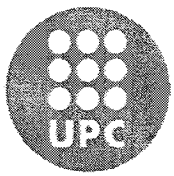



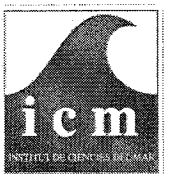


Universitat Politécnica
de Catalunya

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Institut de Ciències
del Mar

# Ecology of the marine cladoceran

# *Penilia avirostris*

Memoria presentada per optar al
grau de Doctor en Ciències del
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Dacha Atienza Ariznavarreta

Vist-i-palu del director y co-director de la tesi

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*El que no sabe y no sabe que no sabe,*
*es un necio, evítalo.*

*El que no sabe y sabe que no sabe,*
*es un discípulo, enséñale.*

*El que sabe y no sabe que sabe,*
*está dormido, despiértalo.*

*El que sabe y sabe que no sabe,*
*es un maestro, síguelo.*

*A mis padres y a Andrés*

*quienes siempre confiaron en mi*

*y me motivaron...*

*...Lo hemos conseguido*

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# SUMMARY

The main objective of this Thesis was to get new insights into the ecology of *Penilia avirostris* in order to characterize and better understand the ecological role of this marine cladoceran on the planktonic food web, the energy flow, and biogeochemical cycles. The hypothesis behind is that in spite of the sporadic and opportunistic appearance of *P. avirostris* populations, its occurrence will have an important impact on the circulation of organic carbon on the planktonic marine food webs. This Thesis covers a wide view of *P. avirostris* including topics on distribution, seasonal pattern, grazing, metabolism, life cycles, and impact on the plankton communities in the water column. *P. avirostris* showed a clear seasonal patter in the NW Mediterranean, being an important component of the mesozooplankton during the stratification period. Specific horizontal and vertical patterns were identified. Higher abundances of *P. avirostris* appeared in the southern region due to the presence of low salinity waters, high chlorophyll concentrations, and higher temperatures. Lower abundances occurred on high salinity waters (>38, oceanic waters) or with low temperatures (<22 °C). *P. avirostris* appeared in the upper 60 m of the water column, being more abundant between 20 and 30 m. Life cycle of this marine cladoceran is characterized by two types of reproduction, parthenogenesis and gametogenesis. Parthenogenesis seems to be the responsible for the explosive growth of *P. avirostris* populations when conditions are favourable. Brood size varied between 1 and 8 embryos female$^{-1}$, and developmental time was estimated around 2 days. Both parameters rendered in higher birth and population growth rates. Shift between parthenogenetic reproduction to gametogenesis was associated to the disappearance of *P. avirostris* from the water column. The results indicate that *P. avirostris* feeds on particles on a wide size range, mostly on nanoplankton (2-20 μm), including larger prey such as dinoflagellates and ciliates. Autotrophic and heterotrophic bacteria were not grazed on, but picoflagellates (<2 μm) were significant contributors to diet, indicating a narrow lower prey size threshold. Although this marine cladoceran is considered to behave like a filter feeder, our results indicate that it can display variable selectivity patterns not depending only on prey size. During oligotrophic conditions daily rations of *Penilia avirostris* ranged between 26% and 157% body carbon d$^{-1}$. Trophic impact of *P. avirostris* accounted, on average, for <10% of the standing stock of each of the microbial groups considered. The results indicate that *P.avirostris* does not excrete phosphorus, but it does ammonia, with the consequent higher recycling of N when it dominates the water column. Finally, gross growth efficiencies (GGE) varied between 16 and 58% which is not different to other zooplankters, such as copepods, and the ecological success of this species is related more with the life cycle and feeding performance than with GGE.

# RESUMEN

Esta tesis tiene como objetivo principal profundizar y ampliar el conocimiento que se tiene de la ecología de *Penilia avirostris,* para poder entender y caracterizar su papel ecológico dentro de las tramas tróficas planctónicas, los flujos de energía y los ciclos biogeoquímicos. La hipótesis de trabajo fue que a pesar del carácter esporádico de esta especie, su presencia en el plancton tendría un impacto importante en la circulación del carbono orgánico y las tramas tróficas marinas. Esta tesis cubre una amplia variedad de los aspectos ecológicos de *P. avirostris*, tales como su distribución tanto especial como horizontal, variación estacional, alimentación, metabolismo, ciclo de vida e impacto sobre las comunidades planctónicas. Las poblaciones de *P. avirostris* en el Mediterráneo NorOccidental presentan una marcada estacionalidad, llegando a ser una parte importante del mesozooplankton durante los períodos de estratificación térmica. Se pudieron identificar patrones claros de distribución horizontal y vertical. Las mayores abundancias de *P. avirostris* se registraron en la zona sur debido a la presencia de aguas con bajas salinidades, altas concentraciones de clorofila y altas temperaturas. Las menores abundancias se encontraron cuando había aguas oceánicas (salinidades >38) o bajas temperaturas (<22 °C). En cuanto a la distribución vertical, este cladócero marino se encontró en los primeros 60 m de la columna de agua, siendo más abundante entre los 20 y 30 m. El ciclo de vida de *P. avirostris* está caracterizado por dos tipos de reproducción, la partenogénesis y la gametogénesis. Cuando las condiciones son favorables, las poblaciones de esta especie presentan unos crecimientos explosivos que parecen estar relacionados con la partenogénesis. El número de embriones varía entre 1 y 8, mientras que su desarrollo tarda alrededor de 2 días. La combinación de ambos se traduce en altas tasas de nacimientos y de crecimiento poblacional. La rápida desaparición de las poblaciones de *P. avirostris* está relacionada con el cambio en el tipo de reproducción, de partenogénesis a gametogenesis. Los resultados evidencian que el rango de tamaño de las partículas ingeridas por *P. avirostris* es muy amplio, siendo en su mayoría nanoplancton (2-20 μm) e incluyendo presas de mayor tamaño como ciliados y dinoflagelados, mientras que las bacterias autotróficas y heterotróficas no son ingeridas, aún cuando los picoflagelados (<2 μm) son parte importante de su dieta. Estos resultados sugieren que el umbral de las presas de pequeño tamaño es muy estrecho. Aunque *P. avirostris* se comporta como un organismo filtrador, esta tesis muestra evidencias que indican que puede haber selección de presas, y no necesariamente en base a su tamaño. El consumo de carbono diario por parte de *P. avirostris* varió entre 26 y 157% en las aguas oligotróficas y su impacto trófico sobre las comunidades planctónicas fue <10%. Los resultados evidencian que *P. avirostris* no excreta fosfato, mientras que si excreta amonio, por lo que su presencia contribuye a un mayor reciclaje de N frente al P. Por ultimo, los valores de eficiencias de crecimiento para *P. avirostris* variaron entre 6 y 58%, no siendo suficientemente diferentes de aquellas publicadas para otros grupos del zooplancton. Por lo tanto, el éxito ecológico de esta especie en condiciones oligotróficas parece estar relacionado con su ciclo de vida, así como con su funcionamiento alimenticio.

# General Introduction

# GENERAL INTRODUCTION

## Cladocerans in aquatic environments

Cladocerans are likely the most well studied group of zooplankton in freshwaters, where they are usually the dominant zooplankter and are represented by more that 600 species (Schram 1986). They can be found from cold to the tropical systems, from sea level to alpine ponds, inhabiting lakes, ponds, slow-moving streams, and rivers. Cladocerans are important for aquatic ecosystem functioning because they are the primary herbivores in lakes (Edmonson & Litt 1982), and they are also valuable diet items for fishes (Dodson & Hanazato 1995). In agreement with such relevance, the taxonomy, ecology and biology of freshwater cladocerans, especially from *Daphnia*, have been extensively studied in many diverse aspects and very deep insight has been acknowledged. Life cycles: Lampert & Sommer 1997, Mauchline 1998; morphology: Brendelberger 1991; cyclomorphosis: Jacobs 1987 and references therein; migratory behaviour: Ringelberg *et al.* 1991; population dynamics: Threlekeld 1987; grazing behaviour: Lampert 1987; impact on nutrient recycling: Sterner 1990, Hessen & Lyche 1991; and genetics: Hebert 1980, Hebert & Cristescu 2002.

In contrast to the freshwater environment, cladocerans have not been very successful invading marine systems (Aladin & Potts 1997). Actually, only 8 species can be considered truly marine (Table 1), and their distribution is highly variable and restricted to coastal and neritic areas, and semi-enclosed ecosystems. All species are characteristic of stratified waters of temperate and warm ecosystems, and when conditions are optimum, they can dominate zooplankton (Kim et al. 1989). Their very seasonal and ephemeral character has hindered their study, and actually very few works have addressed their ecological role in the marine environment (Wong et al. 1992, Lipej et al. 1997, Broglio et al. 2004). This Thesis will focus on the only genus of marine cladocerans (*Penilia*) and its only representative (*P. avirostris*), which alike *Daphnia* in freshwaters, is filter feeding and dominates the summer zooplankton communities in neritic waters of many seas. Below, I will review the present knowledge on the biology and ecology of this species, and the main deficiencies in knowledge, which will be addressed in the different chapters of the Thesis, will be highlighted.

Table 1. Cladocerans prevalent in neritic and oceanic systems.

**Branchiopoda**

| | |
|---|---|
| Order Onychopoda Sars, 1865<br>Family Podonidae Mordukhai-Boltovski, 1968 | *Evadne nordmanni* Lovén, 1836<br>*Evadne spinifera* P.E. Müller, 1867<br>*Podon intermedius* Lilljeborg, 1853<br>*Podon leuckartii* (G.O. Sars, 1862)<br>*Pseudoevadne tergestina* Claus, 1877<br>*Pleopis polyphemoides* (Leuckart, 1859)<br>*Pleopis schmackeri* Poppe, 1889 |
| Order Ctenopoda Sars, 1865<br>(replace superfamily Sidoidea Brooks, 1959)<br>Family Sididae Baird, 1850 | *Penilia avirostris* Dana, 1852 |

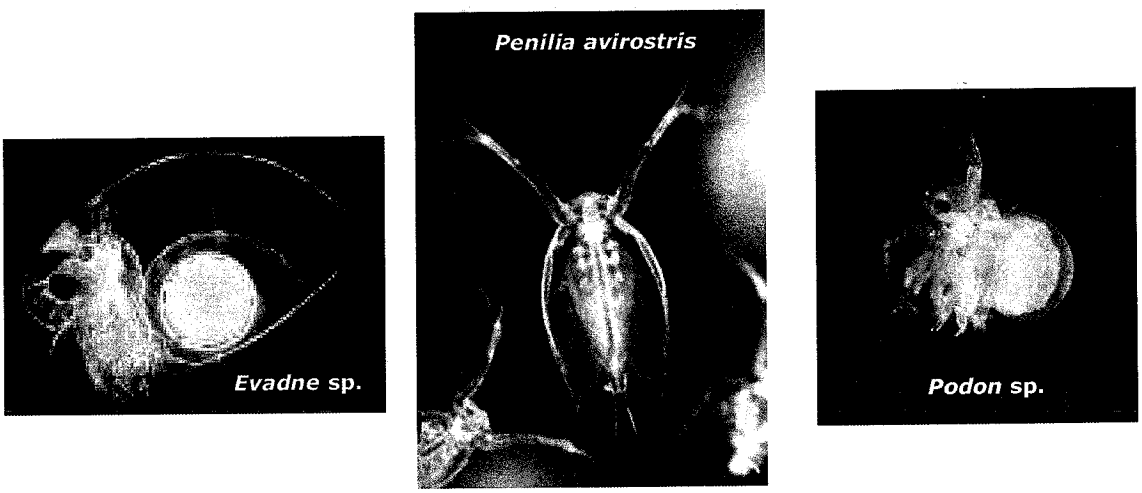



Figure 1. Marine cladocerans.

