acartia tonsa Dana is a eurythermic and euryhaline calanoid copepod species (Lance 1963; Lance 1964; Jeffries 1967) which occurs in a wide range of geographic areas, from temperate to subtropical waters. Within these regions, the distribution is restricted to habitats characterized by relatively high food levels like estuaries and upwelling near shore environments (Paffenhöfer & Stearns 1988).

Acartia tonsa is often the dominant component of the zooplankton assemblage in terms of abundance and biomass (Durbin & Durbin 1981, Escaravage & Soetaert 1995, McManus & Foster 1998, Cervetto et al. 1999, Marques et al. 2006), and may be considered a key species in the flux of matter and energy from low to higher trophic levels. It is an important grazer and omnivorous species, as well a source of food for several marine and estuarine invertebrates and fish larvae.

In Ria de Aveiro (Portugal), *A. tonsa* has an annual average density of 30% of the total mesozooplankton abundance (Morgado 1997). In this south European estuarine ecosystem, the species occurs throughout the year over a temperature range of 15 to 22ºC and at salinities between 12 and 28 PSU (see Chapter 5).

Comprehensive information on the effect of temperature on growth rates and development times of juvenile stages (nauplius and copepodite stages) of *Acartia tonsa* is not available. The influence of temperature at non-limiting food conditions on the growth of this species is mostly limited to studies of egg production (adult growth, e.g. Parrish & Wilson 1978, Kiørboe et al. 1985, Kleppel et al. 1985), which have recently proved not to be equivalent to the somatic growth of the juveniles (e.g. Koski et al. 1998, Hirst & Bunker 2003). In addition, differences between growth rates and development times of nauplii and copepodites have not been investigated.

The goal of the present study was to obtain basic information on growth rates of *Acartia tonsa* from the Ria de Aveiro, Portugal, necessary for the calculation of potential copepod

production rates in the estuary. The specific objectives of the study were i) to determine the effect of temperature on the post-embryonic development and ii) to define a temperature-dependent growth model for *A. tonsa* from the Ria de Aveiro. To accomplish these objectives, we followed the development and growth of the species from egg to the adult stage, under food-saturated conditions, at four different temperatures within the ambient range in the Ria de Aveiro.

4.1.3 Materials and Methods

4.1.3.1 Zooplankton collection and laboratorial stock cultures

Zooplankton was collected in the Canal de Mira, Ria de Aveiro, Portugal (40º 36' N, 8º 44'W) on August 2004 by horizontal tows made with a 200 μm mesh size plankton net fitted with a large plastic bag as a cod end. After collection, the samples were diluted in water from the sampling site and brought into the laboratory within less than 1 hour. Around 2000 adult females of *Acartia tonsa* were sorted out under a binocular microscope and transferred to 15 l polyethylene buckets containing natural filtered seawater (0.45 μm, 20 PSU).

The copepods were kept at 18ºC in a constant temperature room under a light:dark cycle of 12h:12h with gentle permanent aeration. They were fed *ad libitum* a mixture of the marine diatom *Thalassiosira weissflogii* and the cryptophyte *Rhodomonas* sp., which were obtained from cultures that were growing exponentially. Phytoplankton cultures were grown in f/2 medium at 20ºC under a 12:12 light:dark cycle.

In order to establish laboratorial stock cultures of *A. tonsa*, eggs produced by the field collected females were collected from the bottom of the buckets by siphoning, cleaned from adults and detritus and then incubated at the same conditions. These cultures were left for an acclimation period of at least 2 generations before the start of the growth experiments.

4.1.3.2 Growth experiments conditions

Development of the copepods was followed from egg hatching to maturity, at 10, 15, 18 and 22ºC, using 2 replicates per temperature. For each replicate, approximately 10 000 eggs were obtained from the acclimated stock culture and incubated in polyethylene buckets containing 10 l of filtered seawater (0.45 μm, 20 PSU) at the same conditions described above, except for food that was adjusted according to development phase.

Food levels were always > 1000 μg C l^{-1}, which is at least twice the threshold concentration defined by Berggreen et al. (1988) for *Acartia tonsa* maximum growth. The size of the algae was 2 to 5% of the prosome length, which is considered to be within the optimum particle size for copepods (Berggreen et al. 1988). To maintain an optimal particle size of the food, the diet offered to nauplii consisted of only *Rhodomonas* sp. (mean equivalent spherical diameter, ESD=6.7 μm). Copepodites were fed a mixture of *Rhodomonas* sp and *Thalassiosira weissflogii* (ESD=14.5 μm) in equal amounts of carbon. Both algae are considered nutritionally good for copepod growth and development owing to the presence of poly-unsaturated fatty acids and sterols (Koski et al 1998, Klein Breteler et al. 1999, Koski & Klein Breteler 2003). Food concentration was checked daily by means of microscopic counting of algae density using a Neubauer chamber. The algal volume (μm^3) was converted to carbon concentration (μg C l^{-1}) through the relationship defined by Strathmann (1967). When necessary, food concentration was adjusted by adding fresh algae from cultures in exponential growth phase.

4.1.3.3 Identification, length and weight of nauplii and copepodites stages

Identification of developmental stages was based on the ICES Plankton Identification Leaflets (Ogilvie 1956) and on the descriptions of *Acartia tonsa* copepodite stages from Sabatini (1990). Total body length of naupliar stages (BL, μm) and prosome length of copepodite stages (PL, μm) were measured with an inverted microscope (100x magnification) with a calibrated eyepiece micrometer. Dry weight (DW, μg) for nauplii

and copepodites was estimated by the regression defined by Berggreen et al. (1988), $Log_{10}\ DW = 3.31\ Log_{10}\ BL\ —8.498$ (NI – NVI) and $Log_{10}\ DW = 2.92\ Log_{10}\ PL – 7.958$ (CI – CVI).

4.1.3.4 Stage durations and development time

After every 24 hours (except for the 10°C experiment that was surveyed every 48h) each culture bucket was sampled by pouring 100-300 ml of water through a 75 μm sieve in order to yield at least 30 individuals for staging and measuring. The original volume of the culture was kept constant by adding filtered sea water. In all experiments, time 0 was defined as the time when the eggs were harvested (eggs 0-24h old) and incubated.

The duration of each development stage (stage duration, SD) in each replicate was calculated as the difference of the median development time (MDT) of two successive stages. The MDT of each stage was defined as the time when 50% of the organisms in a culture had passed that stage (Landry 1975, 1983) and was estimated by fitting a gamma distribution function to the cumulative proportion of that stage over time (Klein Breteler et al. 1994). Development time at each temperature was calculated as total time from hatching to the moment when 50% of the culture reached the adult stage.

Development rates for nauplius (NI to NVI) and copepodites (CI to CV) were obtained by averaging the reciprocal of the respective stage durations. The functional relationship between temperature and development time was then estimated by the Belehradek´s function: $D = a\ (T + c)^{-2.05}$, where D is median development time (days), T is temperature (ºC) and a and c are coefficients (McLaren 1963, 1965). The effects of temperature and development stage on stage duration and size were analysed with a two-way factorial analysis of variance (ANOVA).

4.1.3.5 Weight-specific growth rates

The effect of temperature, stage and development time on body weight was analyzed through an analysis of covariance (ANCOVA), were the natural logarithm of dry weight (Ln DW) was the dependent variable and cumulative development time was taken as a covariate.

The slopes of the separate regression lines relating the logarithm of body weight to development time were taken as weight-specific growth rates (*g*). The relationship between the natural logarithm of *g* and temperature, for each development phase, was also analysed with ANCOVA.

All data were tested for normality of distribution and homogeneity of variances previous to the statistical analyses, and deviations from the assumptions of ANOVA and ANCOVA were not detected. All analyses were done using Statistica 6.0 statistical package.

4.1.4 Results

4.1.4.1 Stage durations and development times

Average stage durations were significantly shorter at higher temperatures, (ANOVA: $F_{3, 44}$ = 344.1, P < 0.001, Table 4.1) and the duration of single developmental stages were different within each temperature (ANOVA: $F_{10, 44}$ = 344.1, P < 0.001, Table 8). The duration of the first naupliar stage (NI) was usually shortest (except at 10ºC) while longer stage durations were consistently noted for NII and late copepodite stages (CIV and CV).

There was a significant interaction between temperature and developmental stage ($F_{30, 44}$ = 3.614, P < 0.001), showing that the temperature effect was different on different developmental stages. NII was always significant longer than the other stages, except at

the highest temperature. However, at 22ºC the individuals develop fast and a daily observation is not enough to obtain a precise estimate of stage duration.

Table 4.1 Stage duration (days) of *Acartia tonsa* from southern Europe estimated at four temperatures and under saturating food conditions (> 1000 µg C l^{-1}). Mean value (± standard deviation) and number of observations (n).

	10ºC		**15ºC**		**18ºC**		**22ºC**	
Stage	Mean (± SD)	n	Mean (± SD)	n	Mean (± SD)	n	Mean (± SD)	n
N I	4.05 (±0.038)	2	1.53 (±0.021)	2	1.05 (±0.016)	2	0.93 (±0.009)	2
N II	6.02 (±1.133)	2	2.13 (±0.192)	2	1.44 (±0.053)	2	0.73 (±0.168)	2
N III	2.80 (±1.393)	2	1.59 (±0.235)	2	1.06 (±0.001)	2	0.86 (±0.154)	2
N IV	3.26 (±0.239)	2	1.42 (±0.171)	2	0.94 (±0.010)	2	0.70 (±0.025)	2
N V	2.93 (±0.068)	2	1.54 (±0.423)	2	0.97 (±0.115)	2	0.31 (±0.014)	2
N VI	3.15 (±0.175)	2	1.60 (±0.103)	2	1.08 (±0.207)	2	0.65 (±0.112)	2
C I	3.13 (±0.106)	2	1.44 (±0.290)	2	1.30 (±0.034)	2	0.82 (±0.047)	2
C II	3.19 (±0.147)	2	1.60 (±0.235)	2	1.11 (±0.065)	2	0.73 (±0.021)	2
C III	3.13 (±0.016)	2	1.60 (±0.258)	2	1.11 (±0.003)	2	0.76 (±0.067)	2
C IV	3.72 (±0.286)	2	1.74 (±0.294)	2	1.25 (±0.171)	2	0.83 (±0.227)	2
C V	5.23 (±0.407)	2	2.52 (±0.251)	2	2.07 (±0.086)	2	1.50 (±0.345)	2

Development rates (day^{-1}) increased with temperature, for nauplius (mean ± SD) they were 0.29±0.071, 0.62±0.080, 0.93±0.130 and 1.64±0.800, and for copepodites 0.28±0.055, 0.58±0.113, 0.77±0.171 and 1.16±0.284 at 10, 15, 18 and 22°C, respectively.

The functional relationship between temperature and development time for *Acartia tonsa* was fitted to the Belehradek´s function (Fig. 4.1) and could be described by the expression: $D = 5491.85\ (T + 0.96)^{-2.05}$ ($r^2 = 0.99$, $P < 0.001$). Predicted development times decreased with increasing temperature, 40.6, 18.8, 13.2 and 8.9 days at 10, 15, 18 and 22ºC, respectively.

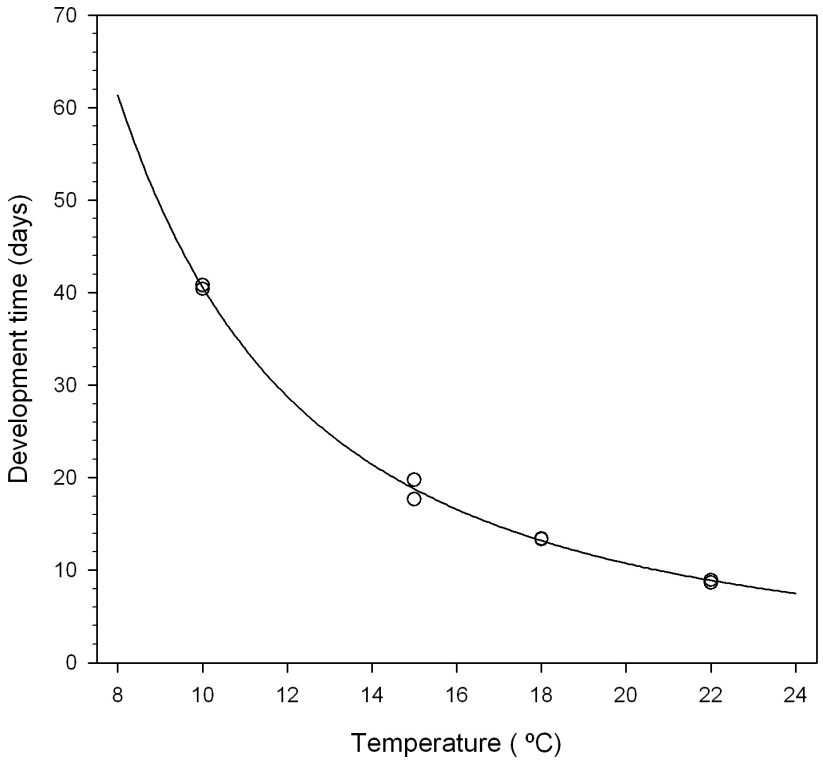

Figure 4.1 Relationship between temperature and development time for *Acartia tonsa* (Ria de Aveiro, Portugal). The fitted curve is described by the Belehradek´s function (see results).

4.1.4.2 Body length of nauplii and prosome length of copepodites

Temperature had a significant effect on body length of nauplii (ANOVA: $F_{3, 2785} = 337.2$, $P < 0.001$, Fig. 4.2), mainly after stage NIV, and on the prosome length of early (CI to CIII) and late (CIV to CVI) copepodites (ANOVA: $F_{3, 2180} = 1937.9$, $P < 0.001$ – CI to CIII and $F_{3, 3990} = 1869.2$, $P < 0.001$ – CIV to CVI, Fig. 4.3). From stage CIV onwards sexes were separated and females were significantly larger than males (ANOVA: $F_{1, 3983} = 8011.6$, $P < 0.001$, Fig. 4.3).

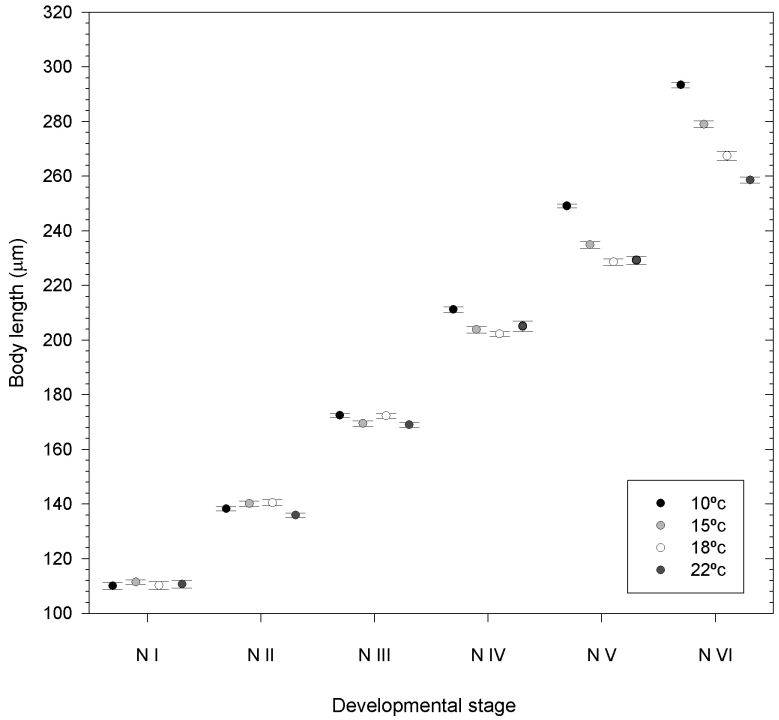

Figure 4.2 Mean total body length (µm) of *Acartia tonsa* naupliar stages reared at 10, 15, 18 and 22 ºC. Error bars indicates 95% confidence limits.

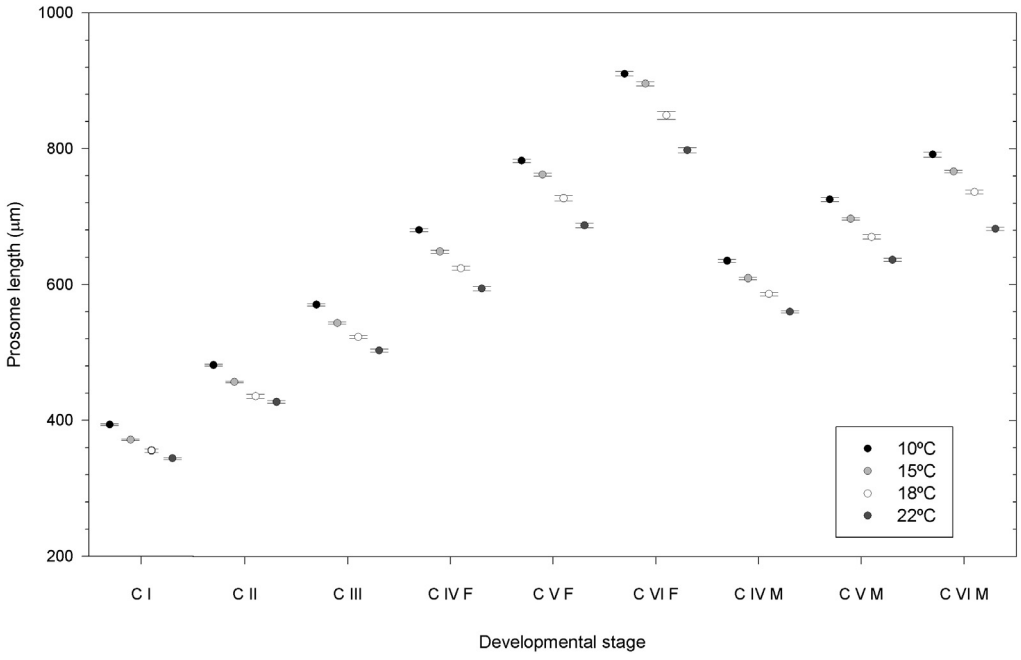

Figure 4.3 Mean prosome lengths (µm) of *Acartia tonsa* copepodites and adult males (M) and females (F) reared at 10, 15, 18 and 22 ºC. (CI to adults, males and females separated from stage CIV onwards) Error bars indicate 95% confidence limits.

4.1.4.3 Weight-specific growth rates

The parameter estimates of the relationships between the natural logarithm of dry weight and cumulative development time (Fig. 4.4) are given in Table 4.2, where the slope of each line represents the weight-specific growth rate. The ANCOVA testing for the homogeneity of slopes among the regression lines (Table 4.3) indicated that the growth rates increased with temperature, and were always higher for nauplii than for copepodites (from $P < 0.006$ to $P < 0.001$). At 10, 15, 18 and 22ºC, the weight-specific growth rate was estimated to 0.185, 0.371, 0.539, 0.880 d^{-1} for nauplii and 0.125, 0.265, 0.358, 0.488 d^{-1} for copepodites, respectively.

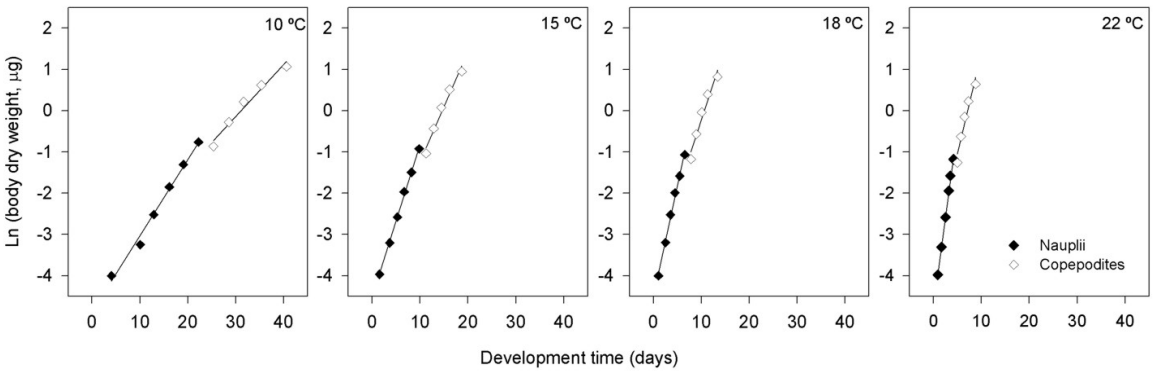

Figure 4.4 Linear regressions of Ln-transformed body weight (µg) on cumulative development time (d). Nauplii (filled symbols) and copepodites (open symbols) are separated and females and males are pooled. Parameter estimates for each regression line are given in Table 4.2.

Table 4.2 Estimates of regression parameters (coefficient ± SE) of the natural logarithm of dry weight versus average development time for nauplii and copepodites of *Acartia tonsa* incubated at 10, 15, 18 and 22ºC under food satiated conditions. The slope of each regression line represents weight-specific growth rate (g).

Temperature (ºC)	Stage	Intercept	Slope	r^2	*P*
10	N	-4.89 ± 0.15	0.19 ± 0.01	0.99	< 0.001
	C	-3.90 ± 0.41	0.13 ± 0.01	0.97	0.0024
15	N	-4.53 ± 0.05	0.37 ± 0.01	1.00	< 0.001
	C	-3.88 ± 0.37	0.27 ± 0.02	0.97	0.0019
18	N	-4.51 ± 0.08	0.54 ± 0.02	1.00	< 0.001
	C	-3.81 ± 0.47	0.36 ± 0.04	0.96	0.0038
22	N	-4.78 ± 0.07	0.88 ± 0.02	1.00	< 0.001
	C	-3.48 ± 0.48	0.49 ± 0.07	0.94	0.0053

Table 4.3 Results from ANCOVA (homogeneity of slopes) comparing *g* (weight-specific growth rate) within growth phase (effect of temperature) and within a given temperature (effect of growth phase).

Within Growth phase, effect of temperature

	Homogeneity of slopes (Temperature x Covariate)				
	df	***F***	***P***	**Conclusion**	**Pair wise comparisons**
Nauplii	3, 16	253.9	<0.001	slopes not parallel, significant differences between temperatures	22ºC > 18ºC > 15ºC > 10ºC
Copepodites	3, 12	22.8	<0.001	slopes not parallel, significant differences between temperatures	22ºC > 18ºC > 15ºC > 10ºC

Within Temperature, effect of growth phase

	Homogeneity of slopes (Stage x Covariate)				
	df	***F***	***P***	**Conclusion**	**Pair wise comparisons**
10 ºC	1, 7	15.2	0.006	slopes not parallel, significant differences between nauplii and copepodites	Nauplii > Copepodites
15 ºC	1, 7	20.9	0.003	slopes not parallel, significant differences between nauplii and copepodites	Nauplii > Copepodites
18 ºC	1, 7	16.4	0.005	slopes not parallel, significant differences between nauplii and copepodites	Nauplii > Copepodites
22 ºC	1, 7	29.7	0.001	slopes not parallel, significant differences between nauplii and copepodites	Nauplii > Copepodites

4.1.4.4 Temperature-dependent growth models

The temperature-dependent growth models for nauplii and copepodites of *Acartia tonsa* (Fig. 4.5) were described by the linear relationship between the natural logarithm of weight-specific growth rates (Ln *g*) and temperature (T). The fitted equations were *Ln g = -2.962 + 0.130 T* (r^2 = 0.99, *P* < 0.001) for naupliar stages and *Ln g = -3.134 + 0.114 T* (r^2 = 0.97, *P* < 0.001) for copepodite stages.

The ANCOVA showed that the slopes were parallel ($F_{1, 4}$ = 1.36, *P* > 0.05), and that the intercepts were significantly different ($F_{1, 5}$ = 47.9, *P* < 0.01), indicating that nauplii of *A. tonsa* grow faster than copepodites at equivalent temperatures. Q_{10} values estimated by the equation $Q_{10} = e^{(10 \times slope)}$ (Hirst & Bunker 2003) and from the slope of the relationship between g and temperature were equal to 3.66 and 3.12 for nauplii and copepodites, respectively.

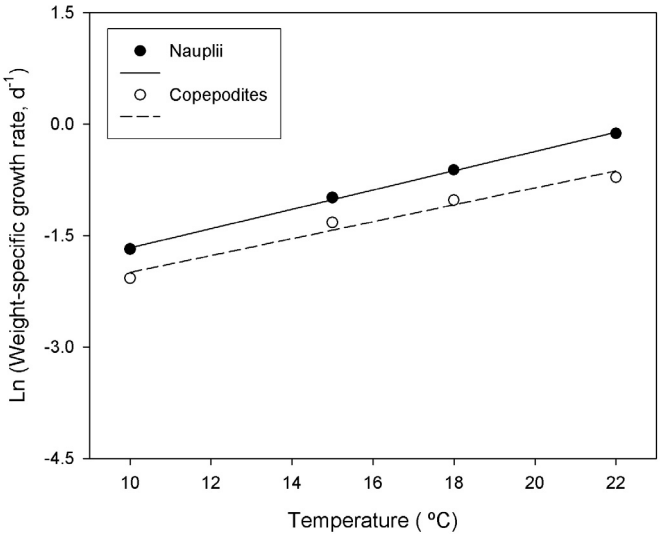

Figure 4.5 Regression between weight-specific growth rate (*g*, d^{-1}) and temperature (ºC) for nauplii (filled symbols and continuous line) and copepodites (open symbols and dashed line) of *Acartia tonsa* from Ria de Aveiro (Portugal).

4.1.5 Discussion

In this study, we successfully reared and followed the development of *Acartia tonsa* from hatching to adult stage at temperatures ranging from 10 to 22ºC at food saturated conditions. The results reported in this paper increase our knowledge about the biology of this species by describing the effects of temperature on the rates of development and growth of juvenile stages and by evaluating the differences between the performance of nauplii and copepodites.

4.1.5.1 Stage duration and development time

Development of *Acartia tonsa* was nearly isochronal with stage durations almost constant from NIII to CIII. Exceptions were the short duration of first naupliar stage (except at 10ºC) and the prolonged stage durations of the second naupliar stage (NII) and late copepodite stages (CIV to CVI). In the short first naupliar stage individuals consume the lipid reserves obtained from the eggs and moult quickly to the first feeding stage (Landry 1975, Peterson 2001).

The prolonged durations of the NII and late copepodites stages are normally attributed to weight losses and inefficient food capture of NII and to maturation processes including external morphological differentiation and the sexual organs (ovary and testis) formation (Landry 1983, Peterson 2001) of the late copepodite stages. Development times decreased exponentially with increasing temperature, with a 4-fold decrease from 10 to 22ºC. An intraspecific comparison with *A. tonsa* development times obtained at different temperatures by other studies (e.g. Landry 1983, Berggreen et al 1988, Paffenhöfer 1991) indicated a good fit to the relationship defined for this southern Europe population (Fig. 4.6A). An exception is the development time estimated by Zillioux-Wilson (1966) at 17ºC which is much higher than in our study. A possible explanation for that difference could be related to the type of eggs that were used by Zillioux-Wilson (1966).

In addition to subitaneous eggs, *A. tonsa* can also produce diapause eggs (e.g. Marcus 1991) in response to unfavourable environmental conditions. Diapause eggs are in a state of arrested development with reduced metabolic activity and will hatch only after they have completed an obligatory refractory phase (Castro-Langoria & Williams, 1999) and under favourable conditions (Grice & Marcus, 1981). Consequently, the hatching of diapause eggs will take more time than the subitaneous eggs and, ultimately, the generation time will be longer.

Based on the interspecific comparison represented in figure 4.6B, it is clear that at high temperatures (> 14ºC) *A. tonsa* tend to develop faster than the majority of marine calanoid copepod species. By developing faster, *A. tonsa* populations will be able to maintain itself within highly advective environments like estuaries, and often dominate the estuarine zooplankton community.

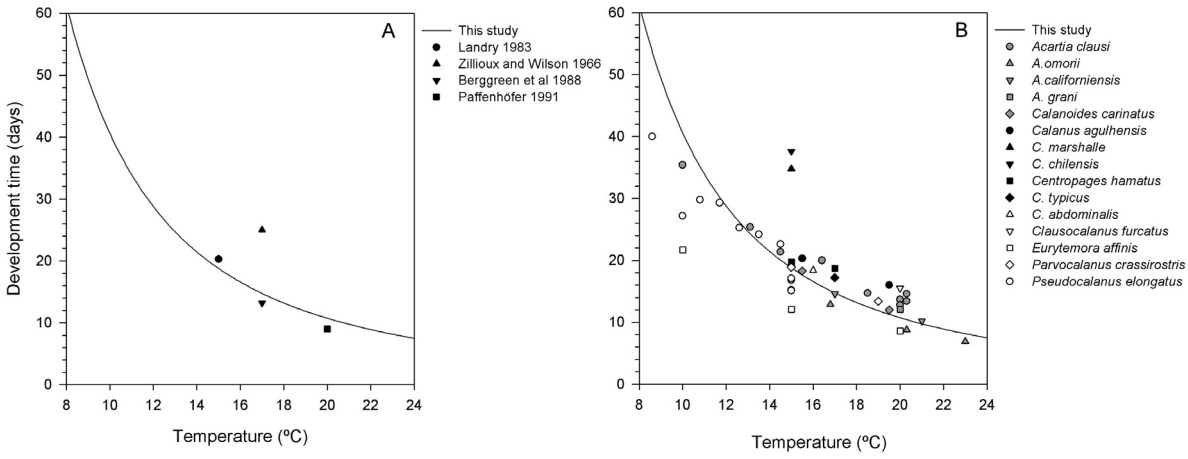

Figure 4.6 Intraspecific (A) and interspecific (B) comparison between development times estimated for *Acartia tonsa* of Ria de Aveiro (Portugal) with previous data obtained from the literature. The data for intraspecific comparison was taken from table 1 of Peterson 2001.

4.1.5.2 Body and prosome length

An increase in body length of nauplii with lower temperatures was only found in late stages (NV and NVI). We did not test differences in body weight between instars and temperatures since those values were taken from the length-weight relationships (Berggreen et al. 1988).

Females were significantly longer than males in all copepodite stages where sex was determined (CIV to CVI), as has been reported for other calanoid species (e.g. *Acartia clausi*, Durbin & Durbin 1978; *Calanus sinicus*, Huang et al. 1993; *Centropages abdominalis*, Slater and Hopcroft 2005). The justification for a shorter length of males compared to females, is that they tend to develop faster in order to increase the probability of mating with receptive females (Hart 1990).

The faster development of males could also be due to physiological constraints, since maturation may be less energetically demanding for males than for females (Peterson 2001). Constrains on the minimum size of females may also be imposed by the selective advantage of producing large numbers of eggs, which increases with body size.

4.1.5.3 Weight-specific growth rates

The weight-specific growth rates increased exponentially with temperature over the range tested in the present study. This exponential relationship has been found in several species of marine calanoid copepods (Mauchline 1998) growing within their normal thermal range.

For *Acartia tonsa* from Ria de Aveiro, copepodite growth was 39 to 45% slower than naupliar growth, with the difference becoming more pronounced at higher temperatures. This conclusion disagrees with the findings of Miller et al (1977), where nauplii and copepodites grew at the same rates. The reason for this difference is because those estimates were based on the assumption of equal stage duration for all developmental

stages (isochronal development), which is not the case in most copepod species including *A. tonsa*.

The divergence between growth phases is probably indicative of a selective adaptation of nauplii to a high mortality risk (Kiørboe & Sabatini 1995). *A. tonsa* would grow faster during the most vulnerable development phase, allowing a greater proportion of individuals to reach the adult stage and reproduce.

The slower grow of copepodites compared to nauplii demonstrated in this study does not fit the common pattern described by Kiørboe & Sabatini (1995). They compiled fecundity, growth and development rates for sac- and broadcast-spawning planktonic copepods, and concluded that in both spawning types nauplii develop faster (shorter development times by a factor of 2) but grow slower (weight increase lowered by 20 to 40%) than copepodites. The discrepancy may be due to different optimal size of prey for nauplii and copepodites.

Nauplii of certain species (e.g. *Calanus pacificus*) may not grow faster than early copepodites due to a low ability in handling and ingesting small food particles (Fernandez 1979) but also the food abundance and composition will determine food intake and, in the end, the growth of a given species. In our study, prey size was optimal for both nauplii and copepodites and concentrations saturating.

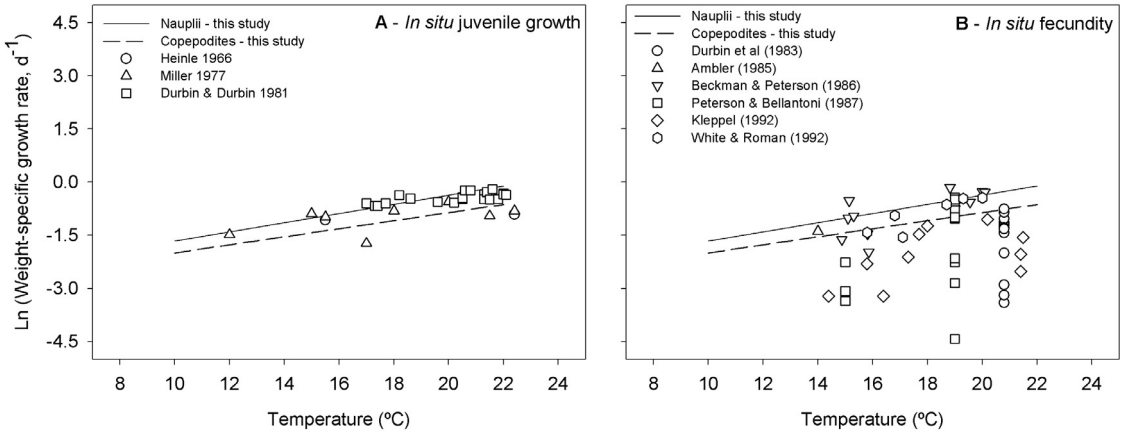

Figure 4.7 *Acartia tonsa* weight-specific growth rates. The *in situ* juvenile and adult growth rates estimated at temperatures within the range 10º - 22ºC were obtained from the literature available. Continuous and dotted line represents the temperature-dependent growth model for nauplii and copepodites, respectively.