## Distribution patterns

Marine cladocerans are widely distributed and seasonally abundant in continental shelf waters, estuaries, and at times, in the open ocean. Among all marine cladocerans, *Penilia avirostris* Dana has received special attention because is the only species exhibiting a circumglobal distribution in coastal waters. The worldwide distribution of *P. avirostris* was reviewed by Della Croce (1964, 1974), who found the species in a sort of environments ranging from eutrophic, near shore or estuarine

waters, to oligotrophic coastal waters (Figure 2). More recently, further studies have supported the view that this species is an important component of the zooplankton community of many tropical, subtropical and temperate waters (between 30% and 92% of total mesozooplankton abundance, Grahame 1976, Clarke & Roff 1990, Checkley et al. 1992, Tang et al. 1995, Marazzo & Valentin 2003a, Rose et al. 2004, Eskinazi-Sant'Anna & Björnberg 2006), being found in the Atlantic (Paffenhöfer 1983b, Marazzo & Valentin 2001, Vicent et al. 2002), the Mediterranean (Alcaraz 1977, 1981, Siokou-Frangou 1996, Lipej et al. 1997, Calbet et al. 2001, Fernández de Puelles et al. 2003, Siokou-Frangou et al. 2004, Umani et al. 2005), the Pacific (Kim & Onbé 1995, Onbé & Ikeda 1995, Tang et al. 1995) and the Indian Ocean (Della Croce & Venupogal 1973, Santhakumari 1991). It is even evident that, lately, *P. avirostris* is extending its distribution to higher latitudes (e.g. Johns 2005, Johns et al. 2005).

*Penilia avirostris* has been found in environments ranging in temperature between 8 to 31 °C, although typically their populations are associated to warm waters. Regarding salinity, they can live from near freshwater up to 49 (Della Croce 1964, Onbé 1985, Turner et al. 1988, Kim et al. 1994). Within these ranges of salinity and temperature no clear patterns of abundance can be depicted. For instance, in the western Mediterranean, *P. avirostris* (Thiriot, 1972-1973) is abundant at salinities <35 and temperatures between 14-21 °C that are lower than those occurring at periods of high abundance in both Chesapeake Bay (United States: 18-24 °C; Paffenhöfer 1983), and Tolo Harbour (Japan: 24-28 °C, salinity between 30-35; Tang et al. 1995). On the other side, Komazaw & Yoshinari 2002 showed that this marine cladoceran lived in Onagawa Bay (Japan) at temperature from 10 to 20 °C and salinities ranging between 19 and 33.

Another feature of zooplankton that has important ecological impact is the vertical distribution on the water column and of the possibility of vertical migration. In the water column, the distribution of marine cladocerans, and specifically *P. avirostris*, is restricted to upper waters, typically above the thermocline (Bainbridge 1958, Thiriot 1968, Onbé 1974, Saito & Hattori 2000). Paffenhöfer (1983b) showed that *P. avirostris* was more abundant in the thermocline, Checkley and collaborators (1992) showed that this specie was mainly distributed between 20-30 meters depth, which agrees with findings by Bird (1983), Falavigna da Rocha (1983), Paffenhöfer et al. (1984), and Mullin & Onbé (1992). However, Onbé (1977) and Kim et al. (1994) reported that *P. avirostris* in the Inland Sea of Japan and Tolo Harbour in China (respectively) appeared within the upper 10 m of water column. Some of these studies described the vertical distribution of this marine cladoceran as a function of temperature (Paffenhöfer 1983b), food concentrations (Bird 1983, Paffenhöfer 1983b, Paffenhöfer et al. 1984), and reproduction (Mullin & Onbé 1992), but to date there is no agreement on the main factors controlling the distribution of *P. avirostris* on the vertical scale.

As for other zooplankters, vertical migrations of low amplitude are reported to be conduct by marine cladocerans (Kim et al. 1994). The information about *P. avirostris* migratory activity is contradictory, Checkley et al. (1992), Mullin & Onbé (1992) and Onbé & Ikeda (1995) reported that this marine cladoceran was distributed uniformly in the upper 30 m without differences between day and night. However, Kim et al. (1994) reported that *P. avirostris* perform low amplitude migrations, being more abundant in the surface waters during day. This behaviour is known as reverse

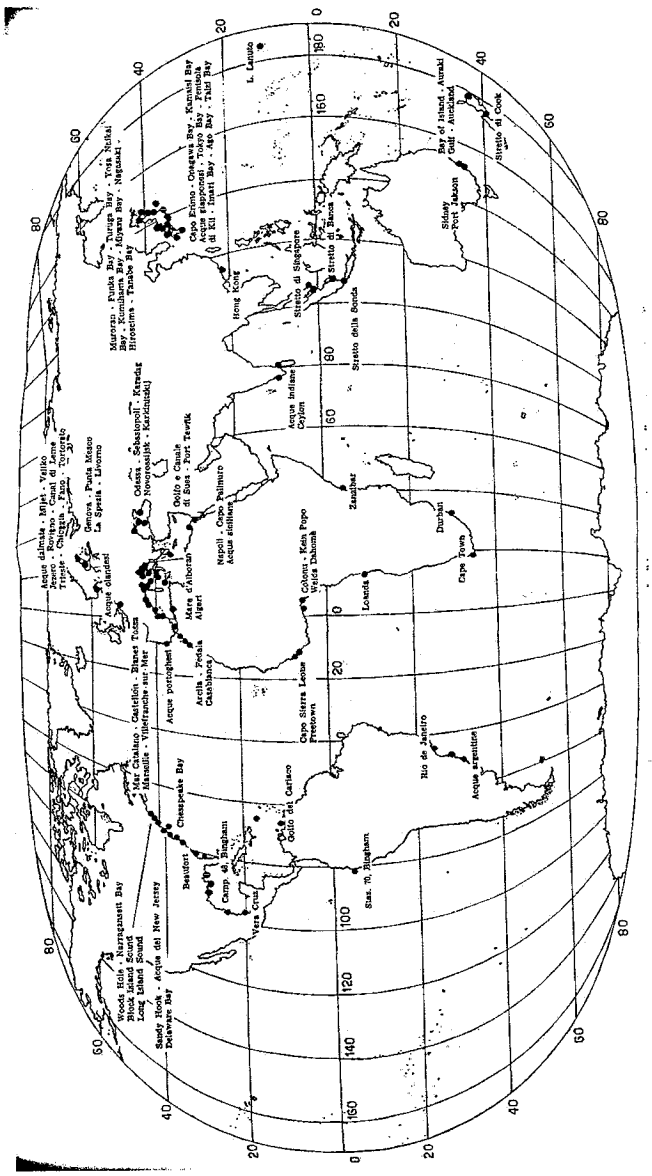


Figure 2. Geographical distribution of *Penilia avirostris* Dana (modified from Della Croce 1964).

vertical migration, and was previously reported for some podonids (Onbé 1974, Onbé 1977, Checkley et al. 1992, Mullin & Onbé 1992, Onbé & Ikeda 1995, Saito & Hattori 2000). Reverse migration have been described for small zooplankton species that are vulnerable to invertebrate predation (e.g. chaetognaths and euphausiids) (Lampert 1993, Ohman et al. 1983).

In summary, the evidence for daily vertical migration, and the factors controlling the distribution of *Penilia avirostris* in the horizontal and vertical scales are still controversial. Therefore, in this thesis (Chapter I) will be address the horizontal and vertical distribution of this species in the Catalan Sea (NW Mediterranean) in an attempt to elucidating the influence of some environmental cues on these variables.

## POPULATION DYNAMICS AND LIFE CYCLES

In temperate regions *Penilia avirostris* occurs seasonally with special relevance during summertime (Della Croce 1964, Alcaraz 1977, 1981, Paffenhöfer et al. 1984, Onbé & Ikeda 1985, Onbé et al. 1996, Lipej et al. 1997, Calbet et al. 2001, Fernández de Puelles et al. 2003). The seasonal occurrence of *P. avirostris* in the NW Mediterranean has been studied in different locations and years. In general, it seems that the population starts to develop by the end of June, or early July (Alcaraz 1970, Calbet et al. 2001, Fernández de Puelles et al. 2003). By the end of July, early August a dense population is usually achieved. The population starts to decline after September. Other authors found the same trend in other areas of the Mediterranean (Siokou-Frangou 1996, Lipej et al. 1997, Ribera D'Alcalà et al. 2004) and other temperate regions (Alcaraz 1981, Onbé & Ikeda 1995).

At first sight it seems evident that temperature drives the seasonal patterns of *Penilia avirostris* (Gieskes 1971a, Onbé & Ikeda 1995). However, other factors, such as food abundance and predation pressure (Marazzo & Valentin 2003b) could be also relevant. Actually, understanding population dynamics involves not only the challenges of describing the pattern of changes in population numbers, but also, identifying the possible mechanisms that cause the observed pattern. The rapid appearance and dominance of *P. avirostris* populations has been related to higher population's growth rates and birth rates together with its life cycle (Egloff et al. 1997).

Cladocerans have life cycles similar to those of Rotifera. Its life cycle is characterized by an alternation between gamogenesis and parthenogenesis. The typical life history of cladocerans begins with the hatching of a parthenogenetic female from a resting egg. This female produces a series of subitaneous eggs that develop without fertilization and produce further parthenogenetic females. When a considerable population has been built up and certain ecological conditions, the

nature of which has given rise to much discussion, have apparently fulfilled, male-producing females may appear in the population (Smith 1963, Sanders & Wickham 1993, Boersma & Vilverberg 1996). The males fertilize some of the parthenogenetic females, which then produce large resting eggs that undergo a prolonged diapause (Figure 3). Populations of cladocerans usually are monocyclic, which means that this alternation of reproductive modes occurs once during the annual cycle (Hutchinson 1967).

Parthenogenetic reproduction allows the rapid expansion of cladoceran populations during times of high food availability and low population density (Lynch & Gabriel 1983), under such conditions cladocerans can build up large populations in a short period of time (Lampert 1978, Sommer et al. 1986). Together with parthenogenesis, high population growth rates are responsible for the rapid expansion of cladoceran populations (Egloff et al. 1997).

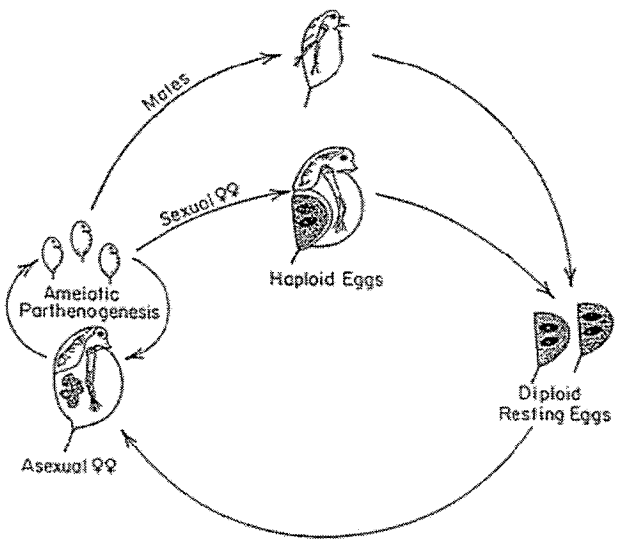



Figure 3. Reproductive cycle of Daphnids (Figure from http://daphnia.cgb.indiana.edu/).

Birth and growth rates are achieved by a combination of brood size and developmental times, parameters that were not extensively reviewed in the literature. Regarding brood size, it is known that *Penilia avirostris* can carry between 1 and 13 embryos per female (1-5 embryos female$^{-1}$, Della Croce 1964; mean 4.4 embryos female$^{-1}$, Tang et el. 1995; 1-13 embryos female$^{-1}$, Kim et al. 1994; 4-8 embryos female$^{-1}$, Valentin & Marazzo 2003a). Developmental time, on the other hand, has been less studied, and only three direct estimations are available, all reported from 2 to 3 days the period for complete embryo development (Paffenhöfer & Orcutt 1986, Mullin & Onbé 1992, Valentin & Marazzo 2004).

In daphnids and *Penilia avirostris* populations, neonates are reproductively immature and molt typically three times before the first brood of eggs is produced (Pavlova 1959). Subsequently, as each new generation leaves the brood chamber, the eggs of the next generation are deposited in the vacant chamber. As a consequence of brood size and the short generation time, the early phases of population growth

could be spectacular. Concerning population growth rates, detailed information is available in relation to *Podon* and *Evadne*, with some extensive studies done by Gieskes (1970), Platt (1977), Poggensee & Lenz (1981), Platt & Yakamura (1986) and Fofonoff (1994). The only published values that we are aware of are 0.42 $d^{-1}$ (Mullin & Onbé 1992), 1.49 $d^{-1}$ (Marazzo & Valentin 2003b) and 2.6 $d^{-1}$ (Valentin & Marazzo 2003). Finally, laboratory research has revealed that food and temperature are important influences for the growth rate on daphnids, with food generally limiting growth and brood size, whereas temperature controls frequency of molting, egg developmental time, and physiological lifespan (e.g. Slobodkin 1954, Herbet 1978, Coull & Bell 1983, DeMott 1983).