The growth of nauplii and copepodites at saturated food conditions and *in situ* conditions of food are similar (Fig. 4.7A). *Acartia tonsa* juvenile developmental stages in the field grow, with some exceptions, at nearly the same rates as the ones defined at non-limiting food conditions (Fig. 4.7A). Although our conclusion is based on few data points for a single species, Hirst & Bunker (2003) also found that juvenile copepods in the field grow at rates close to maximum laboratory determined rates.

Despite the variability of *in situ* food conditions, *A. tonsa* juveniles are able to maximize their growth, which is more limited by the ambient water temperature. The short development time is one of the reasons for the dominance of *A. tonsa* in the estuarine zooplankton. In contrast, fecundity of *A. tonsa* is frequently limited by food availability (Fig. 4.7B) and *in situ* egg production is generally lower than the juvenile growth rates (Peterson et al. 1991).

To estimate production of *Acartia tonsa* in an estuarine ecosystem requires the use of methods to measure juvenile growth *in situ* (e.g. artificial cohorts). But since juvenile growth often seems to be saturated, female egg production will be a key parameter. Biomass of females is often a significant fraction of total biomass, but measures of

production based on egg production extrapolated to the entire population will probably underestimate total production.

4.1.6 References

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Chapter 5

Environmental forcing of *Acartia* populations

Section 5.1

Spatial and temporal scales of environmental forcing of *Acartia* populations (Copepoda: Calanoida) in the Canal de Mira (Ria de Aveiro, Portugal)

5.1.1 Abstract

The specific objectives of this study were to describe seasonal and longitudinal distribution patterns of *Acartia* spp populations on a southern European estuary and to determine the importance of the hydrological factors on its distribution. Zooplankton was sampled from August 2000 to June 2002 at 6 locals distributed along a transect defined in Canal de Mira (Ria de Aveiro, Portugal). At each sampling moment vertical profiles of salinity and temperature were performed and water samples for chlorophyll *a* and suspended particulate matter analysis were also collected. During the study period, two contrasting hydrological years were verified: an abnormal wet 2000-01 and an extremely dry 2001-02. Those extreme situations revealed to have a significant effect on the longitudinal distribution of zooplanktonic distribution as well on the salinity regimes verified at each period. The monthly mean abundance of adult males and females of *Acartia clausi* and *Acartia tonsa* and juvenile stages (nauplii and copepodites) of *Acartia* spp for each local and month were combined on a 3 way data matrix and then decomposed on 3 two-way matrix corresponding to 3 different modes: biological, time and space mode. Cluster analysis applied on each mode revealed the existence of three distinct biological groups and three different zones. At each mode, a classical PCA was also performed and the values of the first principal component represented 2-dimensionally. Strong seasonal variations were found in zone 1 and zone 2. Zone 3, was characterized by the occurrence of the lowest zooplankton abundance levels and by weak seasonal variations. A different longitudinal pattern was found between the periods Nov-00 to Apr-01 and Nov-01 to Apr-02, with the displacement of highest abundance levels from middle estuary to near the mouth as a consequence of advective process. The congeneric populations showed to be segregated in space: *A.clausi* population was

restricted to zone 1 (downstream stations) whereas *A.tonsa* population dominated in region between station 2 and station 5 (middle estuary). The distribution of larvae and juveniles of *Acartia* spp. followed the distribution of the adults. Significant spearman correlations between hydrological parameters and copepod abundances revealed to differ from zone to zone. The statistic methodology here applied proved to be a valuable tool for the discrimination of spatial and seasonal distribution patterns, to define estuarine sections based on the faunistic composition and to evaluate delayed effects of phytoplanktonic biomass the abundance of copepods.

Key words: *Acartia clausi*; *Acartia tonsa*; Canal de Mira – Ria de Aveiro (Portugal); Environmental forcing; spatial and seasonal distribution; three-mode principal component analysis (PCA)

5.1.2 Introduction

Estuaries are transitional areas where continental, marine and atmospheric processes interact and create one of the most dynamic ecosystems found on Earth. At tidal, daily, seasonal and year-to-year time scales, the estuarine ecosystems are characterized by the occurrence of pronounced fluctuations in its physical and chemical properties (e.g. water temperature, salinity, dissolved oxygen and water flow).

A common feature to all estuarine ecosystems is the existence of longitudinal environmental gradients (Elliot & McLusky 2002), one of the most important being related with salinity. In estuaries located in the temperate zone salinity tends to decrease from downstream to upstream sections and the magnitude of seawater dilution is dependent on the level of river flow (Pritchard 1952, 1967, Hansen 1967).

As a consequence of the high instability on environmental conditions in estuaries, the organisms are subjected to strong ecological pressures which will be reflected on population dynamics (mortality, reproduction, production) and distribution.

For zooplanktonic populations like calanoid copepods, the distribution is mainly governed by water temperature, salinity and food supply, being the relative position of different populations along the salinity gradient explained by interspecific differences in tolerance to environmental conditions (Gaudy et al. 2000, Lawrence et al. 2004) and biotic interactions (Soetaert & Rijswijk 1993).

Estuaries are also strong advective environments as a direct consequence of the tidal currents (ebb and flood) and river flow, imposing an additional factor of stress for calanoid copepods (Soetaert & Rijswijk 1993). Copepods of the genus *Acartia* are retained in estuaries due to the combined effect of the swimming behaviour that allow regulation of its vertical distribution (Kouassi et al. 2001), the production of diapause eggs (Castro-Longoria 2001) in response to unfavourable environmental conditions and life cycle traits such as short generation times (Leandro et al. 2006a) and high reproductive potential. These life cycle strategies adopted by *Acartia* spp., allow them to be among the most abundant in the estuarine zooplanktonic communities and also some of the most widespread organisms (Day et al. 1989).

In estuaries located in the North Atlantic, the presence of 2 or 3 *Acartia* species, namely *Acartia tonsa*, *A. clausi* and *A. bifilosa*, is common. However, *Acartia* species are commonly segregated in space (Castro-Longoria 2003, Lawrence et al. 2004, Marques et al. 2006) and time (Durbin & Durbin 1981, Lawrence et al. 2004) as a result of physiological constrains (Gaudy et al. 2000, Calliari et al. 2006) or interspecific relationships such as predation (Tiselius et al. 1997) or competition (Tester & Turner 1991).

The aims of the present study were (1) to estimate the abundance of *Acartia* spp. populations (*A.clausi* and *A.tonsa*) in a Southern European estuary (Canal de Mira, Ria de Aveiro, Portugal), (2) to describe seasonal variations in the longitudinal distribution of development stages (nauplii, copepodites and adults) of *Acartia* spp. along the estuarine gradient and (3) to evaluate the significance of hydrological parameters (temperature, salinity, chlorophyll *a* and suspended matter) on copepod abundance at different time scales. In order to fulfil such purposes, the abundances of *Acartia* populations and environmental conditions were followed during 23 consecutive months at 6 sites distributed from the lower estuary to the upper estuary.

We used 3-mode PCA to analyse simultaneously spatial and temporal changes of multispecies assemblages. This analysis identified three distinct zones along the estuary based on abundances of the two *Acartia* species. For each zone time-lagged correlations between environmental variables and *Acartia* abundance indicated that different zones were forced by different combinations of variables. Additionally, the study also showed the existence of delayed effects of phytoplanktonic biomass on the abundance of *Acartia*. Based on these results we suggest that the identification of the spatial ambit on which the environmental factors operate along the estuarine gradient is crucial to understand the control of zooplanktonic processes.

5.1.3 Materials and Methods

5.1.3.1 Study area

The study area was Canal de Mira, a sub-estuarine system of Ria de Aveiro - Portugal (latitude 40º 38’ N, longitude 8º 44’W; Fig. 5.1). This complex mesotidal shallow coastal lagoon is separated from the sea by a sand bar and classified according to Pritchard (1967) as a bar-built estuary. Tides are semidiurnal with an average range of 2.1 m with ca. 1 and 3 m ranges in extreme neap and spring conditions.

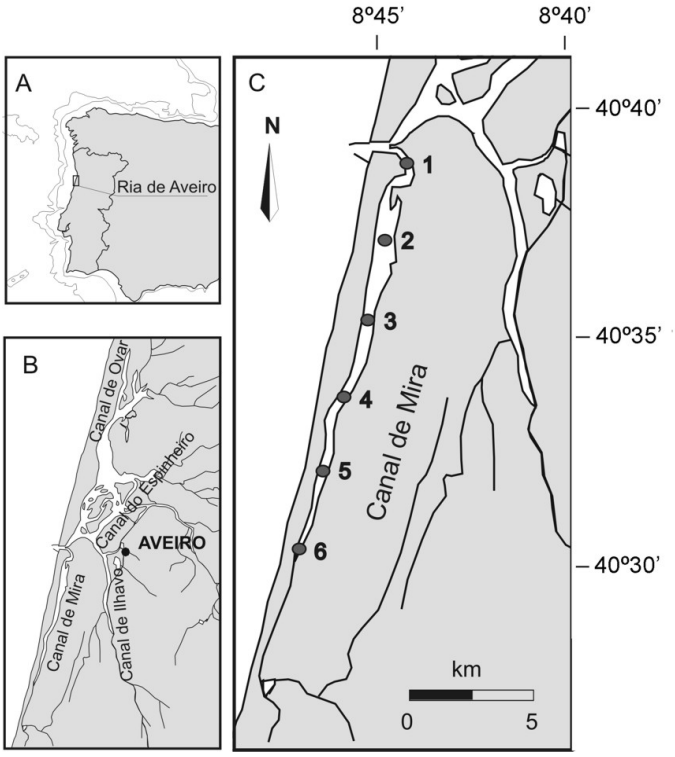

Figure 5.1 Location of Ria de Aveiro coastal lagoon (A) and Canal de Mira (B). Circles and numbers indicate the position of the sampling sites (C).

Canal de Mira runs to south-southwest from near the mouth for 25 km and parallel to the coastline. The average depth is about 1 m, but reaches 4 m at its deepest, at the lower section of the channel. In the upper section, the average depth is less than 0.5 m. This sub-estuarine system behaves like a tidally and seasonally poikilohaline estuary (Moreira et al. 1993) and no significant thermal or salinity stratification occurs, except during high peaks of freshwater discharge.

Freshwater inputs result mainly from rainfall and runoff from the margins. In Canal de Mira, the tidal prism relatively to the mouth is about 10% (Dias et al. 2000). In the area close to the mouth of this channel, the estimated residence time is lower than 2 days, revealing a strong marine influence. At the upstream, the residence time is higher than 1 week (Dias 2001).

5.1.3.2 Sampling

Field sampling campaigns were performed always at the same moment of the tidal cycle (two hours after the beginning of flood). Fortnightly campaigns were done between August 2000 and February 2002 and monthly campaigns between March 2002 and June 2002. Six stations, with a space interval of 2 nautical miles, were distributed along a transect defined from downstream (station 1) to upstream (station 6) (Fig. 5.1).

Zooplankton samples were collected by towing a 125 μm zooplankton net with a mouth diameter equal of 25.4 cm, equipped with a General Oceanics flowmeter. Total volume filtered varied from 0.2 to 3.7 m^3. The tows were done against the tidal current at 0.5 m below the water column surface and at 0.5 m above the bottom. The samples were preserved in 4% formaldehyde for later copepod species identification (*Acartia tonsa* and *Acartia clausi*) and enumeration of nauplii, copepodites and adults.

Larval and juvenile developmental stages of *Acartia* spp. (NI to NVI or CI to CV) were not distinguished. In the laboratory, samples were diluted to a known volume and aliquots were taken until getting a minimum of 200 individuals per stage (nauplii, copepodites and adults). Sorting and counting was made on a stereomicroscope Olympus SZH10. Abundances of all stages were expressed in individuals m^{-3}.

5.1.3.3 Hydrological parameters

Temperature and salinity profiles were recorded using an AANDERAA STD-Sensor Model No.3230 on the same time and location where zooplankton was collected. Water samples were taken with Van Dorn bottle at mid-water for determination of chlorophyll *a* (chl*a*), suspended particulate matter (SPM) and particulate organic matter (POM). Three replicates of 500 ml of seawater were filtered through Whatman GF/C filters and the chlorophyll *a* concentration (mg m^{-3}) was determined by spectrophotometry after 24h extraction with 90% acetone (Strickland & Parsons 1968). SPM was estimated as dry weight (60 ºC, 24 h) after filtration on Whatman CF/C filter (3 replicates). The organic

fraction, POM, was taken by the difference between the dry weight and the ash free dry weight (450ºC, 5 h). Chl*a* / SPM ratio was used as an index of phytoplankton availability in each sampling event.

At each sampling event, the mean values of each environmental parameter were obtained by averaging the respective depth profiles. For each hydrological parameter (salinity, temperature, chlorophyll *a*, SPM and Chl*a* / SPM), monthly mean values were estimated by averaging the respective fortnightly campaigns. The effect of year (2000-2001 and 2001-2002, where each year spans the period from July to June), season (winter, spring, summer and autumn) and location (station 1 to 6) on each hydrological parameter was analyzed with a 3-way orthogonal ANOVA.

5.1.3.4 Statistical analyses: spatio-temporal distribution patterns

For all samples, vertical copepod variation was removed by averaging surface and bottom samples. This vertical integration seems to be reasonable since at all sampling events the water column was never stratified. In addition, given the low depth of this estuarine system (average equal to 0.5 m) the potential copepod vertical migration is not significant. The monthly mean abundances of nauplii, copepodites and adults were log transformed using a log (x+1) function prior to statistical analysis.

Three-mode principal component analysis (three-mode PCA) was recently adapted to zooplankton abundance datasets by Beaugrand et al 2000 as a method for investigating associations of species across temporal and spatial gradients. This method allows the investigator to perform an analysis of complex tables by decomposing the variance in a 3-dimensional matrix.

Here, in order to describe spatial and seasonal distribution patterns of copepod populations of genus *Acartia* in Canal de Mira (Ria de Aveiro, Portugal), the monthly mean abundance of adult females and males of *A. tonsa* and *A. clausi* and the juvenile stages of

Acartia spp. (nauplii and copepodites) for each location were combined to generate a 3-way data matrix (6 taxa × 23 months × 6 locations).

This original table was then decomposed on three different modes: species mode (R^p, 6 station × 23 months – 6 taxa), time mode (R^t, 6 stations × 6 taxa - 23 months) and spatial mode (R^s, 23 months × 6 species – 6 stations). At each mode, a classical PCA were performed. Before analysis, the data were scaled for the 3 PCAs to be run at the same total inertia by scaling the log-transformed abundances for each species so that their sums of squares equalled 1 (Beaugrand et al. 2000).

The values of the first principal component of the three different modes were then represented graphically in 2 dimensions, i.e, stations × month year (Species mode), species × station (Time mode) and species × month-year (Space mode). We restricted this graphic representation to the first principal component because the eigenvectors plots of PC1 and PC2 usually showed the occurrence of a Guttman effect (see Fig. 5.5A to C below), which indicates that the same factor affects the first two axes of the ordination (Guttman 1958), and because these first two axes always explained more than 90% of the variation in the dataset.

In addition, for each mode a similarity matrix between variables was built using Eucledian distances. Cluster analysis using the unweighted pair group method average (UPGMA) was applied to the similarity matrix in order to obtain groups of species (species mode), stations (space mode) and month/year (time mode).

5.1.3.5 Statistical analyses: environmental forcing on zooplankton abundance

The changes of hydrological parameters and abundances through time were graphically represented using cumulative residuals (Ibañez et al. 1993, Le Fèvre-Lehoerff et al. 1995, Beaugrand et al. 2000). Cumulative residuals were calculated by subtracting the mean of the series from each value, and then adding the residuals sequentially.

In order to understand the relationships between environmental parameters and changes in copepod abundance Spearman correlations were computed between each time series of cumulative residuals of the hydrological parameters and the time series of cumulative residuals of PC1 form the species mode ordination, which is a measure of species abundances.

Significant correlations were evaluated after applying the Bonferroni correction for multiple comparisons (Sokal & Rohlf 1995). Additionally, in order to detect lagged effects of environmental variables on the community correlations between hydrological parameters and zooplankton abundance were computed as described above but imposing delays of 1, 2 or 3 months between each time series (cumulative residuals and hydrological parameters).

5.1.4 Results

5.1.4.1 Rainfall regime and hydrological parameters

During the study period, two contrasting precipitation regimes were observed (Fig. 5.2). During 2000-2001, the annual accumulated rainfall was equal to 694.4 mm with heavy peaks occurring in December 2000 (monthly accumulated equal to 148.3 mm), January 2001 (122.1 mm) and March 2001 (144.9 mm). During the year 2001-2002, as a consequence of an extremely dry year, annual accumulated rainfall was only 309.3 mm corresponding to about 45% of total precipitation in 2000-2001. In 2001-2002, the highest monthly accumulated rainfall was noted in October with 84.6 mm.

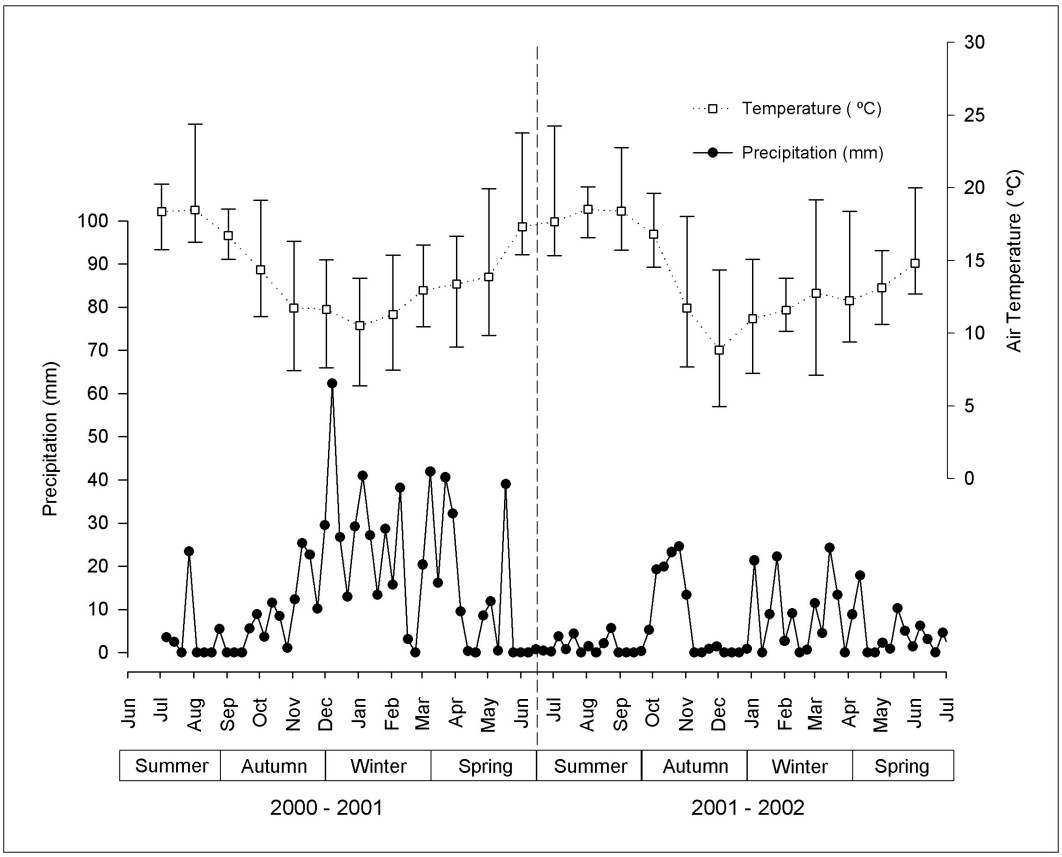

Figure 5.2 Rainfall and air temperature regime in Aveiro from July 2000 to July 2002. Precipitation graph refers to the weekly accumulated rainfall and temperature curve shows the average, maximum and minimum monthly air temperature.

Hydrological gradients (salinity, temperature, chlorophyll *a* and suspended particulate matter) between August 2000 and June 2002 in Canal de Mira (Ria de Aveiro – Portugal) are represented in Figure 5.3.

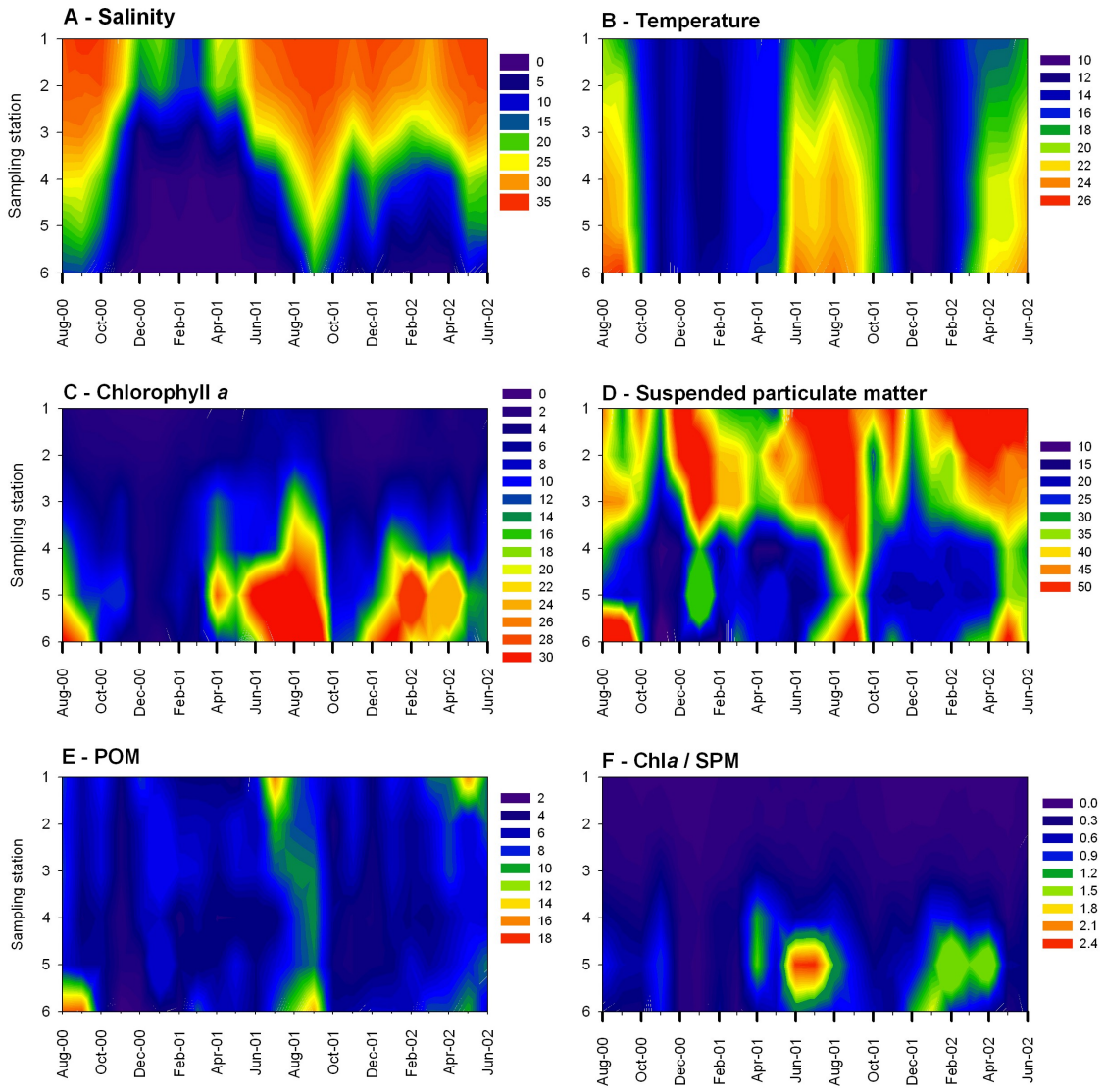

Figure 5.3 Monthly mean values of (A) salinity, (B) water temperature (ºC), (C) chlorophyll *a* (mg m^{-3}), (D) suspended particulate matter (mg l^{-1}), (E) particulate organic matter (mg l^{-1}) and (F) chlorophyll a / SPM ratio (mg g^{-1}) in Canal de Mira (Ria de Aveiro, Portugal) between August 2000 and June 2002.

The effect of year on salinity was highly significant (Year, $F_{1, 96} = 64.65$, $P < 0.001$). The annual average salinity in Canal de Mira (Ria de Aveiro, Portugal) was equal to 14.4 for the period 2000-2001 and 20.9 for the period 2001-2002. A highly significant effect of season and location was also noted (Fig. 5.3A), indicating the existence of markedly seasonal and longitudinal gradients of salinity in Canal de Mira (Ria de Aveiro, Portugal).

Highest salinity values were consistently recorded at downstream stations and during dry seasons (summer and autumn). At lower reaches, salinity ranged from 34.8 (September 2001, station 1) to 12.3 (March 2001, station 1) and at upper reaches from 17.48 (September 2001, station 6) to 0.19 (January 2001, station 6). The interaction Year × Season was also highly significant ($P < 0.001$), reflecting the low values registered in autumn, winter and spring of 2000-2001 (Fig. 5.3A), as a direct consequence of high precipitation levels observed during those periods.

Annual mean water temperature was not significantly different (Fig. 5.3B) between the two study periods (17.0ºC in 2000-2001 and 17.5ºC in 2001-2002). Water temperature showed a markedly seasonal cycle (Season, $F_{3, 96} = 93.99$, $P < 0.001$), with maximal temperature recorded in July 2000 at station 6 (27.9ºC) and minimum in December 2001 (10.7ºC) at station 4. In both study periods, a longitudinal temperature gradient (Station, $F_{5, 96} = 3.81$, $P < 0.01$) was only apparent during the hot season (summer) with water temperature increasing upstream. A significant interaction was found for Year × Season ($F_{3, 96} = 5.71$, $P < 0.001$).

The effect of year on chlorophyll *a* levels was highly significant (Year, $F_{1, 96} = 41.84$, $P < 0.001$) (Fig. 5.3C), with an annual average value equal to 7.73 and 12.34 mg m^{-3} in 2000-2001 and 2001-2002, respectively. Chlorophyll *a* levels were significantly lower at downstream than at upstream stations (Station, $F_{5, 96} = 58.23$, $P < 0.001$). At station 1, the water content in chlorophyll *a* ranged from 6.5 mg m^{-3} (July 2001) to 1.4 mg m^{-3} (February 2001) and from 1.6 (January 2001) to 47.1 mg m^{-3} (September 2001) at upper reaches (station 6).

ANOVA also showed the existence of pronounced seasonal differences (Season, $F_{3, 96} = 27.86$, $P < 0.001$) as well as a significant interaction between Season × Year ($F_{3, 96} = 15.73$, $P < 0.001$). The highly significant interaction Station × Year ($F_{5, 96} = 8.50$, $P < 0.001$) is attributed to the lower chlorophyll *a* concentration measured at upstream stations (St 4

to St 6) throughout 2000-2001 period compared to 2001-2002 reflecting, as previously referred for salinity, the rainier winter and spring of 2000-2001.

Average suspended particulate matter (SPM) in Canal de Mira was significantly higher (Year, $F_{1, 96}$ = 9.8, P < 0.01) in the period 2001-2002 (36.62 mg l^{-1}) than in 2000-2001 (31.38 mg l^{-1}) (Fig. 5.3D). SPM showed significant differences between sampling stations (Station, $F_{5, 96}$ = 21.38, P < 0.001) but did not show significant differences for the interaction Station × Year. The maximum turbidity levels were generally found at lower estuary where they ranged from a maximum of 79.7 (March 2002) to a minimum of 25.0 mg l^{-1}(May 2001). This high SPM can be attributed to the sediment ressuspension from the bottom as a consequence seawater intrusion (tidal wave propagation) and/or wind action. A three-way orthogonal ANOVA also showed the existence of a highly significant seasonality effect on the SPM concentration (Season, $F_{3, 96}$ = 10.17, P < 0.001), as well as a significant interaction between Season × Year ($F_{3, 96}$ = 3.58, P < 0.05) resulting from the higher SPM levels recorded in spring 2001-2002 than in 2000-2001.

5.1.4.2 Zooplankton abundance

The mean monthly abundance of juveniles and adults of *Acartia* spp. estimated by a 125 μm mesh net is represented in Figure 5.4. Analysis of this figure indicates that the juvenile stages of *Acartia* spp. (nauplii and copepodites) were the most abundant forms in the Canal de Mira.

Concerning naupliar stages (Fig. 5.4A), the highest abundances were noted in July 2001 at station 3 and 4 with 5.75×10^4 and 2.40×10^4 ind m^{-3}, respectively, and the lowest in February 2001 at station 4 (4.57 ind m^{-3}) and in January 2002 at station 5 and 6 (5.37 ind m^{-3}). Copepodite abundances (Fig. 5.4B) ranged between 5.75×10^4 and 4.57×10^4 ind m^{-3} in July 2001 at station 3 and 4 respectively, to 1.35 and 2.4 ind m^{-3} in February 2001 at station 4 and in December 2001 at station 6.

Under the sampling conditions used in this study (2h after the beginning of flood at each sampling station) the abundance of *A.tonsa* adults (Fig. 5.4C and Fig. 5.4D) during the sampling period was higher than of *A.clausi* (Fig. 5.4E and Fig. 5.4F) in all sampling stations except at the most downstream station 1.

Abundance peaks of *A.tonsa* adults occurred in summer, late autumn and spring with a maximum abundance (males + females) of 1.55 $\times 10^4$ ind m^{-3} recorded in July 2001 at station 4. The occurrence of *A.clausi* adults in Canal de Mira (Ria de Aveiro – Portugal) was restricted to the most downstream region of the estuary. Abundance peaks of *A.clausi* adults occurred mainly during the hot season (spring and summer) with maximum values in April 2002 and May 2002 at station 1 with 2.5 $\times 10^4$ and 1.02 $\times 10^3$ ind m^{-3}, respectively.

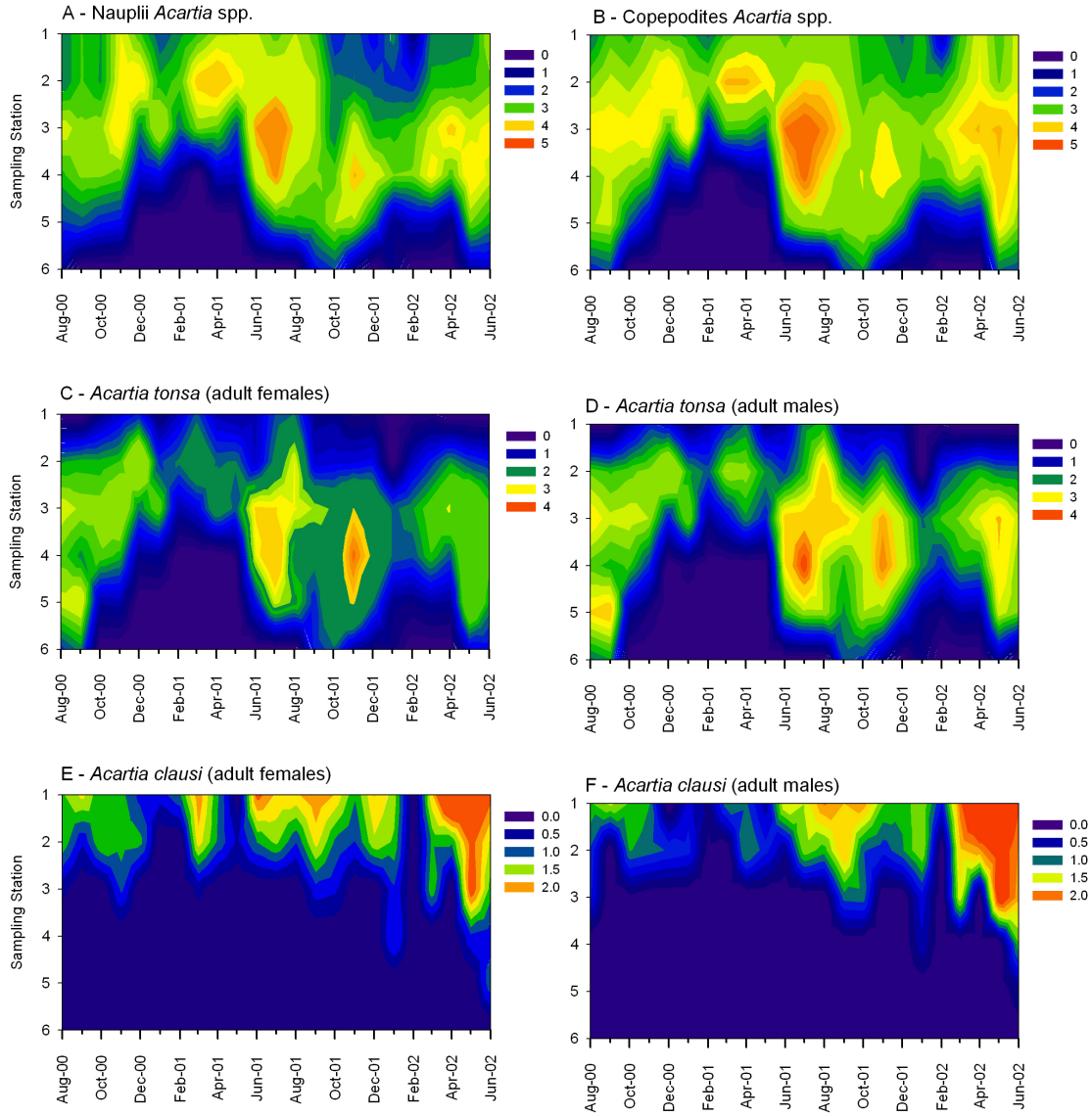

Figure 5.4 Mean monthly abundance in terms of [log (x+1)] of nauplii (A) and copepodites (B) of *Acartia* spp and adult females and males of *A.tonsa* (C and D) and *A.clausi* (E + F). Different scales were applied to the juveniles of *Acartia* spp. and adults of *A.tonsa* and *A.clausi*.

5.1.4.3 Seasonal and longitudinal copepod distribution patterns

Figure 5.5 represents the species mode (A), time mode (B) and space mode (C). In the species mode (R^p), three species clusters were defined at a linkage distance equal to 0.42. Cluster 1 was composed by adult males and females of *A. tonsa*, cluster 2 by *Acartia* spp.

juveniles (nauplii and copepodites) and cluster 3 by *Acartia clausi* (adult males and females). Axis 2 (12.0%) separates *A. tonsa* from *A. clausi* and 85.7% of the total variance was explained by axis 1 (Fig. 5.5A).