As I said before, when certain ecological conditions have apparently fulfiller, males start to appear in the populations, and this event characterizes the beginning of the decrease and disappearance of the population. Cladocerans are known to have environmental sex determination, which means that sex is determined during the embryonic development in response to particular environmental conditions (Innes 1997). After the population maxima, gamogenetic individuals usually appear and cladocerans start reproducing sexually. In freshwater cladoceran it is known that switch to sexual reproduction is related to a deterioration of the environment due to increased population density and reduced food (Innes & Dunbrack 1993). Other environmental factors such as changes in temperature and photoperiod can also influence the switch from asexual to sexual reproduction (Stross 1966, 1987, Carvalho & Hughes 1983, Fitzsimmons & Innes 2006). Stross (1965) suggested that at least two stimulus are necessary to induce the production of males and gametogenic reproduction. During sexual reproduction, females produce broods of diploid males parthenogenetically and sexual females produce haploid resting eggs that are fertilized by males during mating (Hebert 1978). The sexually produced resting eggs are protected and are resistant to freezing and desiccation. The factors triggering the transition from parthenogenesis to gamogenesis in *Penilia avirostris* remain unclear (Onbé 1974, Fofonoff 1994). Description of developmental stages, hatching of resting eggs and other parameters of sexual reproduction are still unknown for *P. avirostris*. Hatching of resting eggs in daphnids is induced by environmental cues (Schwartz & Herbert 1987).

When the resting eggs hatch, the population density is usually low. Males and sexual females produced at this time would have a low probability of encountering each other, resulting in a low mating success. However, several generations of all-female parthenogenetic reproduction greatly increases population density, increasing the encounter rate and probability of mating between males and sexual females. Thus, it is advantageous for parthenogenetic females to be produced when density is low and males and sexual females when density is high (Gerritsen 1980).

In this thesis will be estimate the different population dynamics parameters from natural communities of the NW Mediterranean, and will be try to discern the

effects that different environmental and biological factors may have on them (Chapters II and V). Also, the life history treats of *Penilia avirostris* and their relationship to the ecological success of this marine cladoceran will be discussed (Chapter V).

## FEEDING BEHAVIOR AND TROPHIC ROLE IN NATURAL ECOSYSTEMS

*Penilia avirostris* is a filter-feeder, being functionally similar to the freshwater filter-feeding cladocerans. Gore (1980) conducted one of the most exhaustive studies on the feeding of *P. avirostris,* reporting that this cladoceran can ingest particles (plastic beads) between 1 and 20 μm. Turner and collaborators (1988) described in detail the endopods of the thoracic limbs, which present minute, finely arranged, setae capable to retain particles as small as 2 μm. Accordingly, this species was capable of ingesting heterotrophic nanoflagellates (2-5 μm) and diatoms (4-12 μm). Katechakis et al. (2004), and Katechakis & Stibor (2004) on a mesocosm field study found that this species fed upon prey between 3 and 30 μm, which include nanoflagellates, diatoms and dinoflagellates. Also, Kim et al. (1989, 1994), and Broglio et al. (2004) found similar results. Unfortunately, a great part of these studies either reported ingestion rates using indiscriminative techniques, such as gut fluorescence, or focused on some specific prey, not properly contemplating the whole prey spectra *P. avirostris* may experience.

Regarding the lower size prey range *Penilia avirostris* is able to ingest, some studies (Pavlova 1967, Paffenhöfer & Orcutt 1986, Lipej et al. 1997) suggested that bacteria and small flagellates could be an important source of carbon for this cladoceran. This does not seem strange since Daphnids are important consumers of natural bacteria and small flagellates (Jürgens 1994). However, other studies found *P. avirostris* was not able to efficiently consume pico-sized organisms, such as heterotrophic bacteria (Turner et al. 1988, Katechakis et al. 2004, Katechakis & Stibor 2004). Until now, this controversy remains unsolved. In this thesis, will be explore the ingestion of bacterio- and picoplankton by this marine cladoceran with the purpose of finally ending with this controversy. Also, identifying the food prey of *P. avirostris* is necessary to fully understand the ecological role of this species on its environment.

Being a filter feeder, *Penilia avirostris* is supposed to be unable to actively select their food particles, besides the obvious rejection of those items not entering into the proper size-range as discussed above. However, factors other than size are basic to understand the mechanisms of selective feeding, such as presence or absence of sheath, shape, taste, and assimilability. Also, changes in surface properties, such as hydrophobicity or the electrostatic charge of the cell membranes of both prey and cladoceran phyllopods, have been considered plausible explanations for such changes in selectivity (Lampert 1987, Vanderploeg 1994). Vanderploeg (1994) reviewed such

particle-selection mechanisms in freshwater cladocerans. Very large particles (e.g. filamentous algae) can be rejected by the abdominal claw or just by decreasing the carapace gap. Particles can be rejected even when contained in boluses. Further evidence is given by Lampert & Brendelberger (1996), who reported that *Daphnia* can adjust the area of the filter screens and the appendage beat rate as a function of food concentration. For this reason selectivity in *P. avirostris* was only addressed in relation to food size, and Katechakis et al. 2004 and Katechakis & Stibor (2004) showed that this marine cladoceran had high grazing coefficients for intermediate sizes (15–70 μm, cell dimension). However, selectivity patterns based on different microbial groups were never addressed before for *P. avirostris*. In this thesis will be discussed if this marine cladoceran is able to select food particles, not only in relation to its size, but also in relation to the microbial group (Chapter III).

Cladocerans, and especially daphnids can efficiently control phytoplankton populations (Lampert 1978, Sommer et al. 1986, Lampert et al. 1996), and could be the main responsible for the mortality of protozoans and rotifers in lakes (Pace & Vaqué 1994) and even impact bacterial communities (e.g. Jürgens 1994, Jürgens et al. 1994, Degans et al. 2002), whereas very little information on the impact of marine cladocerans on lower trophic levels is available. *Penilia avirostris* is able to exert its impact on the nano- and microplanktonic communities, and therefore to have a potentially important role, also, on the microbial food webs (Turner et al. 1988, Lipej et al. 1997, Katechakis & Stibor 2004). However, estimates on the impact of *P. avirostris* on the natural communities of its prey are limited. Wong et al. (1992) and Broglio et al. (2004) estimated that this marine cladoceran removed less than 1% of the standing stock of phytoplankton (estimate as chlorophyll concentration), whereas Lipej and collaborators (1997) showed that this impact on phytoplankton stocks was slightly higher, although always <5%. Finally, Broglio et al. (2004) found that the impact on the standing stock of ciliates was <5%. New insights into the natural diet of *P. avirostris* and its implications for the structure and functioning of the planktonic food web will be search in this Thesis (Chapters III and IV).

## CONTRIBUTION TO NUTRIENT CYCLING

Zooplankton needs certain resources and essential nutrients for metabolism and growth. Some of these nutrients are carbon (C), nitrogen (N), and phosphorus (P), the elements most relevant for stoichiometric analyses of aquatic systems and determination of elemental limitation. Cladocerans induce changes on elemental concentrations of nitrogen, phosphorus and carbon by the release of inorganic and organic compounds due to metabolic processes. Numerous investigations studied the ecological stoichiometry in zooplankton, mainly regarding nutrient limitations (e.g. Sterner & Hessen 1994, Gismervik 1997). According to Redfield a ratio of C:N:P of 106:16:1 is believed to allow for phytoplankton growth not limited in nitrogen or phosphorus (Sterner & Elser 2002).

Cladocerans are reported to be relatively rich in phosphorus (P) compared to other organisms (Gismervik 1997), and are able to maintain their mineral composition, e.g. C:P ratio, even if the stoichiometry of their food changes, an effect known as homeostasis (Sterner 1990). Comparatively, copepods are rich in nitrogen (N). *Daphnia* retain phosphorus in their biomass while they preferentially recycle nitrogen (Andersen & Hessen 1991, Hessen & Lyche 1991). Thus, *a priory* cladocerans are able to alter the relative availability P or N for phytoplankton by excretion of nitrogen rich and phosphorus depleted material (Elser & Foster 1998, Touratier et al. 2001). As a consequence of P limited food, growth of cladocerans is reported to be restricted (Elser & Hassett 1994), as cladocerans need phosphorus not only for skeletal tissue, ATP and phospholipids, but also for RNA and DNA production. Elser et al. (2000) stated that *Daphnia magna* face phosphorus deficiency when the C:P ratio of their food exceeds 300, because this leads to a reduced gross growth efficiency. Thus, cladocerans can also have an impact on nutrient stoichiometry of sediment particles (often consisting mainly of faecal pellets), the dissolved fraction and food particles.

In this thesis (Chapter IV) will be assess the effect of *Penilia avirostris* on the recycling of nitrogen and phosphorus, and its possible impact on the natural communities, especially in the Mediterranean Sea.

## AIMS

The main aim of this Thesis is to get new insight into the ecology of *Penilia avirostris* in order to characterize and better understand the ecological role of the marine cladoceran on the planktonic food web, the energy flow, and biogeochemical cycles. The hypothesis behind this Thesis is that in spite of the sporadic and opportunistic appearance of *P. avirostris* populations, its occurrence will have an important impact on the circulation of organic carbon on the planktonic marine food webs.

No complex questions can be placed when we lack the basic knowledge. For this reason, this Thesis covers a wide view of *Penilia avirostris* ecology, including topics on distribution, seasonal pattern, grazing, metabolism, life cycles, and impact on the plankton communities in the water column. This thesis has discussed 5 main questions, which have been addressed through field studies and laboratory experimentation:

1. How environmental factors affect the distribution and dynamics of *Penilia avirostris*?

2. How life cycle characteristics could explain the population dynamics of *Penilia avirostris*?

3. What is the feeding performance and trophic impact of *Penilia avirostris* on natural communities?

4. Does *Penilia avirostris* contributes significantly to nutrient recycling?

5. What are the main reasons for the ecological success of *Penilia avirostris* on summertime natural communities?

## THESIS OUTLINE

**Chapter I** deals with the horizontal and vertical distribution of *Penilia avirostris* in the Catalan Sea (NW Mediterranean). The abundance of this marine cladoceran was recorded in an extensively and detailed survey encompassing four cruises along the Catalan shelf during the seasonal occurrence of *P. avirostris*, together with its vertical distribution and migratory patterns. The distribution patterns of *P. avirostris* in the area will be studied in relation to different environmental variables in order to determine the main factors involved in their distribution.

**Chapter II** describes the seasonal variation, and population dynamics of *Penilia avirostris* in the coastal Catalan Sea (NW Mediterranean), as a model to understand the environmental and biological factors that could affect the development of natural

populations. In this chapter will be discussed how certain aspects of the life cycles (studied here and in Chapter V), together with some physical factors, are responsible for the explosive growth and sudden disappearance of this species from its habitat.

The feeding ecology of *Penilia avirostris* under natural conditions is studied in **Chapter III**. This chapter provides a detailed description of the grazing behaviour, the dietary composition, and the prey selectivity patterns exhibited by this cladoceran species.

**Chapter IV** deals with the trophic impact by *Penilia avirostris* upon the different components of the microbial food web, and discusses the biogeochemical role of this marine cladoceran in the planktonic community. In addition, the possible niche partition between this marine cladoceran and the other summertime dominant zooplankter, the copepod *Oithona nana* is discussed.

**Chapter V** analyses the potential factors involved in the ecological success of *Penilia avirostris* during their seasonal appearance, such as life history parameters, growth and feeding behaviour. This chapter, result of the experimentation in West Atlantic waters will also serve to corroborate and generalize the hypotheses extracted from Chapter III.

Each chapter has its own Introduction, Methodology, Results, and Discussion section; therefore I do not include a general discussion due to the detailed ones that are presented in each chapter. The Thesis ends with the **Main Conclusions** on the ecology of *Penilia avirostris* in the planktonic ecosystems.

# CHAPTER I. HORIZONTAL AND VERTICAL DISTRIBUTION OF *PENILIA AVIROSTRIS* AND *EVADNE* SPP. ON THE NORTHWESTERN MEDITERRANEAN IN RELATION TO HYDROGRAPHIC CONDITIONS

Atienza, D., Saiz, E., Calbet, A., and Sabatés, A.
In preparation

# CHAPTER I. HORIZONTAL AND VERTICAL DISTRIBUTION OF *PENILIA AVIROSTRIS* AND *EVADNE* SPP. IN THE CATALAN SEA (NW MEDITERRANEAN) IN RELATION TO HYDROGRAPHIC CONDITIONS

## INTRODUCTION

The distribution patterns of zooplankton communities in shelfwaters present a high level of spatial and temporal heterogeneity according with the combined effects of a number of physical, chemical and biological factors reflected in mesoscale processes and structures (Sabatés 1990). The Catalan sea (NW Mediterranean) is characterized by the presence of a permanent shelfbreak density front defined by salinity differences between oceanic waters (salinities >38.0) and shelf waters. The shelf is subjected to high spatial and temporal variability due to inputs of continental runoff, mainly from the Rhône River in the northern Gulf of Lions (Masó & Duarte 1989, Masó & Tintoré 1991) and the Ebre River in the south (Salat et al. 2002), which interact with topography and local conditions. The hydrography of this region shows a marked seasonal variability, generally associated with the thermal cycle. Two different periods can be discerned based on the physical characteristics: a mixed period (from October to April) when the water column is vertically homogeneous; and a stratified period (from May to September) when a strong thermocline develops over the whole area. The stratification brings a gradual depletion of nutrients in the surface layer by restricting vertical water movements that could help to replenish the nutrient levels. Consequently, phytoplankton populations are mainly restricted to beneath the thermocline, producing what is known as the deep chlorophyll maximum (Estrada 1985, Estrada & Margalef 1988, Estrada & Salat 1989, Salat 1996). Interaction of light availability and nutrient supply appears to play a key role in the formation and location of a Deep Chlorophyll Maximum (DCM), while chlorophyll patchiness within the DCM may be due to variability in physical forcing.

The present study aims at determining the distribution patterns of two marine cladoceran species, *Penilia avirostris* and *Evadne* spp. (*E. spinifera, E nordmanni* and *Pseudoevadne tergestina*), in the Catalan Sea during the stratification period in relation to environmental factors and mesoscale structures. Contrarily to freshwaters, where cladocerans are the dominant mesozooplanktonic group, in the marine environment cladocerans have been viewed as an unsuccessful group (there are only 8 truly marine species; Egloff et al. 1997). However, in temperate and tropical ecosystems, marine cladocerans can be the dominant zooplankters of summer communities in coastal waters (Turner et al. 1988, Kim et al. 1989). The marine cladocerans *P. avirostris* and *Evadne* spp. are important components of the zooplankton community of many

temperate, tropical and subtropical waters (Della Croce 1964, Tang et al. 1995, Calbet et al. 2001, Marazzo & Valentin 2003, Marrari et al. 2004, Rose et al. 2004), and especially of the NW Mediterranean (Alcaraz 1977, 1981, Siokou-Frangou 1996, Lipej et al. 1997, Calbet et al. 2001, Fernández de Puelles et al. 2003, Siokou-Frangou et al. 2004, Umani et al. 2005). However, the horizontal and vertical distribution of cladoceran assemblages in the NW Mediterranean and the factors regulating them remain largely unexplored, even though salinity and temperature have been suggested to influence their abundance and distribution (Paffenhöfer 1983, Tang et al. 1995, Egloff et al. 1997).

## METHODOLOGY

### Horizontal distribution

The present study was conducted in the Catalan Sea (NW Mediterranean) (Figure 1.1). Four oceanographic surveys were carried out in summer 2003 and 2004: 18-26 July 2003 (CACO1), 10-19 September 2003 (CACO2), 23 June-01 July 2004 (CACO3), and 22-30 July 2004 (CACO4) on board the R/V *García del Cid.* Sampling stations were placed ca. every 5 miles on transects perpendicular to the coast, covering the entire continental shelf. The number of stations on each transect depended on the shelf width (Figure 1.1). Vertical profiles of temperature, salinity, and fluorescence were obtained using a Neil Brown Mark III CTD equipped with a Sea-Tech fluorometer. Fluorescence profiles were transformed to chlorophyll *a* (Chl *a*) through independent calibration equations determined for each cruise.

Microzooplankton samples were taken, after the CTD cast, by vertical hauls, from 100 m (or near bottom when shallower) to surface, using a 40-cm mouth diameter, 53-μm mesh size ring net towed at a speed of 20 m $min^{-1}$. The water volume filtered by the net was measured with a flowmeter. The contents of the cod-end were preserved with borax-buffered formalin (5% final solution). Zooplankton abundance and species composition of cladocerans were estimated by counting and identifying under stereomicroscope at least 300 cladocerans (>4% total sample).

Cladoceran densities were visualized in plots generated using Surfer version 8 (Golden Software), with interpolation by linear kriging. We used an ordination technique to determine to what extent distribution of the marine cladoceran could be described using environmental variables (Clarke & Warwick 2001). Principal Component Analysis (PCA) was run to identify the most relevant environmental factors. The environmental variables used were: temperature and salinity at 5 m, and 20 m, and integrated chlorophyll *a* between 5 m, and 20 m. Depths for the analyses were selected taking into consideration the vertical distribution of cladocerans in the water column (see below). After extraction of PCs factors, Spearman's correlations were conducted between the PCs and the abundance of *Penilia avirostris* and *Evadne*

spp. in order to assess the effect of selected environmental variables on the cladoceran distributions. Statistics were carried out using STATISTICA version 6.0.

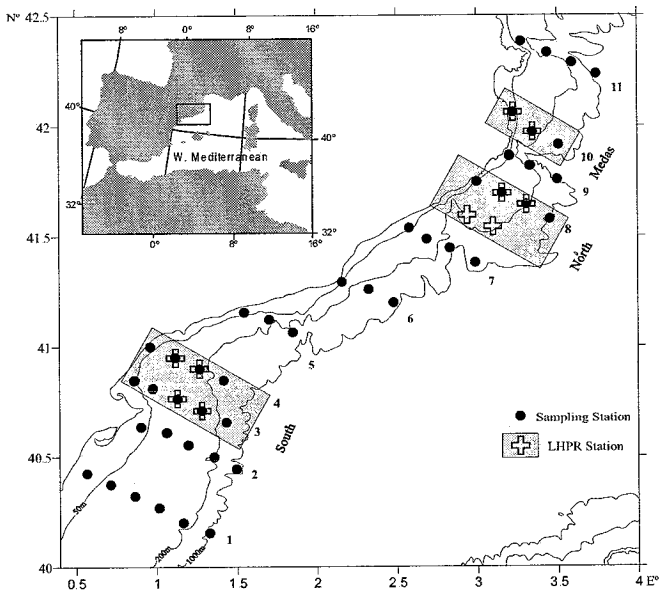



Figure 1.1. Location of the study area in the NW Mediterranean and map showing the major bathymetric features and the location of transects and sampling stations. Transects 3 and 9 were done only during CACO3 and CACO4.

## Vertical distribution

To determine the vertical distribution of cladocerans, stratified microzooplankton samples (9-cm mouth diameter, 53 μm mesh size) were taken using a Longhurst-Hardy Plankton Recorder (*LHPR*; Williams et al. 1983) in 10 stations during the cruise CACO4 (Figure 1.1), 4 of them located in the southern part of the Catalan Sea shelf, and the remaining 6 in the north (2 of these latter close to the Medes Islands). Due to cruise time restrictions stations were sampled only once, not allowing to study day/night changes in distribution at the same location. This constrain was surpassed by taking bathimetrically similar stations in closest parallel transects as replicates. Hence, five of these stations were sampled at day (between 8:00 and 16:00) and five during the night (between 21:00 and 03:00) (Figure 1.1). The tows were hauled obliquely, from surface down to a maximum depth of 100 m, with a vertical resolution of 10 m. The volume of water filtered by the net was recorded by a flowmeter attached to the mouth of the net. All zooplankton samples were preserved with borax-buffered formalin (4% final solution). The abundance of cladocerans was estimated by counting and identifying under

stereomicroscope at least 100 individuals. Samples were concentrated into 50-ml filter seawater and homogenized. An aliquot of 5-ml was taken and counted.

## RESULTS

### Horizontal patterns

*Hydrography*

The hydrographic characteristics at 5 and 20 m depth during the four cruises are shown in Figures 1.2, 1.3, 1.4 and 1.5. We identified general features in relation to temperature, salinity, and chlorophyll *a* concentrations for all the cruises. Temperature was generally higher in the southern part of the sampling area. Lower salinities were usually located in the southern area, associated to the Ebre river outflow, and sometimes in the northern region, associated to other continental discharges. Besides, different intrusions of oceanic waters were identified (e.g. during CACO4, Figures 1.3 and 1.5). Finally, chlorophyll *a* concentrations were higher close to the coastal zone, and decreased towards the oceanic area. In addition, higher values of chlorophyll *a* were associated with the Ebre discharge. In spite of these common patterns, it is necessary to emphasize the particular characteristics of each cruise.

During CACO1 (July 2003, Figures 1.2 and 1.4), there was a gradual decrease in surface water temperature from 26-27 °C in the southern region to 23-24 °C in the north. Deepest waters were higher in the central shelf. Oceanic waters (>38 psu) were identified in the central part of the sampling region only in surface waters. Finally, higher values of chlorophyll *a* were associated to waters of lower salinities and higher temperatures (1-2 μgChl*a* $l^{-1}$ at 5 m and 5-7 μgChl*a* $l^{-1}$ at 20 m). The northern shelf area was characterized by concentrations around 0.7 μgChl*a* $l^{-1}$ in surface waters and ≈ 3 μgChl*a* $l^{-1}$ at 20 m.

During CACO2 (September 2003, Figures 1.2 and 1.4) the temperature varied between 20 and 24 °C. Waters with high salinities (>38 psu) clearly dominated most of the Catalan Sea shelf. It is worth mentioning the superficial intrusion of less saline and warmer waters in the central shelf, in front of Barcelona (probably an intrusion of Atlantic waters). This intrusion will be associated to a decrease on *Penilia avirostris* concentration, as detailed further below. As for CACO1, chlorophyll *a* concentrations were higher near the Ebre river region. The rest of the sampling area showed rather homogeneous concentrations of chlorophyll *a* (varying between 0.6 and 0.8 μgChl*a* $l^{-1}$ at surface and between 3 and 4 0.6 and 0.8 μgChl*a* $l^{-1}$ in deepest waters).

CACO3 (June 2004, Figures 1.3 and 1.5) showed the lowest temperatures registered among all the cruises, with values ranging from 20 to 23 °C in surface

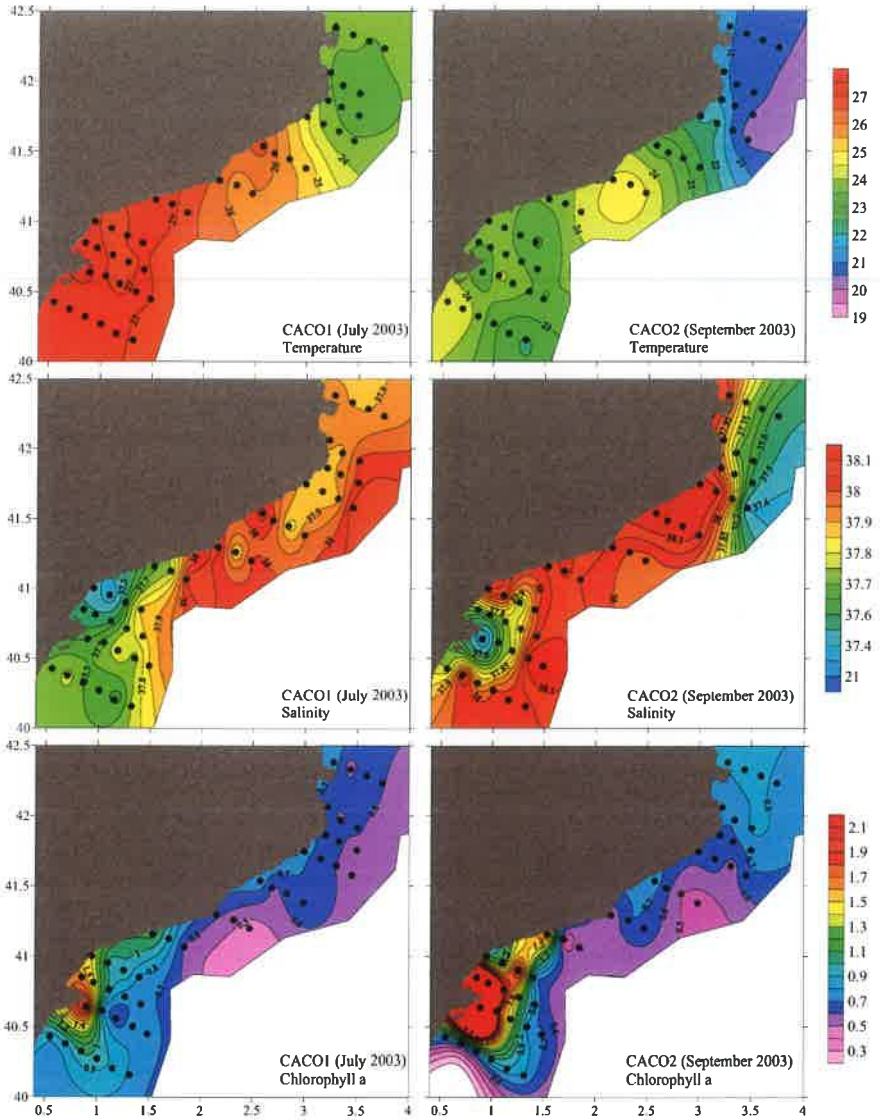



Figure 1.2. CACO1 and CACO2 temperature (°C), salinity (psu), and chlorophyll *a* (µg Chl*a* $l^{-1}$) contours at 5 m showing stations as black dots.