In time mode (R^t), the cluster analysis separated at a threshold equal to 0.52 the months between December 2000 to April 2001 from the remain study period (Fig. 5.5B).

Concerning the space mode (R^s), the cluster analysis defined the existence of 3 distinct zones composed of adjacent stations, at a linkage distance of 1.25 (Fig. 5.5C). Axis 1, explaining 81.8% of the total variance, separates Zone 1 (Stations 1 and 2) and Zone 2 (Stations 3, 4 and 5) from the most upstream Zone 3 (Station 6). Axis 2 (12.9%) showed Zone 1 to be in opposition to Zones 2 and 3. Zones 1, 2 and 3 can be interpreted as corresponding to the lower, middle and upper estuary (Fig. 5.5D).

Regarding to the first principal component in the location-month space (species mode) (Fig. 5.6A), strong seasonal variations were found in Zone 1 and Zone 2. Zone 3 was characterized by the occurrence of the lowest abundances levels and by weak seasonal variations. Abundance peaks occurred in summer, late autumn and spring of both years.

A clearly different longitudinal pattern was found between the periods December 2000 to April 2001 and December 2001 to April 2002, with a displacement of the highest abundance levels from near the mouth (Zone 1) to the middle estuary (Zone 2) during the latter period (Fig. 5.6A).

A clear spatial segregation of *A.clausi* and *A. tonsa* populations is illustrated by Fig. 5.6B, which refers to the first principal component in the species-location space (time mode). *A.clausi* population was found only in Zone 1, whereas *A.tonsa* population dominated in Zone 2. The distribution of juvenile stages of *Acartia* spp. followed the distribution of the adults.

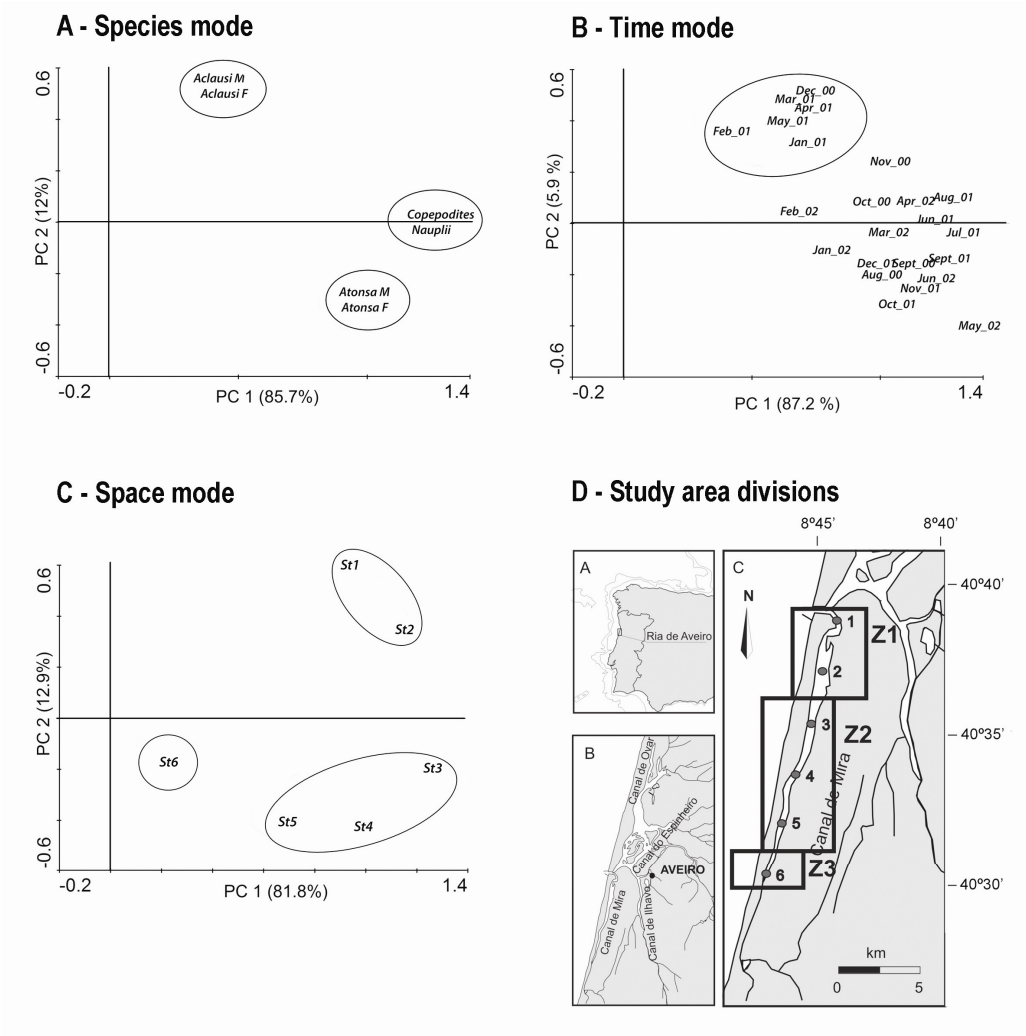

Figure 5.5 Seasonal variability. (A) Species mode (R^p). Correlations between the species and the two first principal components location-month. Percentage of variance explained by the components is given in parentheses. The cluster analysis resulted on the 3 species groups. (B) Time mode (R^t). Correlations between the months and the 2 first principal components species-location. The months were clustered in two periods. (C) Space mode (R^s). Correlations between the locations and the 2 first principal component species-month. The sampling stations were aggregated into 3 zones. (D) Map of the three zones defined in the space mode.

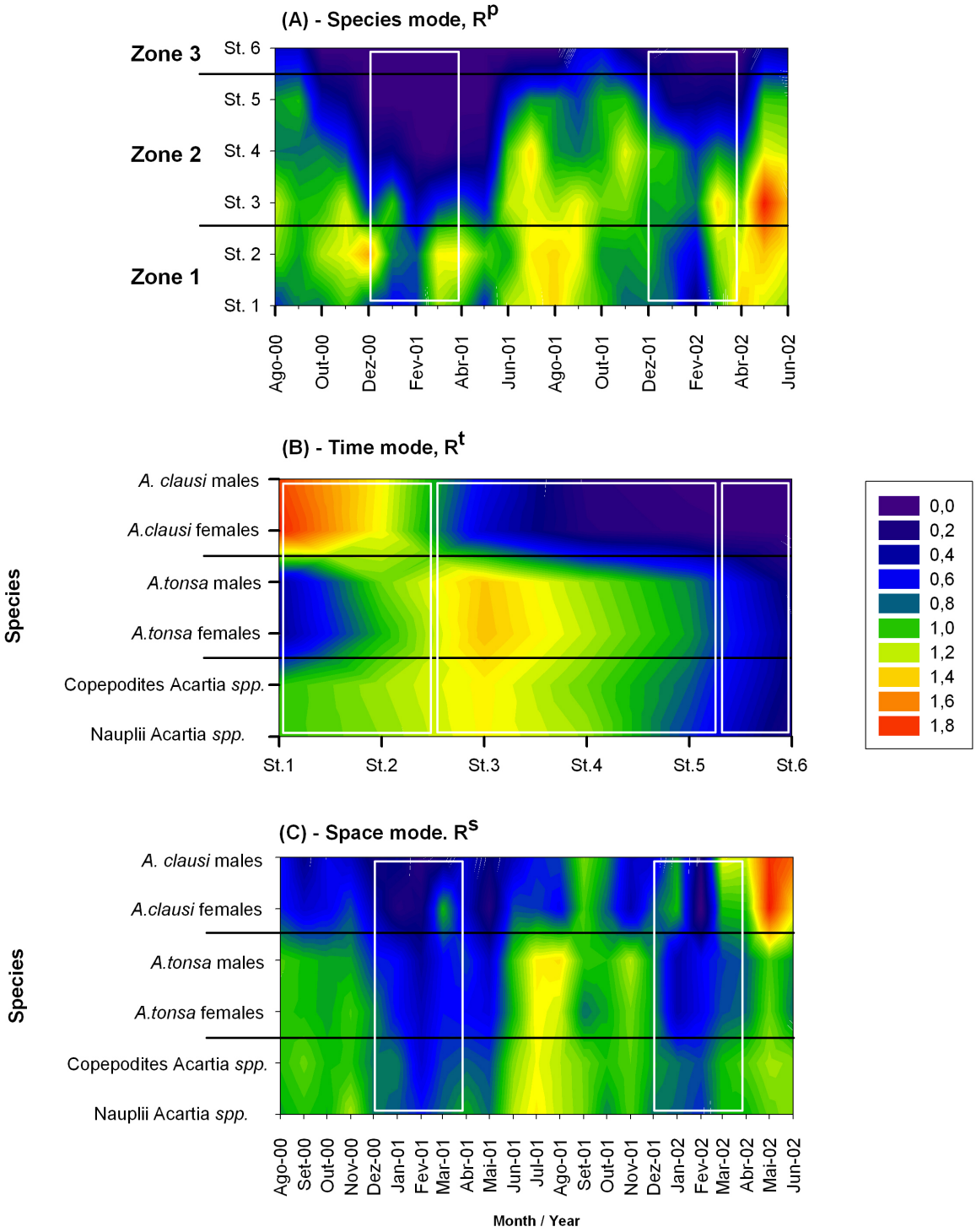

Figure 5.6 Seasonal variability. (A) Species mode (R^p). Variability of the first principal component month-location. The color scale indicates the intensity of the first component. The groups defined by cluster analysis are indicated for months in ordinate and for locations in abscissa. (B) Time mode (R^t). Variability of the first principal component species-location. The groups defined by cluster analysis are indicated for species in ordinate and for locations in abscissa. Space mode (R^s). Variability of the first principal component species-month. The groups defined by cluster analysis are indicated for species in ordinate and for month in abscissa. The white boxes in (A) and (C) represents the two consecutive winter periods and in (B) the 3 zones defined.

Variations in the first principal component in the species-month space (space mode) indicated seasonal changes of the *Acartia tonsa* populations, with low abundances during winter periods and high abundances during summer (Fig. 5.6C). For both species, marked differences were noted between the two springs analysed in this study, with Spring of 2001 being characterized by low abundance levels. Periods of high abundance of *A. clausi* were noted during Summer of 2001 and Spring of 2002. Larval and juvenile stages occurred throughout the year, with highest abundances verified during the summer periods and lowest abundances during winter periods.

5.1.4.4 Environmental forcing of *Acartia* spp. abundance and distribution

Figure 5.7 graphically represents the cumulative residuals of PC1 and hydrological parameters obtained for the three different zones, while Table 5.1 shows Spearman correlation coefficients between cumulative residuals of PC1 and the hydrological parameters at time lags ranging from 0 to 3 months.

In Zone 1, zooplankton abundance was positively correlated with rainfall and negatively with salinity, with similar values for lags 0 to 3 months. In Zones 2 and 3 copepod abundance was positively correlated with salinity and negatively with rainfall, with maximum values reaching over 0.95 occurring at a lag of 0 months for salinity. Zones 2 and 3 also showed positive correlations between zooplankton abundance and temperature peaking at lags of 1 to 2 months, and between zooplankton abundance and chlorophyl *a* peaking at lags of 2 to 3 months. Zooplankton abundance in Zones 2 and 3 was also positively correlated with SPM, POM and chl *a*/SPM ratio. The observed correlation values indicate a quick response of the *Acartia* populations to salinity and a delayed response to temperature and chlorophyll *a*.

Regarding to the correlations among environmental factors in each of the three zones, significant negative associations were found between salinity and rainfall in Zone 1 (-0.91)

and Zone 2 (-0.81), and between chlorophyll *a* and rainfall in Zone 2 (-0.69) and Zone 3 (-0.92). In addition, it were also found positive associations between salinity and SPM in Zone 1 (0.68) and Zone 3 (0.79), between temperature and SPM in Zone 2 (0.65) and Zone 3 (0.73), and between temperature and POM in Zone 1 (0.73), Zone 2 (0.80) and Zone 3 (0.84).

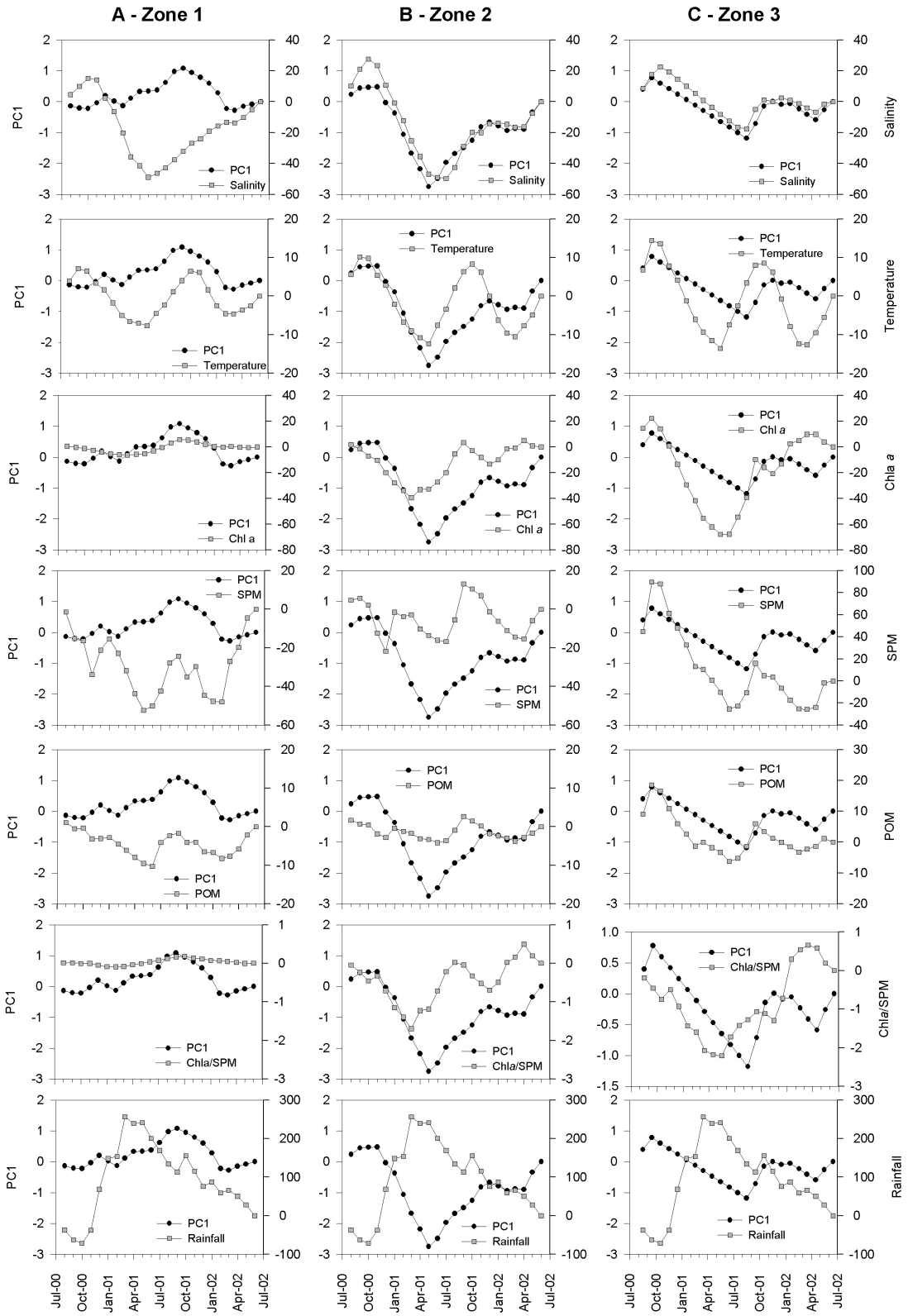

Figure 5.7 Hydrological parameters and the first principal component month-location after cumulative sums treatment for each zone determined from the cluster analysis on location mode.

Table 5.1 Spearman coefficient correlation between the cumulative residuals of the first principal component month-location (PC1) and hydrological parameters (salinity – sal, temperature – temp, chlorophyll a – Chla, suspended particulate matter – SPM, particulate organic matter – POM and Chla/SPM ratio) estimated at the three zones and imposing a delay of 1, 2 or 3 month between time series. * Significant correlation at 0.05 level after Bonferroni correction.

		Lag			
Zone 1	Parameter	0 months	1 month	2 months	3 months
	Salinity	**-0.68***	**-0.72***	**-0.73***	**-0.70***
	Temperature	0.14	0.03	-0.15	-0.41
	Chl a	0.30	0.11	-0.13	-0.37
	SPM	-0.42	-0.35	-0.23	-0.15
	POM	-0.10	-0.06	-0.06	-0.18
	Chla/SPM	0.48	0.35	0.13	-0.17
	Rainfall	**0.62***	**0.63***	**0.67***	**0.69***

		Lag			
Zone 2	Parameter	0 months	1 month	2 month	3 month
	Salinity	**0.95***	**0.80***	0.53	0.26
	Temperature	**0.58***	**0.64***	**0.59***	0.46
	Chl a	0.39	**0.64***	**0.86***	**0.95***
	SPM	0.29	0.28	0.31	0.34
	POM	0.46	**0.46***	0.41	0.34
	Chla/SPM	0.26	0.51	**0.72***	**0.86***
	Rainfall	**-0.87***	**-0.92***	**-0.86***	**-0.72***

		Lag			
Zone 3	Parameter	0 months	1 month	2 month	3 month
	Salinity	**0.94***	**0.84***	0.55	0.26
	Temperature	**0.55**	**0.74***	**0.77***	**0.70***
	Chl a	0.54	**0.74***	**0.79***	**0.75***
	SPM	**0.72***	**0.76***	**0.62***	0.34
	POM	**0.75***	**0.91***	**0.86***	0.57
	Chla/SPM	0.30	0.44	**0.58***	**0.65***
	Rainfall	**-0.64***	**-0.73***	**-0.79***	**-0.72***

5.1.5 Discussion

The present study was set out to investigate the effect of environmental forcing on the distribution and abundance of estuarine populations of *Acartia* spp. The results indicate that, in the Canal de Mira, the estuarine gradient can be subdivided into three zones, each controlled by a combination of different environmental factors.

A clear gradient in salinity was observed in the Canal de Mira, with low values upstream. This gradient is strongly affected by rainfall, which is more intense during winter and spring (Anónimo 1974). This corresponds to the typical situation in temperate estuaries (e. g. Pritchard 1952, 1967, Hansen 1967), and to previous observations showing that salinity and water residence in the Canal de Mira time react quickly to rainfall (Moreira et al. 1993, Dias 2001).

Salinity was positively correlated with suspended particulate matter, which showed high levels at the most downstream stations. Such levels of suspended matter can be attributed either to intruding seawater carrying high loads of suspended sediments, or to sediment resuspension due to turbulence induced by tidal currents or winds in the lower section of the estuary. The strength of the seasonal signal in salinity was different in the two years.

The high values of rain fall during the winter of 2000-2001 caused salinity to decrease to values below 15 at the most downstream station, while in the winter of 2001-2002 salinity did not drop below 30. Concurrently with the decrease in salinity to very low values during the winter of 2000-2001 there was a decrease in the biomass of phyto- and zooplankton.

Phytoplankton populations in particular were completely flushed out from the estuary during December of 2000, a period when values between 0 and 3 mg m^{-3} were observed throughout the estuary, while values above 25 mg m^{-3} were recorded during the summer months in the upstream stations. Water temperature ranged between 10 and 26^{o}C and

also showed a strong seasonal signal. During winter temperatures were generally below 15^{o}C and constant along the estuary, while in summer they ranged from 19^{o}C downstream to 26^{o}C upstream.

The spatial distribution and abundance of estuarine zooplanktonic populations is determined by advection (Kimmerer 2002, Kimmel & Roman 2004) specific tolerance to salinity (Jassby et al. 1995, Cervetto et al. 1999, Gaudy et al. 2000), response of individual and population growth rates to temperature (Huntley & Lopez 1992), food availability (Paffenhöfer & Stearns 1988), predation and competition (Kimmel & Roman 2004).

Acartia tonsa and *A. clausi* from the Ria the Aveiro show significant differences in individual growth rates within the range of temperatures typical of the Canal de Mira (Leandro et al. 2006a, 2006b). Although we did not investigate the effects of food availability and biotic relationships on the populations of *Acartia*, we expected to find a response of the abundance and distribution of these populations to the main environmental conditions, given the clear longitudinal and seasonal gradients prevailing in this system.

The 3-mode PCA employed in this study identified a spatial segregation of the two *Acartia* species inhabiting the Canal de Mira, a division of the estuary into 3 distinct sections, and a distinct regime during the winter of 2000-2001. Abundance peaks of both species were noted during late spring to early autumn, with *A. clausi* distributed exclusively in the most downstream stations (Zone 1) and *A. tonsa* in the middle estuary (Zone 2), while the upstream section (Zone 3) had consistently low copepod abundances that dropped to 0 ind m^{-3} during the winter of 2000-2001.

The spatial segregation pattern of the species is a consequence of the neritic origin of *A. clausi* and the endemism of the estuarine copepod species *A. tonsa* (Soetaert & Rijswijk 1993) and is rooted on the different ecophysiological capabilities of both species (Gaudy et al. 2000).

The results obtained from the correlation analysis between zooplankton abundance, given by the scores on the PC1 axis, and hydrological parameters uncovered different environmental forcings in each estuarine zone. Zooplankton abundance in Zone 1 was positively correlated with rainfall levels and negatively correlated with salinity reflecting an advective transport of zooplanktonic populations inhabiting middle and upper estuarine zones to the lower estuary.

This transport is driven by the increased freshwater flow, with a consequent downstream displacement of their habitat (Kimmerer 2002) and an associated negative effect of salinity in abundance in Zones 2 and 3. Maximum values of correlation between salinity and zooplankton abundance occurred with a lag of 0 months, suggesting a quick response of the system. Negative effects of freshwater flow were reported elsewhere (Deegan 1990, Kaartverdt and Aksnes 1992, Livingston et al. 1997, Howarth et al. 2000) for different estuarine populations and are basically considered to be a distributional response.

Zooplankton abundance in Zones 2 and 3 was positively correlated with water temperature at time lags of 1 to 2 months. The zooplankton of those zones was dominated by *A. tonsa*, which is characterized by having a stronger response to temperature than the majority of marine and estuarine calanoid copepod including *A. clausi* (Leandro et al. 2006a, 2006b) and consequently higher growth rates and lower development times. By developing faster, *A. tonsa* populations will be able to maintain itself within highly advective environments like estuaries, and dominate the estuarine zooplankton community (Leandro et al. 2006a).

The correlations between chlorophyll *a* and zooplankton abundance in Zones 2 and 3, were unexpected, because previous studies very often failed to identify similar patterns (e. g. Mallin 1991, Froneman 2000). An explanation for this lack of non-significant associations between the two variables in previous studies may be related to the fact that

these did not investigate delayed effects of phytoplankton standing stock on zooplankton. The delayed effects disclosed in our study may be interpreted assuming that primary production is maintained at high levels during spring and summer but is grazed down by increasing densities of zooplankton, which lower the initial peak in phytoplankton abundance. This explanation is consistent with the short generation time of *A. tonsa* at the temperatures characteristic of the Canal de Mira during the summer, which is on the order of 15 days (Leandro et al 2006a): if phytoplankton production did not continue then the delay should be at most of 15 days (Plourde and Runge 1993).

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Chapter 6

Biomass and production of *Acartia* populations

Section 6.1

Biomass and production of juvenile stages of *Acartia* (Copepoda: Calanoida) populations from a Southern European estuary (Canal de Mira – Ria de Aveiro, Portugal)

6.1.1 Abstract

This paper presents data about seasonal patterns of biomass and daily secondary production rates of *Acartia* populations inhabiting on a Southern European estuary (Canal de Mira, Ria de Aveiro – Portugal). Zooplankton was sampled between August 2000 and June 2002 at 6 stations distributed throughout a gradient of salinity. Secondary production was estimated by the combination of field data (copepod biomass and water temperature) with specific temperature-dependent growth models previously defined for *Acartia tonsa* and *A.clausi*. During the study period, the average biomass (including all stages) of *A.tonsa* and *A.clausi* was equal to 4.216 mg C m^{-3} and 0.528 mg C m^{-3} respectively. Biomass peaks were verified in spring and summer for both populations and in late autumn for *A.tonsa*. Average daily secondary production rates of *A.tonsa* and *A.clausi* juvenile forms were equal to 1.140 mg C m^{-3} d^{-1} and 0.068 mg C m^{-3} d^{-1}. Average daily P/B ratios for *A.tonsa*, *A.clausi* and *Acartia* spp. were equal to 0.27, 0.13 and 0.25 d^{-1}, respectively.These results demonstrate the key role of *A.tonsa* population on the transfer of matter and energy within the planktonic realm, given that these species account to approximately 50% of the total copepoda biomass in Ria de Aveiro and represents 30-40% of the total daily secondary production. Future studies should combine the estimate of adult and juvenile production in order to evaluate its relative contribution and to obtain a more precise value of secondary production.

Key words: *Acartia tonsa*; *A.clausi*; biomass; secondary production rate; Canal de Mira (Ria de Aveiro – Portugal)

6.1.2 Introduction

Zooplankton is a group of organisms extremely important on the transfer of matter and energy in marine ecosystem. Among zooplankton, copepods are the most abundant organisms comprising as much as 80% of its total biomass (Kiorboe 1998). In North Atlantic estuarine ecosystems, species of *Acartia* genus frequently dominates the pelagic environment (Durbin & Durbin 1981, Lawrence et al. 2004, Marques et al. 2006) and may be considered a key species in the carbon flux.

The impact of a given species on the carbon flux and on higher trophic levels can be assessed by the calculation of its secondary production rate. Zooplanktonic production can be measured by the estimate of growth and mortality in cohorts over consecutive sampling intervals (Parslow & Sonntag, 1979). Nevertheless, for continously reproducing populations as marine and estuarine copepods, cohort´s identification requires an enormous field sampling effort given the constraint of an adequate resolution in time and space. When estimated from field samples, the development time for each copepod stage requires that a single stage must be sampled at least several times during the course of the life cycle, being the number os samples depended on the specific generation time (Huntley & Lopez, 1991). Additionaly, it's also essential to ensure that the same copepod population is being sampled and that the change in number is not a consequence of advective processes. This implies to have a good spatial resolution of the population throughout an appropriate number of sampling stations (Huntley & Lopez, 1991).

Other methodologies for the calculation of secondary production rates are based on the estimate of growth rates, as weight-specific egg production or juvenile somatic growth. During the last decades, the majority of copepod production estimates were derived from egg production studies which have been assumed to be equivalent to the juvenile growth (Kiørboe & Nielsen 1994, Hay 1995, Nielsen & Sabatini 1996). However, recently evidences have point out that under *in situ* conditions adult females' growth rates are lower than the respective juveniles (Hirst & Sheader 2003). As a direct consequence, the measurements of production based on egg production

extrapolated to the entire population have certainly underestimated the total production of a given copepod population.

Concerning to juvenile somatic growth, the data compilation performed by Hirst & Bunker 2003 revealed that juvenile copepods in the field grow at rates close to maximum laboratory rates determined at food saturated conditions. Based on that evidence, realistic estimates of juvenile production can be easily determined by combining *in situ* data (copepod biomass and water temperature) with temperature-dependent growth models.

Nevertheless, the growth models should be species specific and not general growth equations because different copepod species shows different generation times when exposed at the same conditions of water temperature (Leandro et al 2006a). Additionally, the specific growth model should be defined for a particular copepod population since allopatric populations could have developed different genotypes in response to different temperature regimes normally experienced in its natural environment (Leandro et al 2006b).

In previous studies Leandro et al (2006 a, b) addressed the temperature-dependent growth rate of *Acartia* populations inhabiting Ria de Aveiro (Portugal), namely *Acartia tonsa* and *A.clausi*, and defined site- and species-specific temperature-dependent growth models for those copepod populations. In the present study we attempt to describe seasonal biomass patterns of *A.tonsa* and *A.clausi* along a salinity gradient (Canal de Mira – Ria de Aveiro, Portugal) and to estimate daily secondary production rates of non-adult stages. To fulfil such purposes, *Acartia* populations were sampled for 23 consecutive months and secondary production rate estimates obtained by multiplying juvenile copepod biomass by growth rates derived from specific temperature-dependent growth models.

6.1.3 Materials and Methods

6.1.3.1 Study area

The study area was Canal de Mira, a sub-estuarine system of Ria de Aveiro - Portugal (latitude 40º 38' N, longitude 8º 44'W; Fig. 6.1). This complex mesotidal shallow coastal lagoon is separated from the sea by a sand bar and classified according to Pritchard (1967) as a bar-built estuary. Tides are semidiurnal with an average range of 2.1 m with ca. 1 and 3 m ranges in extreme neap and spring conditions. Canal de Mira runs to south-southwest from near the mouth for 25 km and parallel to the coastline.

The average depth is about 1 m, but reaches 4 m at its deepest, at the lower section of the channel. In the upper section, the average depth is less than 0.5 m. This sub-estuarine system behaves like a tidally and seasonally poikilohaline estuary (Moreira et al, 1993) and no significant thermal or salinity stratification occurs, except during high peaks of freshwater discharge. Freshwater inputs result mainly from rainfall and runoff from the margins. In the area close to the mouth of this channel, the estimated residence time is lower than 2 days, revealing a strong marine influence. Upstream, the residence time is higher than 1 week (Dias, 2001).

Based on the abundance and distribution patterns of *Acartia* populations, Canal de Mira was previously divided into three distinct zones (Leandro et al., submmited).: Zone 1 (lower estuary) corresponding to stations 1 and 2, Zone 2 (middle estuary) corresponding to stations 3, 4 and 5 and Zone 3 (upper estuary) comprising station 6 (Fig. 6.1). At each zone, *Acartia* spp. abundance was controlled by different environmental factors (see below) that ultimately have a strong influence on zooplankton production

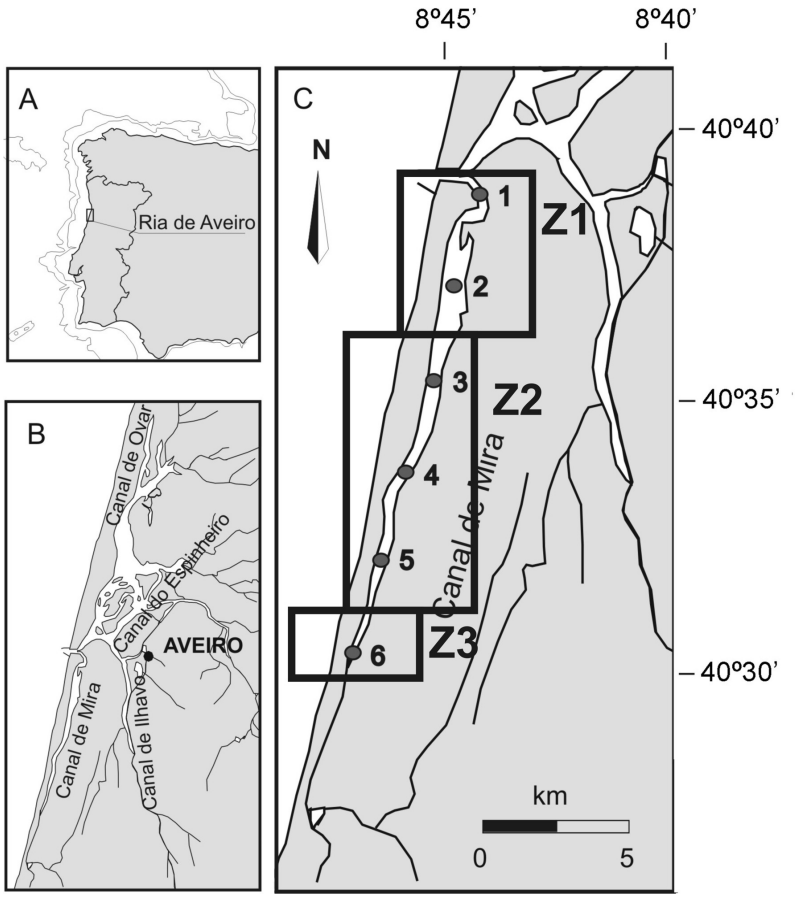

Figure 6.1 Map of Canal de Mira (Ria de Aveiro – Portugal) with the localization of the sampling stations and the 3 zones previously defined by Leandro et al (submitted).

6.1.3.2 Sampling

Zooplankton and environmental data (salinity, temperature, chlorophyll *a* and particulate suspended matter) were collected in Canal de Mira (Ria de Aveiro – Portugal) at 6 fixed locations (Fig. 6.1). The sampling was performed between August 2000 and June 2002 with a mean time interval of 15 days, except for the period between March 2002 and June 2002 when samples were collected monthly.

Copepods were collected by towing a 125 μm zooplankton net with a mouth diameter of 25.4 cm, equipped with a General Oceanics flowmeter. The tows were done against the tidal current 0.5 m below surface and 0.5 m above the bottom, except when the

water column was lower than 1 m. The total volume filtered varied between 0.2 to 3.7 m^3.

The samples were preserved in 4% formaldehyde for later species identification (*Acartia tonsa* and *A. clausi*) and quantification of the different developmental stages, nauplii (NI to NVI), copepodites (CI to CV) and adults (males and females).

6.1.3.3 Copepod biomass

In the laboratory, samples were diluted to a known volume and aliquots were taken until getting a minimum of 200 individuals per stage. Sorting and counting was made on a stereomicroscope Olympus SZH10. Abundances were expressed in individuals m^{-3}. Since Canal de Mira is a relatively shallow estuary (1.2 m) it was assumed the inexistence of vertical preference distribution and bottom and surface samples were pooled. For each location, a mean monthly value was obtained by averaging the fortnightly campaigns except when only 1 campaign per month was performed.

Because larval and juvenile developmental stages (nauplii and copepodites) of *A.tonsa* and *A.clausi* are very difficult to distinguish, it was assumed that its distribution follows distribution of adults. The abundance was estimated by multiplying the respective adult proportion by the total abundance of nauplii or copepodites.