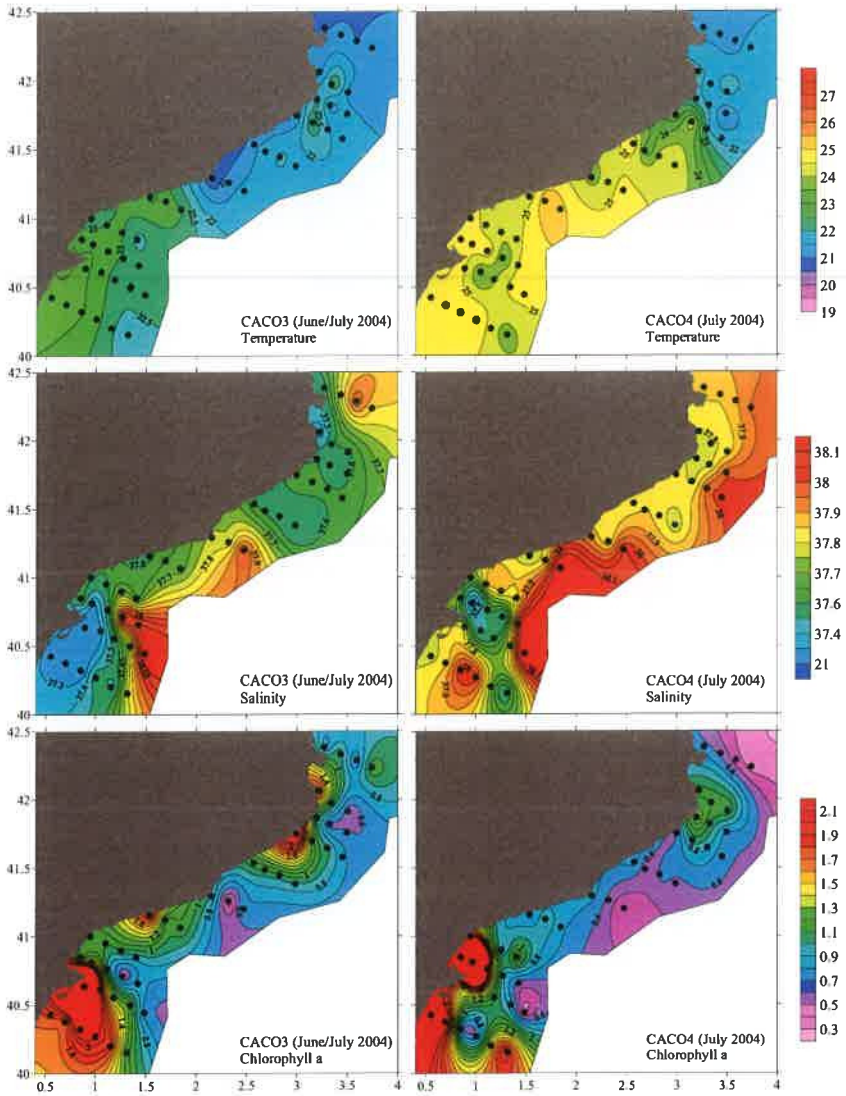



Figure 1.3. CACO3 and CACO4 temperature (°C), salinity (psu), and chlorophyll *a* (μg Chl*a* $l^{-1}$) contours at 5 m showing stations as black dots.

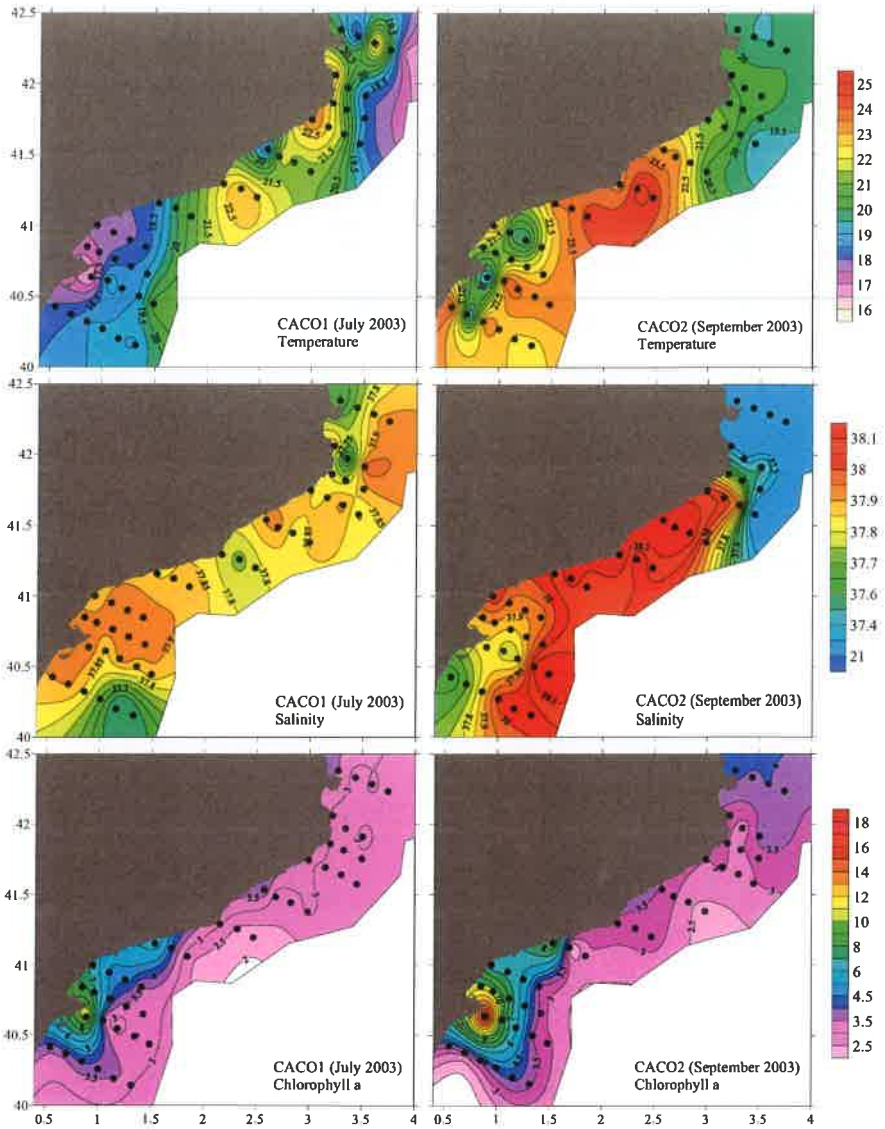



Figure 1.4. CACO1 and CACO2 temperature (°C), salinity (psu), and chlorophyll *a* (µg Chl*a* $l^{-1}$) contours at 20 m showing stations as black dots.

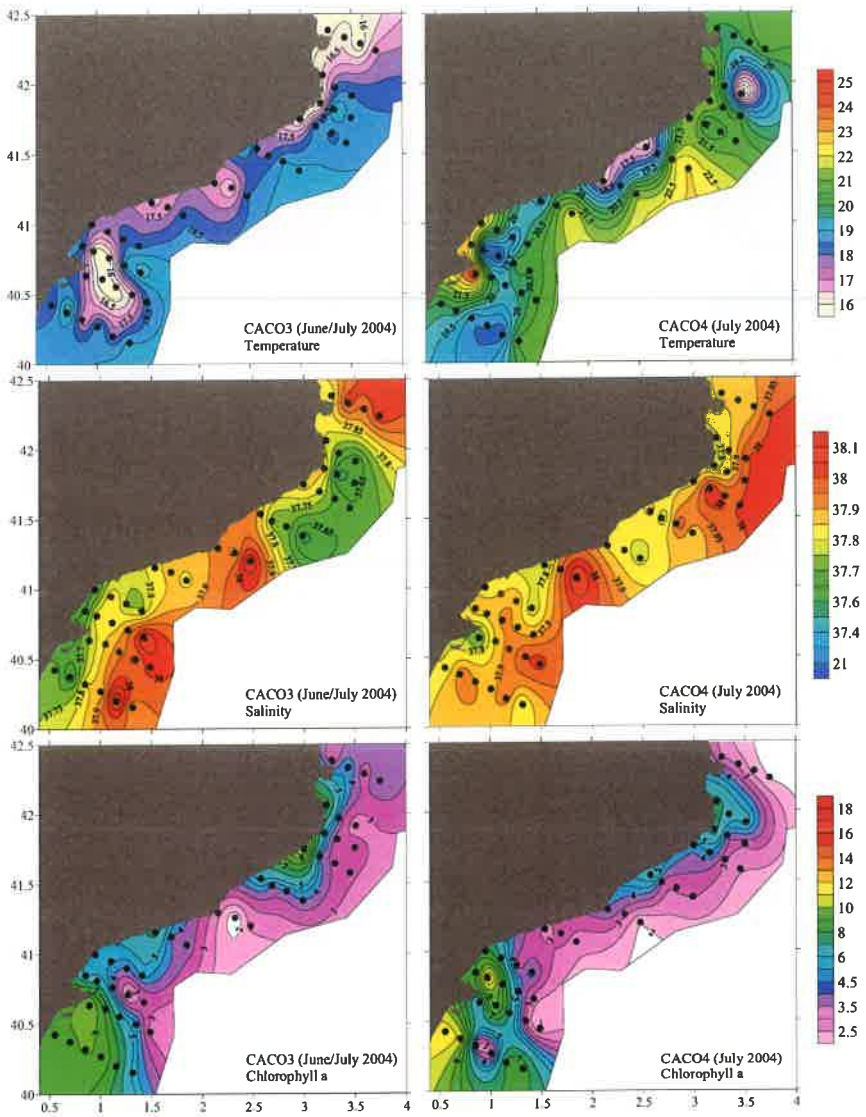



Figure 1.5. CACO3 and CACO4 temperature (°C), salinity (psu), and chlorophyll *a* (µg Chl*a* $l^{-1}$) contours at 20 m showing stations as black dots.

waters and between 16 and 19 °C in deepest waters. Higher temperatures occurred in surface waters on the southern region, showing the beginning of summer conditions. Lower salinity waters were located in the northern area, close to the coast. Intrusion of oceanic water (>38 psu) was detected in the southern region, and probably in the central area. Associated to the lower salinity waters, higher values of chlorophyll *a* were reported (ranging between 1-4 μgChl*a* $l^{-1}$ in surface ans between 4 and 6 μgChl*a* $l^{-1}$ at 20 m), although some sporadic peaks of chlorophyll were detected further north along the coast.

Finally, CACO4 (July 2004, Figure 1.3 and 1.5) was similar to CACO1, being both representative of typical hydrographic characteristics in summer. Surface temperature varied from 24 to 25 °C in the south, with lower values (<24 °C) in the north. Temperature at 20 m varied between 17.5 and 21.5 °C. The presence of waters of higher temperatures (>25 °C) is evident in the central part of the sampling area. Two intrusions of oceanic waters (higher salinities, >38 psu) were located in the central region and in the north area. Higher values of chlorophyll *a* were again found in the southern coastal area, associated with lower salinities (values ranged from 1 up to 6 μgChl*a* $l^{-1}$ in surface, and between 7 and 9 μgChl*a* $l^{-1}$ at 20 m).