The sorted organisms where washed with distilled water and collected on a pre-combusted glass fibber filter (Whatman GF/C). After drying at 60ºC for 24 hours, the weight was taken on a microbalance (Mettler Toledo, sensitivity 0.1 μg). Dry weights were corrected for weight lost during preservation according to Omori and Ikeda (1984) by a factor of 1.3 (corresponding to a loss of 30%) and then converted to carbon weight (μg C) assuming this to be 40% of the dry weight (Omori & Ikeda 1984, Båmstedt 1986). The total biomass of nauplii, copepodites and adults for each

sampling campaign, was obtained by the product of its abundance and respective mean individual carbon weight, expressed in µg C m^{-3}.

6.1.3.4 Copepod secondary production

Daily secondary production rate was estimated by the product of biomass and the growth rate:

$$P = B \times g$$

where *P* is the daily secondary production (mg C m^{-3} d^{-1}), *B* is the biomass (mg C m^{-3}) and *g* is growth rate (d^{-1}) in a given moment. For each *Acartia* species, nauplii and copepodites growth rates were taken from specific temperature-dependent growth models previously defined for copepod populations inhabiting Ria de Aveiro (Portugal) (Table 6.1).

Table 6.1 Temperature-dependent growth model for *A.tonsa* and *A.clausi* of Ria de Aveiro (Portugal)

Species	Nauplii	Copepodites	Reference
A.tonsa	$g = 0.0517\ e^{(0.130 \times T)}$	$g = 0.0364\ e^{(0.114 \times T)}$	Leandro et al. 2006a
A.clausi	$g = 0.0914\ e^{(0.0701 \times T)}$	$g = 0.0591\ e^{(0.0775 \times T)}$	Leandro et al. 2006b

Since copepod biomass of *Acartia* species is not evenly distributed througout Canal de Mira, mean copepod biomass (mg C m^{-3}) and mean daily secondary production rate (mg C m^{-3} d^{-1}) were first estimated for each of the three zones. Average values estimates for Canal de Mira were obtained by weighting the mean biomass and production of both zones by the respective water volume proportion (Table 6.2).

Table 6.2 Estimated area (m^2) and water volume (m^3) for Canal de Mia and each Zone. (Dias, pers. Comm)

	Area (m^2)	Volume (m^3)
Zone 1	2 372 800	4 887 728
Zone 2	4 017 600	3 496 352
Zone 3	592 000	374 352
Canal de Mira	6 982 400	8 758 342

6.1.4 Results

6.1.4.1 Hydrological data

Hydrological data and correlation analysis between environmental parameters and zooplankton abundance were already described in chapter 5. Briefly, it was noted specific environmental forcing on *Acartia* spp abundance and distribution at a given zone of Canal de Mira. At Zone 1 zooplankton abundance showed to be positively correlated with rainfall reflecting an advective transport of zooplankton to downstream. Zone 2 and 3 showed to be positively correlated with water temperature. On these two last zones, it was also noted a delayed effect of chlorophyll *a* levels (1 to 3 months) on zooplankton abundance (see Chapter 5).

6.1.4.2 *Acartia* biomass

The occurrence of *A.clausi* was almost limited to the most downstream region – Zone 1 (Fig. 6.2). In this zone, the mean biomass of *A.clausi* (all stages) was 0.898 mg C m^{-3}. The highest values were registered in April 2002 (5.857 mg C m^{-3}) and May 2002 (2.458 mg C m^{-3}) and juvenile stages (nauplii plus copepodites) accounted to 64% of the total biomass. The mean biomass of *A.tonsa* in Zone 1 was equal to 2.696 mg C m^{-3} with an average contribution of juvenile stages (nauplii plus copepodites) of 73% (Fig. 6.2). Maximal values were observed in December 2000 (13.492 mg C m^{-3}), March 2001 (9.947 mg C m^{-3}) and August 2001 (7.281 mg C m^{-3}).

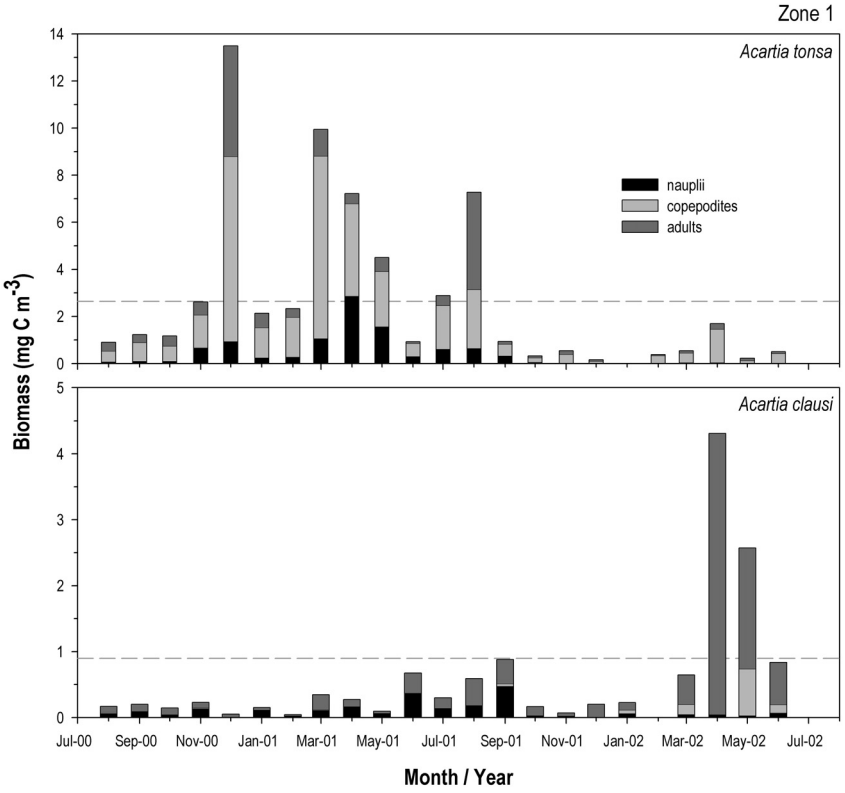

Figure 6.2 Monthly variations in biomass (mg C m^{-3}) of *Acartia* populations in Zone 1 of Canal de Mira (Ria de Aveiro – Portugal) from August 2000 to June 2002. The gray dashed line represents mean biomass over the entire study period.

The highest mean biomass of the *A.tonsa* in Canal de Mira (Ria de Aveiro – Portugal) was recorded in Zone 2 (7.944 mg C m^{-3}), with an average contribution of juveniles of

70% (Fig. 6.3). Biomass peaks occurred in July 2001 (51.515 mg C m^{-3}), November 2002 (22.0 mg C m^{-3}) and May 2002 (178.7 mg C m^{-3}). The mean biomass of *A.clausi* at Zone 2 was very low (0.080 mg C m^{-3}), with juveniles accounting for about 56% (Fig. 6.3). The highest value was noted in May 2002 (1.068 mg C m^{-3}).

Copepod biomass showed the lowest values in Zone 3 (Fig. 6.4). The mean biomass of *A.tonsa* in this zone was 0.427 mg C m^{-3}, with the highest value recorded in September 2001 (7.625 mg C m^{-3}). Juvenile forms account to almost 57% of the total mean biomass. There were no records of *A. clausi* individuals in Zone 3 (Fig. 6.4).

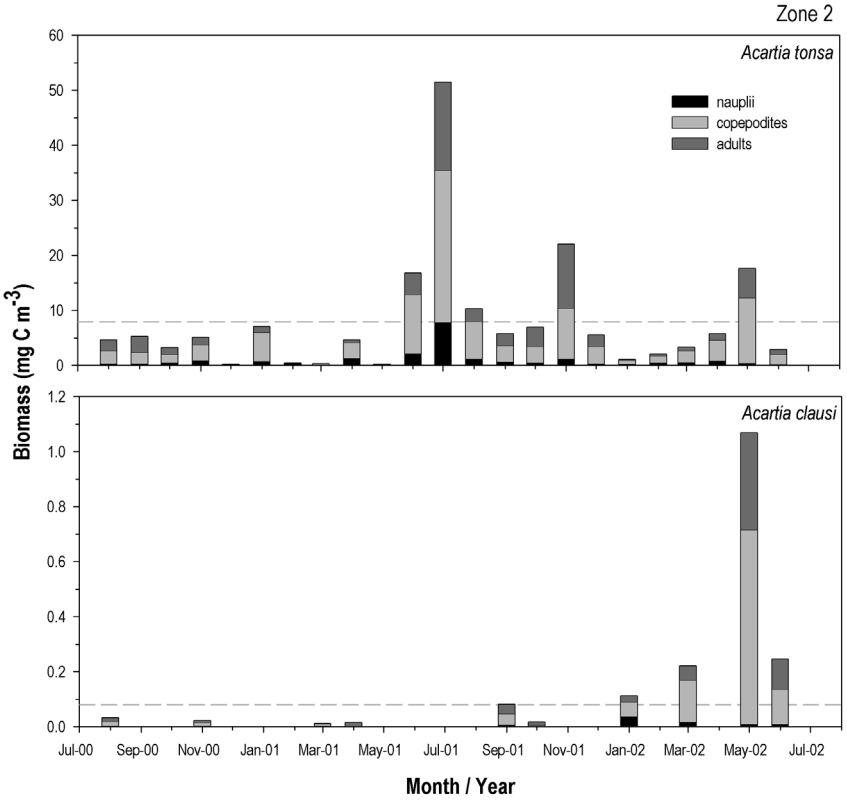

Figure 6.3 Monthly variations in biomass (mg C m^{-3}) of *Acartia* populations in Zone 2 of Canal de Mira (Ria de Aveiro – Portugal) from August 2000 to June 2002. The gray dashed line represents mean biomass over the entire study period.

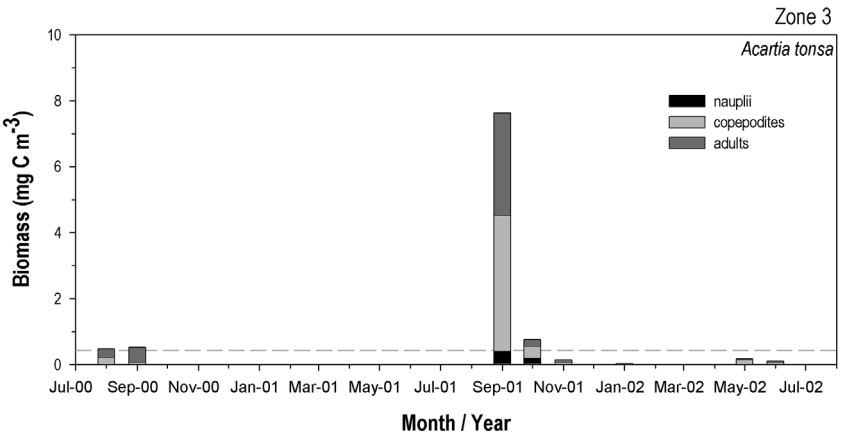

Figure 6.4 Monthly variations in biomass (mg C m^{-3}) of *Acartia* populations in Zone 3 of Canal de Mira (Ria de Aveiro – Portugal) from August 2000 to June 2002. The gray dashed line represents mean biomass over the entire study period.

The average *Acartia* biomass over the entire Canal de Mira (Ria de Aveiro – Portugal) is represented in Figure 6.5. Concerning *A. tonsa*, average biomass was equal to 4.216 mg C m^{-3} (Fig. 6.5), with juvenile stages contribution accounted for 70% (nauplii – 13% and copepodites – 57%). *A.tonsa* biomass showed a consistent seasonal pattern, with highest levels recorded in July 2001 (19.076 mg C m^{-3}), November 2001 (7.773 mg C m^{-3}) and May 2002 (6.125 mg C m^{-3}). Lowest biomass values were regularly observed during the winter.

The average biomass of the *A.clausi* population was equal to 0.528 mg C m^{-3}, with juvenile stages representing 54% (nauplii – 11% and copepodites – 43%) of the total biomass (Fig. 6.5). During the study period, *A.clausi* biomass was higher during spring / summer than autumn or winter, with the highest value recorded in April 2002 (3.268 mg C m^{-3}).

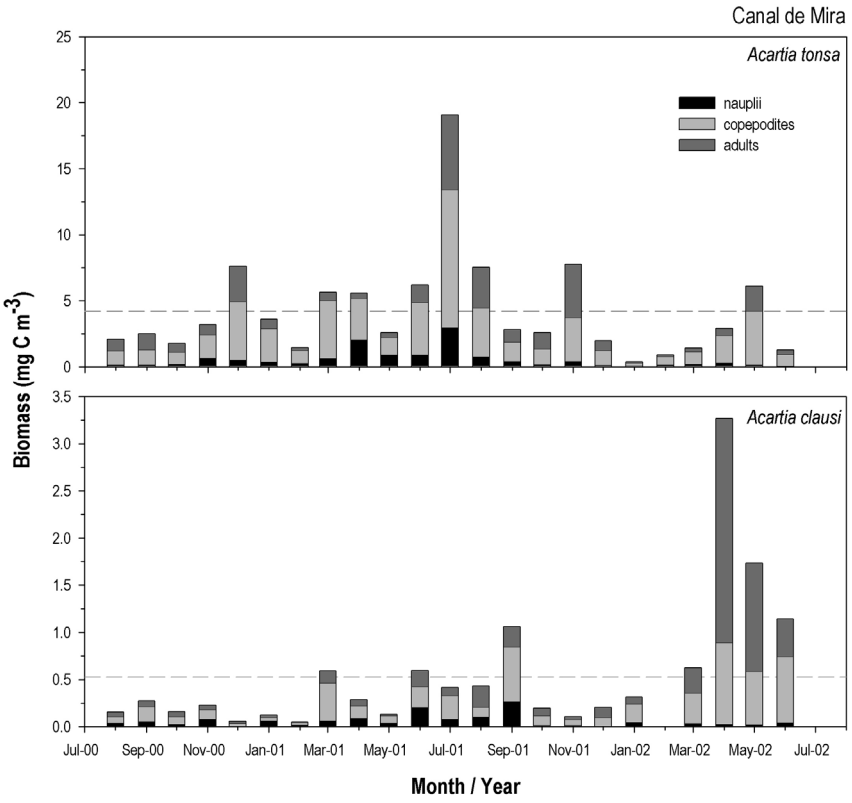

Figure 6.5 Monthly variations in biomass (mg C m^{-3}) of *Acartia* populations in Canal de Mira (Ria de Aveiro – Portugal) during the period between August 2000 and June 2002. The gray dashed line represents mean biomass over the entire study period

6.1.4.3 *Acartia* juvenile production

The mean daily secondary production rate of *A.tonsa* juveniles in Zone 1 (Fig. 6.6) was 0.608 mg C m^{-3} d^{-1}, with highest values in March 2001 (2.139 mg C m^{-3} d^{-1}), April 2001 (2.116 mg C m^{-3} d^{-1}) and December 2000 (2.072 mg C m^{-3} d^{-1}). At Zone 1, the mean secondary production rate of *A.clausi* juveniles (nauplii + copepodites) was equal to 0.113 mg C m^{-3} d^{-1}. The highest value was observed in September 2001 with 0.421 mg C m^{-3} d^{-1}, followed by April 2002 (0.351 mg C m^{-3} d^{-1}), June 2002 (0.315 mg C m^{-3} d^{-1}) and June 2001 (0.226 mg C m^{-3} d^{-1}). With some exceptions, the copepodite stages accounted for more than 50% of the total juvenile production.

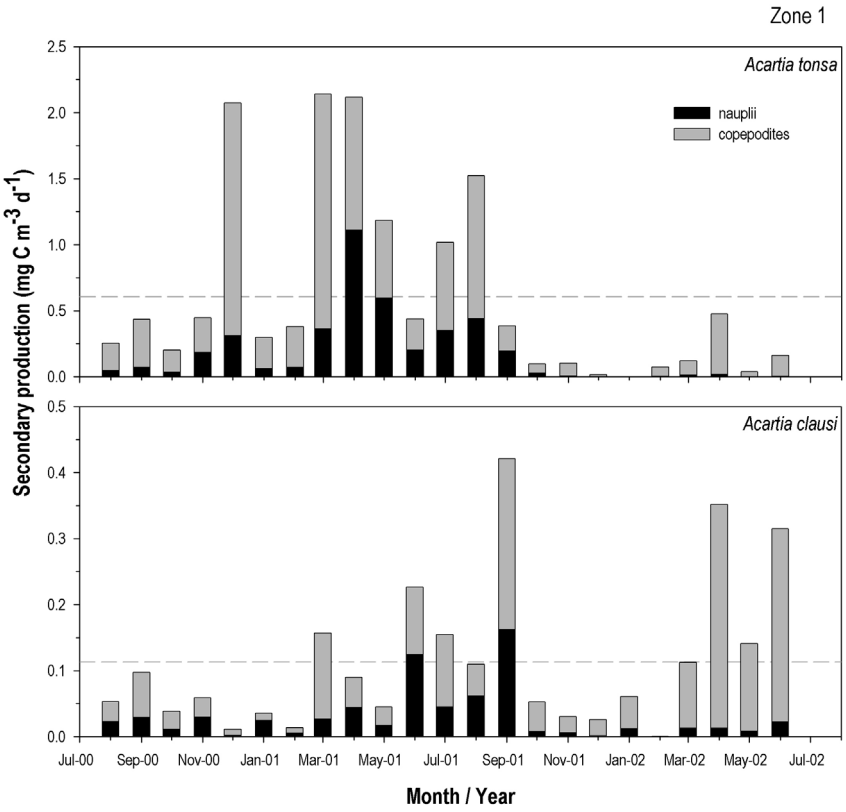

Figure 6.6 Monthly variations in daily secondary production rates (mg C m^{-3} d^{-1}) of *Acartia* populations in Zone 1 of Canal de Mira (Ria de Aveiro – Portugal) from August 2000 to June 2002. The gray dashed line represents mean daily secondary production over the entire study period.

The mean daily secondary production rate of *A.tonsa* juveniles in Zone 2 during the period between August 2000 and June 2002 was equal to 2.343 mg C m^{-3} d^{-1} (Fig. 6.7), with the highest values during the hot season of 2001 – July (19.151 mg C m^{-3} d^{-1}) and June (7.143 mg C m^{-3} d^{-1}).

Copepodites accounted, on average, for almost 75% of the total juvenile production. *A.clausi* mean secondary production rate in zone 2 was residual (Fig. 6.7) and was equal to 0.013 mg C m^{-3} d^{-1}. The missing of the spring/summer peak of 2001 was a consequence of high precipitation levels observed during that period (see Fig. 5.2).

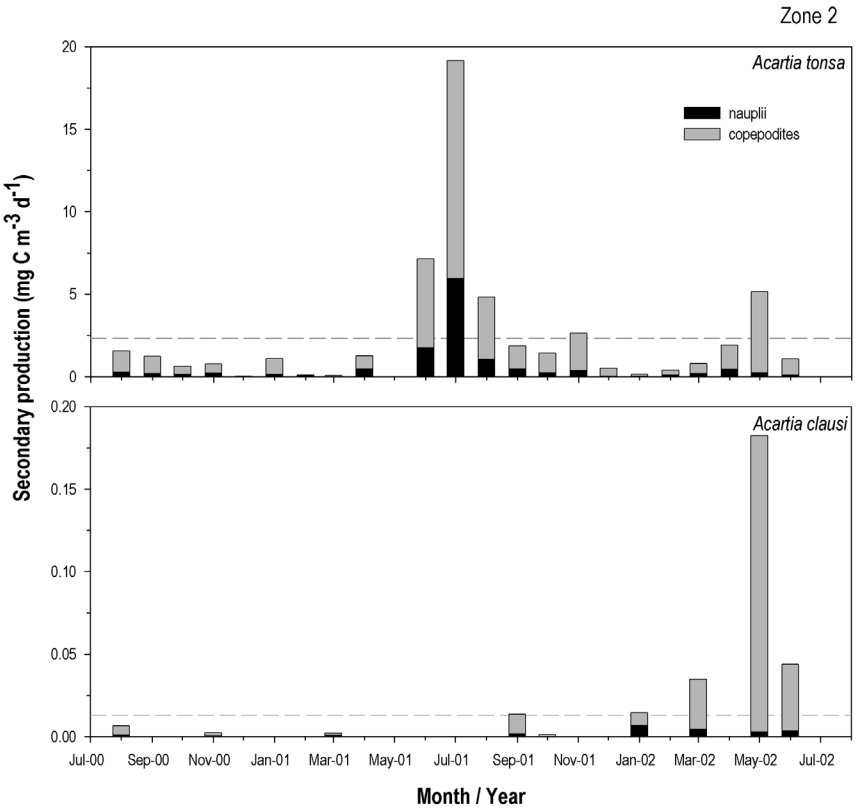

Figure 6.7 Monthly variations in daily secondary production rates (mg C m^{-3} d^{-1}) of *Acartia* populations in Zone 2 of Canal de Mira (Ria de Aveiro – Portugal) from August 2000 to June 2002. The gray dashed line represents mean daily secondary production over the entire study period.

The most upstream region was characterized by the lowest production values of *A.tonsa* juveniles (Fig. 6.8). The mean secondary production rate was 0.150 mg C $m^{-3}d^{-1}$ and was highest in September 2001 (2.845 mg C m^{-3} d^{-1}). The juveniles of *A.clausi* were never observed in this region of Canal de Mira.

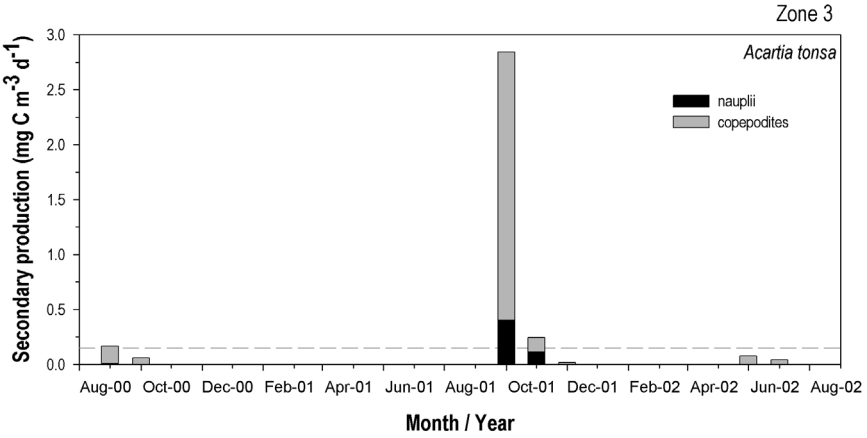

Figure 6.8 Monthly variations in daily secondary production rates (mg C m^{-3} d^{-1}) of *A. tonsa* in Zone 3 of Canal de Mira (Ria de Aveiro – Portugal) from August 2000 to June 2002. The gray dashed line represents mean production over the entire study period.

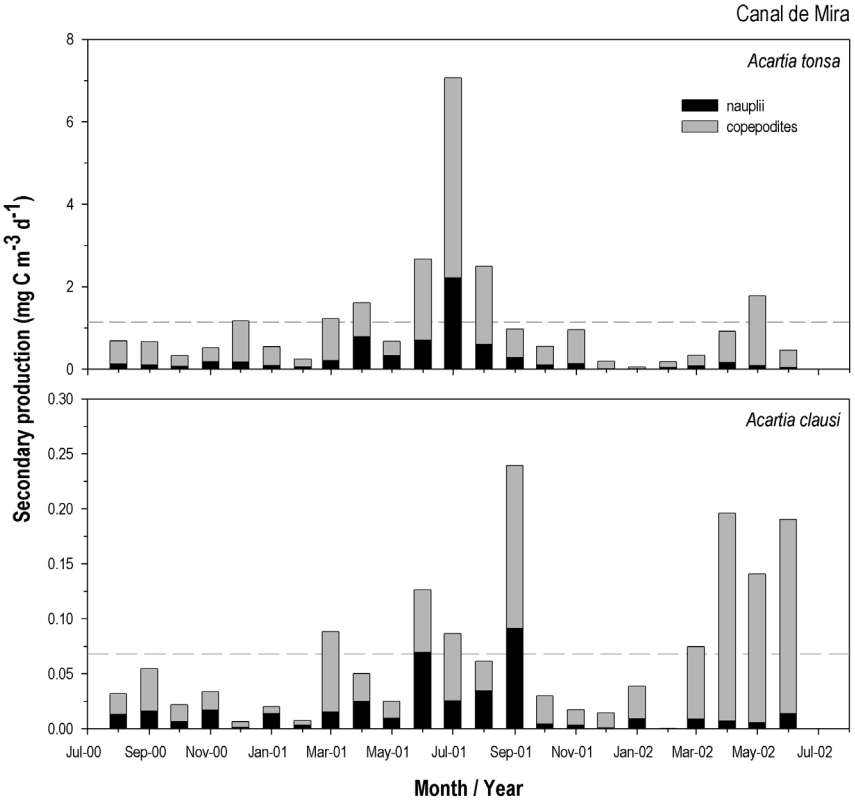

Figure 6.9 Monthly variations in daily secondary production rates (mg C m^{-3} d^{-1}) of *Acartia* populations in Canal de Mira (Ria de Aveiro – Portugal) from August 2000 to June 2002. The gray dashed line represents mean daily secondary production over the entire study period.

The average daily secondary production rate of copepod juvenile stages over the whole Canal de Mira was equal to 1.140 and 0.068 mg C m^{-3} d^{-1} for *A.tonsa* and *A.clausi*, respectively (Table 6.3).

Concerning *A.tonsa*, the highest values were observed during the summer 2001 and ranged from 2.487 (August 2001) to 7.059 mg C m^{-3} d^{-1} (July 2001). The lowest value was observed in January 2002 – 0.048 mg C m^{-3} d^{-1}. For *A.clausi*, daily secondary production was highest in September 2001 (0.239 mg C m^{-3} d^{-1}) and April 2002 (0.196 mg C m^{-3} d^{-1}). Average daily P/B ratios for *A.tonsa*, *A.clausi* and *Acartia* spp. were equal to 0.27, 0.13 and 0.25 d^{-1}, respectively.

Table 6.3 Average daily and annual secondary production rate of *Acartia* populations inhabiting Canal de Mira – Ria de Aveiro (Portugal). (Assuming 365 days per year and a mean depth equal to 1.25 m estimated from figure 2 of Moreira et al. 1993).

	mg C m^{-3} d^{-1}	mg C m^{-2} d^{-1}	mg C m^{-3} yr^{-1}	mg C m^{-2} yr^{-1}
Acartia tonsa	1,140	1,426	416,1	520,54
Acartia clausi	0,068	0,085	24,82	31,05
Acartia spp.	1,208	1,511	440,92	551,59

6.1.5 Discussion

In this study, it was described the spatio-temporal distribution patterns of biomass and juvenile production of *Acartia* populations in a southern European estuary. Secondary production rate, in terms of juvenile production, was obtained by combining *in situ* data on abundance with specific temperature-dependent growth models defined at food saturated conditions. This methodology is assumed to give realistic estimates based on recent studies that concluded that growth rates of juveniles under *in situ* conditions are close to maximum laboratory rates determined at food saturated conditions (Hirst & Bunker 2003).

Seasonal and longitudinal distribution of *Acartia* biomass followed previously defined patterns for estuaries located on temperate regions (e.g. Durbin & Durbin 1981, Kiørboe & Nielsen 1994, Irigoien & Castel 1995). The average biomass of the estuarine copepod community of Ria de Aveiro was estimated by Leandro et al. (2007) as equal to 8.345 mg C m^{-3}. Comparing with the data obtained in the present study for *A.tonsa* – 4.216 mg C m^{-3}, is evident the high importance of this population in the estuarine copepod community. This species alone, represents nearly 51% of the total copepod biomass.

The relative contribution of juvenile forms (nauplii and copepodites) to the respective total copepod biomass accounted to more than 54% in *A.clausi* and 70% in *A.tonsa*. This fact, in conjugation with the highest growth rates of juveniles compared to the adults (Hirst & Bunker 2003), supports the growing evidence that measurements of secondary production, based on fecundity rates and extrapolated to the entire population, certainly underestimate the total copepod production.

In this study, the average daily juvenile secondary production of *Acartia* populations was estimated as equal to 1.208 mg C m^{-3} d^{-1}, with *A.tonsa* representing more than 94%. Nevertheless our approach was based only on juvenile forms, *Acartia* production revealed to represent 32.6 (Huntley & Lopez model) to 41.7% (Hirst & Bunker model) of the total copepod community production of Ria de Aveiro (Leandro et al. 2007). The

daily average P/B ratios obtained for *Acartia* populations inhabitinhg this southern european estuary were whithin the range of published values for *Acartia* species (Durbin & Durbin 1981, Escaravage & Soetaert 1995, Irigoien & Castel 1995) and confirmed that these calanoid copepods populations have a key role on the transfer of matter and energy within the planktonic community. The P/B ratio estimated with the present data, suggests that nearly 25% of the biomass produced daily by *Acartia* populations will be available for higher trophic levels.

Future studies should consider the evaluation of both adult and juvenile production of *Acartia* populations simultaneoulsy in order to understand the relative contribution to the total copepod production. By this way, it will be also possible to validate two different approaches that are frequently used to express secondary production.

6.1.6 References

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Chapter 7

Concluding Remarks

This last chapter synthesizes the main conclusions derived from the studies performed in this thesis, namely: the characterization and description of spatio-temporal patterns of abundance, biomass and production of the estuarine copepod community of Ria de Aveiro; the comparative study about growth and development rates of *Acartia clausi* allopatric populations; the definition of a specific temperature-dependent growth model for nauplii and copepodites of *Acartia tonsa*; the evaluation of environmental forcing on the distribution and abundance of *Acartia* populations at different time scales and the assessment of the relative contribution of *Acartia* spp to the carbon fluxes in the planktonic realm of Ria de Aveiro (Portugal).

Concerning the study of the copepod community of Ria de Aveiro, the use of different mesh sizes showed to affect significantly the estimates of nauplius concentration, with the 64 μm net producing values 13.9 times higher on average than the 125 μm net. The estimates of the concentration of copepodites and adults were not affected by mesh size. The production values calculated for the copepod community of Ria de Aveiro were based on the concentration values measured by the 64 μm net for nauplius and on the average of the two nets for copepodites and adults. If the production were calculated based only on the 125 μm net, the estimate would be 69% of that obtained including the data for nauplii collected with the finer mesh.

This study was the first estimate of biomass and secondary production of the copepoda community for Ria de Aveiro, which represents 63.6 and 62% of the total zooplanktonic abundance and biomass, respectively. Secondary production rates were obtained by the application of two general growth equations, Huntley & Lopez (1992) and Hirst & Bunker (2003), and were within the range of the data previously obtained for temperate estuarine ecosystems. However, some differences between the estimates given by the two methods were noted, reflecting the assumptions incorporated in each model. On the whole, the variation of copepod abundance, biomass and chlorophyll *a* concentration followed those expected for temperate coastal waters (Kiørboe and Nielsen, 1994; Escaravage & Soetaert, 1995; Peitsch, 1995), namely highest values during spring/summer and lowest during winter season.

In order to estimate secondary production two key variables are necessary: biomass and growth rate. Several methodologies are available to estimate growth rates of copepods, being the simplest one the application of temperature dependent growth models (see introduction). However, a general growth model does not consider that different species may respond differently to temperature, leading to erroneous values.

The need for site-specific growth model definition was confirmed by the study concerning the growth and development of *Acartia clausi* populations. Allopatric populations exhibited different development rates when reared at the same temperature. It also indicated the existence of differences in growth rates between populations, particularly when reared at high temperatures, with the north population (acclimated to cold temperatures) growing faster than the south population (warm acclimated).

Additionally, the two populations showed different ontogenetic responses to temperature shifts. The north population had a shorter naupliar phase over all temperatures and increased the growth of copepodites at the highest temperature. This suggests that the two populations have developed slightly different survival strategies to adapt to their main area of occurrence in response to specific environmental characteristics.

Some controversy exists about the extrapolation of growth rates data obtained in laboratorial experiments to *in situ* conditions. Here, it was demonstrated that, at least for estuarine copepods, juvenile developmental stages in the field grow, with some exceptions, at nearly the same rates as the ones defined at non-limiting food conditions (see Fig. 4.7). This evidence supports the assumption that juvenile grow is more limited by ambient water temperature than food conditions.

Although such conclusion is based on few data points for a single species, Hirst & Bunker (2003) also found that juvenile copepods in the field grow at rates close to maximum laboratory determined rates. In contrast, fecundity of *A. tonsa* is frequently

limited by food availability and *in situ* egg production is generally lower than the juvenile growth rates (Peterson et al. 1991).

The use of 3-mode PCA to simultaneously analyse spatial and temporal changes of multispecies assemblages, allowed the identification of three distinct zones along the estuary, based on abundances of the two *Acartia* species. For each zone time-lagged correlations between environmental variables and *Acartia* abundance indicated that different zones were forced by different combinations of variables. Moreover, the study also showed the existence of delayed effects of phytoplanktonic biomass on the abundance of *Acartia*. Given these results it is suggested that the identification of the spatial ambit on which the environmental factors operate along the estuarine gradient is crucial to understand the control of zooplanktonic processes.

Finally, secondary production studies demonstrated the relative high importance that *Acartia spp.* populations, namely *A.tonsa*, assume on the transfer of matter and energy within the estuarine copepod community of Ria de Aveiro (Portugal). It should be noted that the secondary production rates of *Acartia* populations were obtained by the application of site- and species-specific temperature-dependent growth models. This strategy was supported on the previously referred results that indicated the existence of differentially allopatric growth and demonstrated that development and juvenile growth under controlled an *in situ* conditions are similar.

Although adult forms were not included in the estimate of secondary production and some under-estimate are associated with nauplii data since it was obtained by the use of the 125 μm mesh net (see chapter 2 and previously conclusions), *Acartia tonsa* production represents between 32.6 and 41.7% of the total copepod community production of Ria de Aveiro, depending on the method used for the estimate of copepod community production (Huntley & Lopez or Hirst & Bunker model, respectively).

It should also be noted that juvenile biomass over the entire Canal de Mira (Ria de Aveiro – Portugal) always accounted to more than 70% (nauplii 13% and copepodites

57%) and 54% (nauplii 11% and copepodites 43%) of, respectively, *A.tonsa* and *A.clausi* total biomass. These results, and recent evidence showing that *in situ* adult females growth rates are lower than the respective juveniles (Hirst & Sheader 2003), sustain the fact that the measurements of production based on egg production when extrapolated to the entire population certainly underestimate the total production.

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