All these hydrodynamic features could be generalized into a clear zonation with three visibly separate hydrographic areas (Figure 1.6). The central shelfarea (CS) is characterized by a high mesoscale activity due to the dynamic coupling of coastal and oceanic waters. In this region, the incursions of offshore waters to the coast (CACO1, CACO2, and CACO4) or the high influence of continental waters (CACO3) are common during the period of maximum stratification. The other two regions, northern (NS) and southern (SS) shelf areas are characterized by specific hydrodynamics, such as the influence of Ebre runoff on the southern shelf associated to a surface layer of lower salinities and higher chlorophyll *a*, and the influence of the Liguro-Proveçal-Catalan current (northern shelf).

All these hydrodynamic features could be generalized into a clear cross-shore zonation with three visibly separate hydrographic areas (Figure 1.4), a central area (CA) characterized by a high mesoscale activity due to the dynamic coupling of coastal and oceanic waters. In this region, the incursions of offshore waters to the coast (CACO1, CACO2, and CACO4) or the high influence of continental waters (CACO3) are common during the period of maximum stratification. The other two regions, northern (NA) and southern (SA) areas are characterized by specific hydrodynamics, such as the influence of Ebre runoff on the SA associated to a surface layer of lower salinities and higher chlorophyll *a*, and the influence of the Liguro-Proveçal-Catalan current (NA).

The PCA analysis for the four cruises generated 12 principal components, the first two of which had eigenvalues >1 and explained 69% (CACO1), 86% (CACO2), 84% (CACO3), and 69% (CACO4) of the variance of the original data set. These

PCs were obtained as a weighted linear combination of the original variables and were used for further analysis. Table 1.1 shows the importance of each factor to explain the variance of the data set and the importance of each environmental variable on each PCs. In CACO1, PC1 was related to chlorophyll *a* and salinity at 5 m. In contrast, salinity at 20 m was significant on PC2. In CACO2, PC1 was clearly associated to temperature and salinity, whereas PC2 was significantly influenced by chlorophyll *a*. In CACO3 PC1 was associated to salinity, and also with temperature at 20 m and chlorophyll *a* at 5 m, whereas temperature at 5 m and chlorophyll *a* at 20 m were the main factor on PC2. Finally, in CACO4 PC1 was significant influence by the salinity and also by chlorophyll *a* and salinity at 5 m. PC2 was significantly associated to salinity at 20 m.

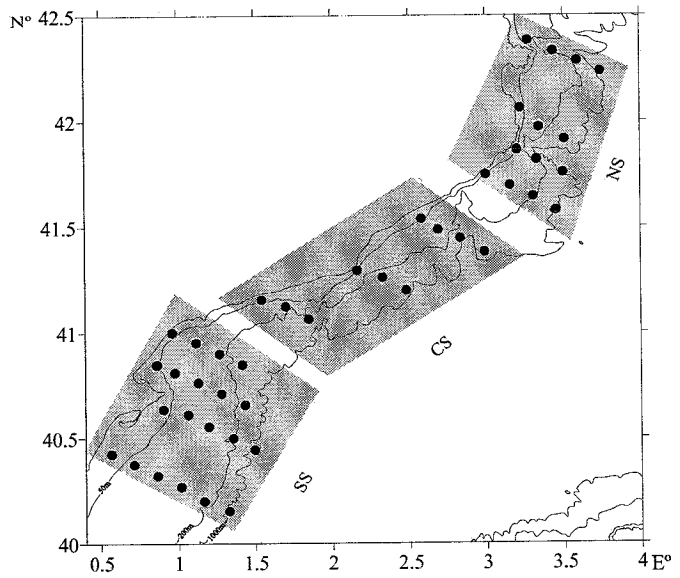



Figure 1.6. Geographical location of the hydrographical areas identified in the NW Mediterranean. The hydrographical areas are indicated as (SS) southern shelf, (CS) central shelf, and (NS) northern shelf.

Table 1.1. Principal component analysis. PC: principal component. Loadings of the individual variables along the PC1, and PC2. T: temperature; Sal: salinity; Chla: chlorophyll *a* concentration; int: integrate.

| | **CACO1** | | **CACO2** | | **CACO3** | | **CACO4** | |
|---|---|---|---|---|---|---|---|---|
| | **PC1** | **PC2** | **PC1** | **PC2** | **PC1** | **PC2** | **PC1** | **PC2** |
| T (5 m) | 0.68 | 0.11 | **0.90** | 0.08 | 0.13 | **0.75** | -0.53 | -0.24 |
| T (20 m) | -0.67 | -0.03 | **0.78** | -0.28 | **0.91** | 0.09 | 0.31 | -0.68 |
| Sal (5 m) | **-0.79** | 0.18 | **0.93** | 0.11 | **0.98** | 0.14 | **0.81** | 0.34 |
| Sal (20 m) | 0.08 | **0.98** | **0.90** | 0.02 | **0.97** | 0.18 | 0.15 | **0.87** |
| Chla (int 5m) | **0.91** | 0.14 | -0.01 | **0.99** | **-0.98** | 0.07 | **-0.92** | 0.09 |
| Chla (int 20m) | **0.88** | 0.21 | 0.05 | **0.98** | 0.07 | **0.82** | **-0.92** | 0.06 |
| **Variance (%)** | **53** | **17** | **51** | **33** | **63** | **20** | **46** | **23** |

*Horizontal distribution patterns of Penilia avirostris and Evadne spp.*

The cross-shelf distribution of marine cladocerans changed markedly over the four surveys (Figures 1.7 and 1.8). In general, *Penilia avirostris* was one order of magnitude more abundant than *Evadne* spp., varying between 100 and 200000 ind $m^{-2}$ and from 300 and 21000 ind $m^{-2}$, respectively.

*Penilia avirostris* was present in all the studied areas, with higher abundances in CACO4 (800-200000 ind $m^{-2}$), and lower densities reached in CACO3 (200-60000 ind $m^{-2}$). A quite homogenous distribution of *P. avirostris* was observed during CACO1 (Figure 1.7) and CACO4 (Figure 1.8), while particular patterns were observed for the other two cruises. During CACO2 the organisms showed low densities in the central shelf (600-5000 ind $m^{-2}$), moderated in the northern shelf (400-35000 ind $m^{-2}$), and high in the southern shelf (2500-110000 ind $m^{-2}$) (Figure 1.7). Through CACO3 organisms were clearly more abundant in the southern shelf area, being nearly absent in the central and northern shelfs (Figure 1.8).

*Evadne* spp. extended also its abundance to all the sampling area, reaching higher densities in CACO3 (600-14000 ind $m^{-2}$) and lower in CACO2 (200-11000 ind $m^{-2}$). Generally, higher abundances were achieved in the coastal area, and decreased towards oceanic waters, although the pattern was quite erratic. Homogeneous distribution of *Evadne* spp. was observed in CACO1 and CACO 2 (Figure 1.7). During CACO3 and CACO4 the organisms were nearly absent in the central shelf (Figure 1.8).

We explored the possible relationships between the PCs scores and the abundance of the marine cladocerans (Table 1.2). In CACO2, and CACO4 we did not find any significant correlation between PCs scores and the densities of *Penilia avirostris* and *Evadne* spp. However, in CACO3, the abundance of *P. avirostris* and *Evadne* spp. was negative correlated to PC1, indicating that densities of these marine cladocerans were positive correlated to chlorophyll *a* concentrations at 5 m negative correlated to the other significant factors. Besides, in CACO1 the abundance of *Evadne* spp. was positive correlated to PC1, indicating that densities of this marine cladoceran was positive correlated to chlorophyll *a* and negative correlated with the salinity at 5 m.

## Vertical patterns

*Hydrography*

Vertical profiles of temperature, salinity and chlorophyll *a* concentration of the LHPR stations in CACO4 are shown in Figure 1.7. We only present the averaged values for day and night stations in each of the three sampling areas. In general, the stratification of the water column was evident, with a thermocline well established in all the sampling regions. The thermocline was located between 20 m and 30 m depth

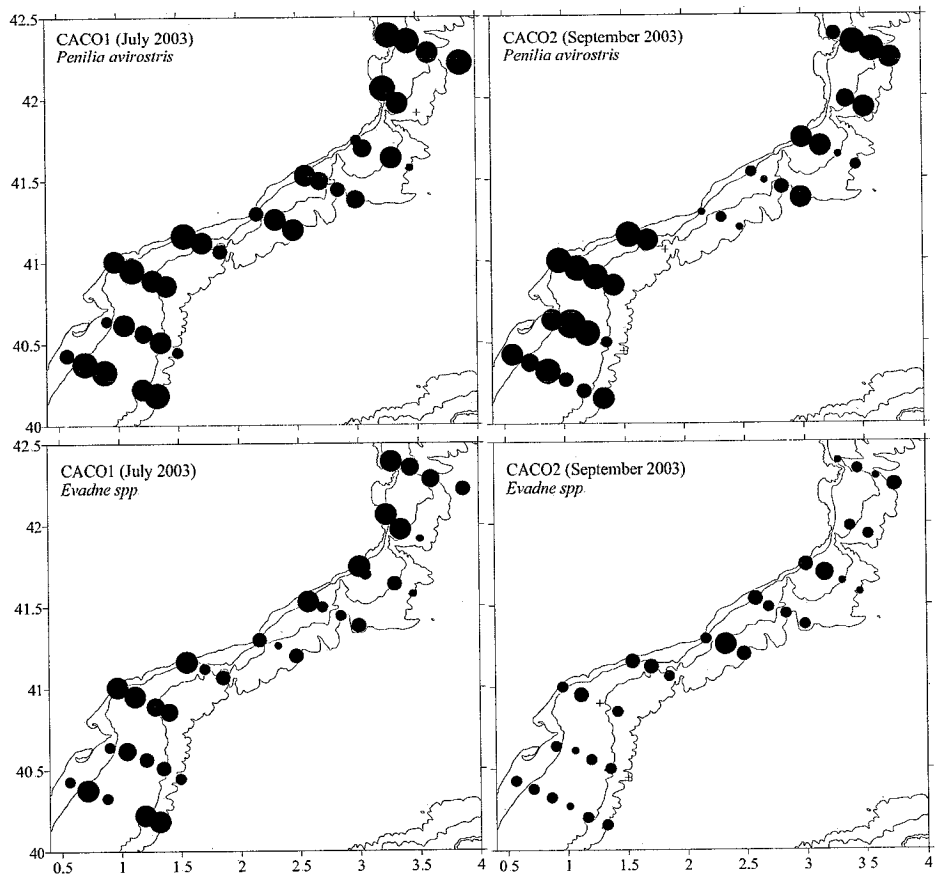



Figure 1.7. Abundance and distribution of *Penilia avirostris* and *Evadne* spp. in the Catalan Sea. CACO1 and CACO2 cruises.

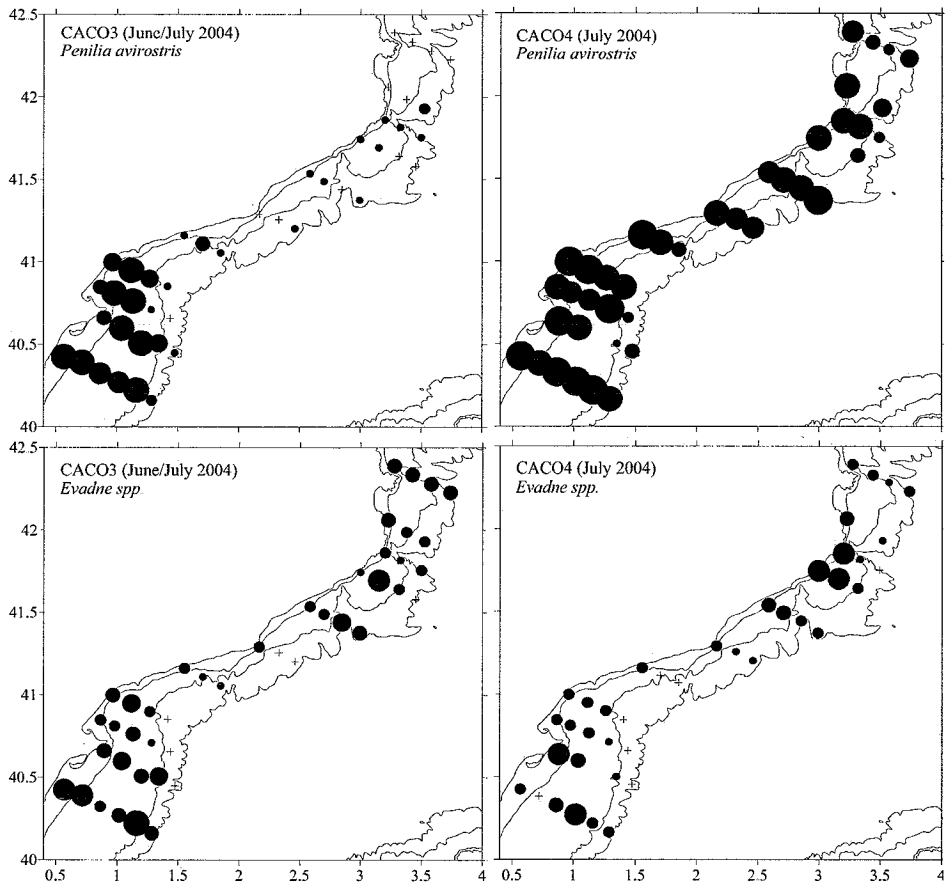



Figure 1.8. Abundance and distribution of *Penilia avirostris* and *Evadne* spp. in the Catalan Sea. CACO3 and CACO4 cruises.

in the southern region, between 20 m and 40 m depth in the northern stations, and between 30 m and 60 m depth in Medes Islands (Figure 1.9). Water temperature above the thermocline ranged between 23 and 25 °C in the southern stations, between 22 and 23 °C in the northern ones, and between 21 and 22 °C in Medes Islands region (Figure 1.9). Three different salinity profiles were evident: a) the occurrence of waters with low salinities (<37.5 psu) clearly associated to the Ebre runoff and with a clear halocline in the south; b) a homogenous salinity profile without the establishment of the halocline in the north; and c) waters with a well established halocline in Medes Islands (Figure 1.9). A clear deep chlorophyll maximum was present in all the sampling areas, being located between 60 and 80 m depth. Notice that the vertical profile of Medas Islands is the same for both, day and night.

Table 1.2. Spearman's correlation matrix between *Penilia avirostris* and *Evadne* spp. and the factor scores of PCA analysis. * $p < 0.05$; ** $p < 0.01$.

| | CACO1 | | CACO2 | | CACO3 | | CACO4 | |
|---|---|---|---|---|---|---|---|---|
| | r | p | r | p | r | p | r | p |
| ***Penilia avirostris*** | | | | | | | | |
| PC1 | 0.23 | 0.19 | 0.06 | 0.75 | -0.49 | ** | 0.15 | 0.34 |
| PC2 | -0.25 | 0.14 | 0.30 | 0.07 | -0.08 | 0.61 | 0.17 | 0.29 |
| ***Evadne spp.*** | | | | | | | | |
| PC1 | 0.16 | 0.35 | 0.21 | 0.22 | -0.40 | ** | 0.44 | 0.78 |
| PC2 | -0.36 | * | -0.15 | 0.38 | 0.09 | 0.55 | -0.04 | 0.82 |

*Vertical distribution patterns of Penilia avirostris and Evadne spp.*

Both cladocerans were mainly distributed in the upper 60 m of the water column in all the stations (Table 1.3, Figure 1.10), above or associated to the thermocline. *Penilia avirostris* showed the deeper distribution (maximum at 20-40 m), whereas *Evadne* spp. were slightly more superficial (maximum at 0-20 m) (Figure 1.8). No clear migration pattern can be depicted out of the few stations sampled, although there are evidences of nocturnal deepening of the *P. avirostris* populations in northern stations, and of *Evadne* spp. in the Medes Islands. Conversely, *P. avirostris* from the southern stations seemed to migrate upwards during night.

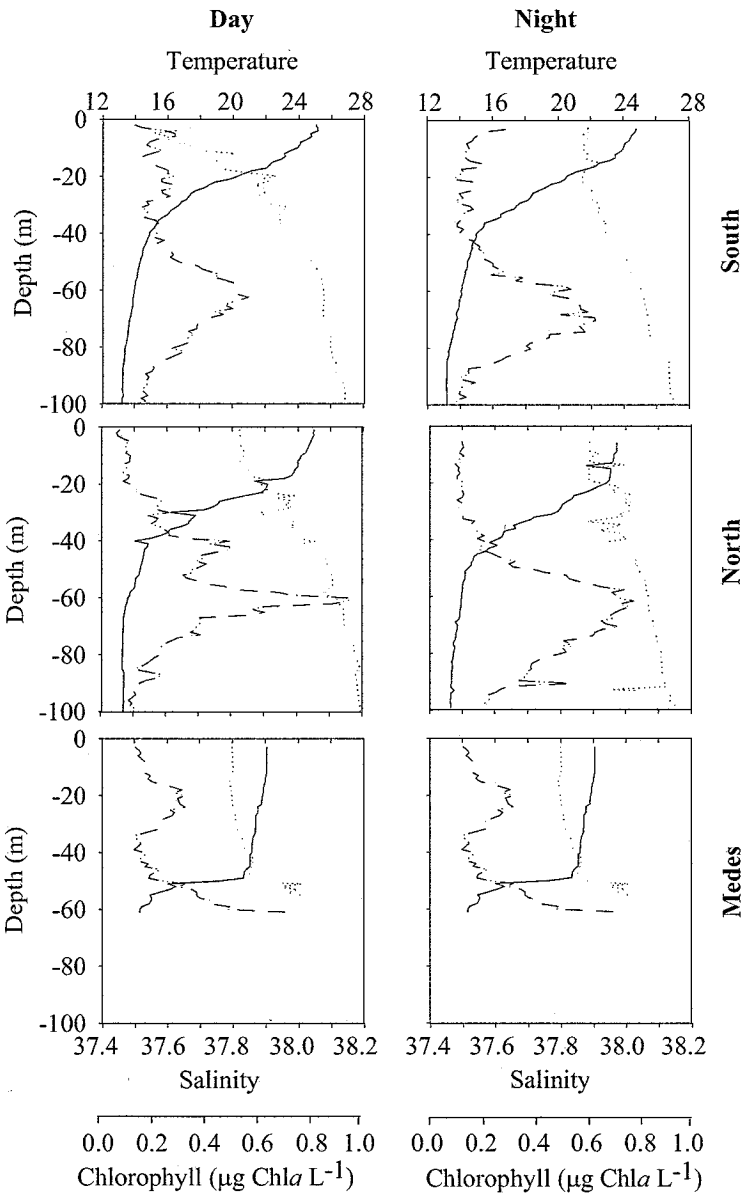



Figure 1.9. Temperature, salinity, and chlorophyll *a* profiles for the LHPR stations on the study area.

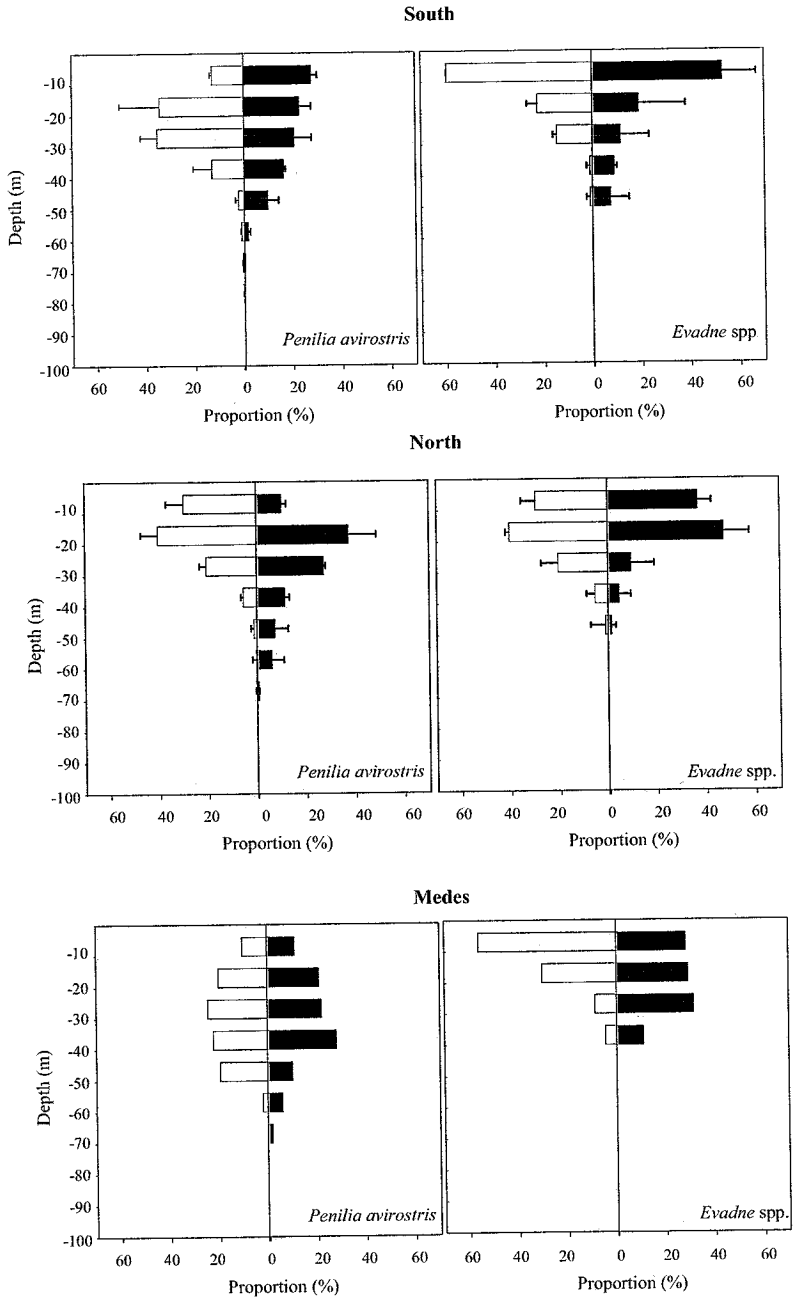



Figure 1.10. Vertical distribution of *Penilia avirostris* and *Evadne* spp during daytime (open bars) and nighttime (filled bars) in July 2004 (CACO4).

Table 1.3. Vertical abundance of *Penilia avirostris* and *Evadne* spp. from July 2004 (CACO4).

| Region | Depth range (m) | *Penilia avirostris* | | *Evadne* spp. | |
|---|---|---|---|---|---|
| | | Day | Night | Day | Night |
| **South** | 0-10 | 2835 | 10175 | 383 | 218 |
| | 10-20 | 7771 | 8564 | 137 | 100 |
| | 20-30 | 7730 | 7506 | 98 | 40 |
| | 30-40 | 2800 | 6025 | 11 | 38 |
| | 40-50 | 507 | 3747 | 11 | 39 |
| | 50-60 | 263 | 724 | 0 | 0 |
| | 60-70 | 130 | 101 | 0 | 0 |
| | 70-80 | 45 | 0 | 0 | 0 |
| | 80-90 | 14 | 0 | 0 | 0 |
| | 90-100 | 0 | 0 | 0 | 0 |
| | | TOTAL | 22094 | 36841 | 641 |
| **North** | 0-10 | 3216 | 3278 | 502 | 225 |
| | 10-20 | 4576 | 11545 | 576 | 279 |
| | 20-30 | 2330 | 9131 | 339 | 91 |
| | 30-40 | 648 | 3941 | 96 | 45 |
| | 40-50 | 109 | 2865 | 12 | 16 |
| | 50-60 | 145 | 2411 | 0 | 0 |
| | 60-70 | 54 | 206 | 0 | 0 |
| | 70-60 | 54 | 0 | 0 | 0 |
| | 60-70 | 0 | 0 | 0 | 0 |
| | 70-80 | 0 | 0 | 0 | 0 |
| | 80-90 | 0 | 0 | 0 | 0 |
| | 90-100 | 0 | 0 | 0 | 0 |
| | | TOTAL | 11134 | 33377 | 1525 |
| **Medas** | 0-10 | 1295 | 1300 | 334 | 300 |
| | 10-20 | 2477 | 2401 | 180 | 309 |
| | 20-30 | 3000 | 2520 | 53 | 332 |
| | 30-40 | 2750 | 3183 | 28 | 116 |
| | 40-50 | 2404 | 1164 | 0 | 0 |
| | 50-60 | 298 | 687 | 0 | 0 |
| | 60-70 | 0 | 219 | 0 | 0 |
| | 70-80 | 0 | 0 | 0 | 0 |
| | 80-90 | 0 | 0 | 0 | 0 |
| | 90-100 | 0 | 0 | 0 | 0 |
| | | TOTAL | 12224 | 11474 | 594 |

## DISCUSSION

One of the most significant findings of the present study is the characterization of strong coupling between the distribution of *Penilia avirostris* and *Evadne* spp. and the coastal hydrography. The study site had been previously characterized for its hydrography and our results agree with previous reports and descriptions of the Catalan Sea shelf during summer months (Estrada & Salat 1989, Masso & Duarte 1989, Estrada 1996, Salat 1996). The presence of more saline waters (>38 psu) in the central area of the studied region is associated with the incursions of oceanic waters that are typical in this region (Salat 1996), in relation with the lesser signature of the shelfbreak front in the upper layers. Also, during the four cruises we found evidence of a well-developed thermocline at around 20-40 m depth and the occurrence of a deep chlorophyll maximum (DCM) below the thermocline. The presence of a DCM is generally known feature in most oligotrophic areas in the period of water column stratification (Estrada & Salat 1989). Additionally, we found a surface peak of chlorophyll in the southern region of the sampling area. This indicates a more productive layer above the thermocline, due to the influx of nutrients in this area by the Ebre runoff.

In this hydrodynamic environment, *Penilia avirostris* and *Evadne* spp. represented an important numerical component of the zooplankton communities. The presence and dominance of these marine cladocerans had been previously recorded by other authors in the same area (Alcaraz 1977, 1981, Siokou-Frangou 1996, Lipej et al. 1997, Calbet et al. 2001, Fernández de Puelles et al. 2003, Siokou-Frangou et al. 2004, Umani et al. 2005).

### Horizontal patterns

Cladocerans in the study region were not randomly distributed. Moreover, even though individuals of both species were caught in the majority of samples taken during the four cruises, patterns varied based on species, area, and year. The most remarkable feature of the spatial distribution results in the high level of variability observed in the distribution patterns of both marine cladocerans. This variability is strongly associated to the surface hydrographical characteristics, explained by a combination of geophysical and hydrological factors: the intrusion of oceanic waters in the central area, and occurrence of low-salinity water from the Ebre runoff (Salat 1996).

The fact that in most cases the analysis of PCs scores showed no correlation with the cladocerans distribution is a consequence of the particular characteristics of the Catalan Sea shelf during the study, with the intrusions of higher salinity and higher temperature waters from one side, latitudinal differences in temperatures on the other, which mask the effect of particular variables such as temperature or salinity in the analysis. For this reason, we think the data from our study is best

discussed taking each cruise separately and arranging them sequentially to characterize the evolution of the populations of both cladocerans on a seasonal basis: CACO3 (end of June), CACO1 (mid July), CACO4 (end of July), and CACO2 (mid September).

For *Penilia avirostris*, CACO3 corresponds to the beginning of their seasonal appearance in the Catalan Sea shelf (see Chapter II). Temperature shows a clear latitudinal gradient during the cruise, warmer waters located in the southern area, where chlorophyll concentration is also high due to the Ebre river influence. *P. avirostris* population has still not developed in the upper shelf area, likely due to the lower temperatures in the neritic waters in the area. Interestingly, an intrusion of offshore waters appears remarkably close to the Ebre delta, a typical local phenomenon due to the interaction of the Catalano-Liguro-Provençal current and the topography (Tintoré et al. 1990). This intruding of offshore waters is coincident with an absence of *P. avirostris* in the upper bound of the Ebre river shelf (Figure 1.8).

In CACO1 (mid July) *Penilia avirostris* is distributed all along the Catalan Sea shelf, with no remarkable features, and corresponds to a period of full development of *P. avirostris* population in the area (Chapter II). In Figure 1.2 an intrusion of offshore waters can be observed in the central shelf and the slope of the upper shelf, but this does not reflect on changes in *P. avirostris* distribution because this intrusion is very superficial, as evidenced on the isoline plots at 20 m depth (Figure 1.4).

CACO4, that took place at the end of July, occurs also on a period of full development of *Penilia avirostris* population in the area (Chapter II). Figure 1.3 shows also an intrusion of offshore waters in the central and southern shelfs, but remains mostly at the shelfbreak not affecting much *P. avirostris* distribution (except in a few stations) (Figure 1.8).

Finally, in CACO2 that took place in a later period of the season, *Penilia avirostris* appears distributed mainly in the northern and the southern shelfs, whereas the central shelf appears occupied by offshore waters that have displaced *P. avirostris* away. The intrusion of offshore waters also affects the more offshore stations in the Ebre river area, which showed a lower presence of *P. avirostris*.

*Evadne* spp. horizontal distribution evidenced that this species has a wide range of optimum temperature and salinity. The differences observed in the distribution of this specie in relation to *Penilia avirostris* could be related to the tolerance of *Evadne* spp. to lower temperatures (< 22 °C) as in CACO3. Also, the presence of this marine cladoceran was restricted to the coastal areas, being less abundant throughout the slope, probably associated to the influence of oceanic waters. Generally, the species that comprise this group are considered as stenohalines and thermophils (Tregouboff 1963, Specchi 1965, Gieskes 1971a, Alcaraz 1977). Regarding *Evadne* spp. the pattern is not so clear. This group of cladocerans seems to appear earlier in the season, as also corroborated by other studies (Alcaraz 1977, 1981, Calbet et al. 2001), and

maximized its abundance during the highest temperature period (July 2003); although its abundance never reach that of *Penilia avirostris*. During CACO2 (September 2004) we found the lowest abundance, likely evidence of the decline of the population. As for *P. avirostris* hydrography acts as confounding factor when unveiling the distribution patterns of the species in a seasonal basis. Moreover, the group *Evadne* spp. encompasses 3 species that could well have different seasonality and distribution. For instance, Isari et al. (2006) described the distribution of marine cladocerans in the North Aegean Sea (Eastern Mediterranean) during the same periods than CACO1 (July 2003) and CACO2 (September 2003). They found that *Pseudoevadne tergestina* was more abundant in more coastal areas, whereas, *Evadne spinifera* was less abundant than the other two species, and appeared only present in those stations associated to the frontal area, with colder and more saline waters. Alcaraz (1977) described the beginning of *Evadne nordmanni* populations at spring (from March to July, and peaked in April), when waters were still cold and the thermocline was not well established. As waters became warmer, and the thermocline more stable, this species disappeared and *P. tergestina* and *E. spinifera* started to increase their presence. The former species achieved its maximum abundance between August and September, and the latter were more abundant between June and July. The same succession in *Evadne* species was observed by Alcaraz (1981) in Deva and Gueraria harbors (Basque country), Gieskes (1971a) in the North Sea, and by Onbé & Ikeda (1995) in Toyama Bay (Japan). In our surveys it is possible that the distribution shown during CACO3 corresponded to *E. nordmanni*, and *E. spinifera*. CACO1 and CACO4 probably showed the distribution of *E. spinifera* and *P. tergestina*. And finally, CACO2 could show the distribution of *P. tergestina*. All these species also had particular distributions in relation to salinity and temperature. *P. tergestina* and *E. spinifera* are typical warm water species, being associated with temperatures ranging 21.7 and 23.4 °C in Otsuchi Bay (Japan) (Onbé et al. 1996). On the other hand, *E. nordmanni* seems to be associated to colder waters, between 16.3 and 18.2 °C, (Bryan & Grant 1979). Differences in their salinity preferences are also reported, *E. spinifera* occurring exclusively in waters of high salinities (Onbé 1977, Onbé & Ikeda 1995, Onbé et al. 199), whereas *E. nordmanni* is known as euryhaline.

Until now, we have highlighted salinity and temperature as the main parameters that determine the distribution of marine cladocerans. The importance of this parameters have been reported to influence abundance and distribution of cladoceran species, thereby partly defining a unique species-specific niche, which might additionally be specific for distinct areas (Egloff et al. 1997). In our study, *Penilia avirostris* abundance was maximum in the southern shelf area, where high temperatures, low salinities, and higher surface chlorophyll *a* concentrations were achieved. In contrast, abundance of this marine cladoceran was lowest in high salinity water masses (intrusion of oceanic waters) or where temperatures were below 22 °C. Favorable conditions for the establishment of abundant populations of this marine cladoceran have been reported in the literature at temperatures ranging between 12

and 30 °C, with an optimum of 24-28 °C and salinity range of 30–35 in different areas (Leveau 1965, Alcaraz 1970, Specchi & Fonda 1974, Iwasaki et al. 1977, Paffenhöfer et al. 1984, Onbé & Ikeda 1995, Tang et al. 1995). Alcaraz (1977) described the horizontal distribution of *P. avirostris* in Gibraltar (in the Atlantic and Mediterranean sides), and the main results indicated that there is a clear decreasing gradient from south to north in the Mediterranean coast, that coincides with the positive gradient on salinities. These previous findings agree with the patterns observed in this study.

Hydrography has been found to affect brood size via body size (Moraitou-Apostopoulou et al. 1986), prenatal mortality (Platt & Yamamura 1986), birth rates (Onbé 1978), developmental times (Onbé 1978), and death rates (Fofonoff 1994) as well as hatching of resting eggs (Onbé 1985). However, other factors such as food have been considered to influence the distribution of marine cladocerans, although available literature is contradictory. Paffenföfer (1983) and Kim et al. (1989) reported that the presence of *Penilia avirostris* was previously associated to the concentration of particulate matter, being more abundant with increasing concentrations of particulate matter, being more abundant in eutrophic waters. Tang et al. (1995) showed that *P. avirostris* densities usually increased as chlorophyll *a* concentrations decreased. Besides, Paffenhöfer & Orcutt (1986) suggested that *P. avirostris* is well adapted to the low food concentrations of oligotrophic waters, and they showed that higher concentrations of food, equivalent to those found in the Ebre influence (SA), are negative to the biology of this species. We found that higher abundances of *P. avirostris* were always associated to high productive waters on the southern region, and the combination of higher chlorophyll concentrations but also the relative warm and less saline waters are favorable conditions for the establishment of dense populations of this marine cladoceran. Finally, water stability (which is obviously associated to temperature and salinity) also seems to be important in the apparition and distribution (Alcaraz 1981) of marine cladocerans, as shown by the clear relationship between vertical stability and cladoceran abundance (Gieskes 1971a).

In summary, *Penilia avirostris* and *Evadne* spp. were widely distributed in the NW Mediterranean; however special features are necessary to understand their individual horizontal distribution. *P. avirostris* was more abundant in the southern region of the sampling area, and during summer surveys (CACO1, end of June-July; and CACO4, July). The spatial distribution pattern exhibited by this marine cladoceran is highly influenced by warm waters (between 22 and 25 °C), with lower salinities (between 37.5-37.9), and a stable thermocline. Also, higher concentrations of chlorophyll *a* seem to be related to higher abundance of *P. avirostris*. In contrast, *Evadne* spp. did not showed any clear pattern, probably due to the fact that it was not a single species, and the different species exhibited a wide range of optimum temperatures and salinities. Besides, we suggested the temporal evolution of *P. avirostris* population in the Catalan Sea arranging the different cruises. This species starts its seasonal presence by the south (CACO3) at the end of June (when temperature reaches values

>22 °C), extending their distribution further north by mid June. Near the end of July *P. avirostris* in an important component of the zooplanktonic communities with abundances ranging 2 $10^4$-$10^5$ ind $m^{-2}$. In September its abundance starts to decline, apparently by a combination of decrease of temperatures, changes in seston composition, and intensification in the hydrodinamism, typical of the season.

**Vertical patterns**

Vertical distribution of zooplankton is regulated by both biotic and abiotic factors such as temperature, oxygen, food availability, interspecific competition, and predation (Dumont 1972, Kerfoot et al. 1985). In the present study, despite the different hydrographic characteristics between the three different areas, vertical distribution patterns tended to be similar.

The averaged highest concentrations of *Penilia avirostris* were found above or associated to the thermocline independent from the sampling area and the time of the day. These results agree with the observations of Paffenhöfer (1983) and Checkley et al. (1992) who showed that the abundance of this marine cladoceran increased with increasing depth, being more abundant between the thermocline and 30 m. However, Onbé (1977) and Kim et al. (1994) reported that *P. avirostris* in the Inland Sea of Japan and Tolo Harbour in China (respectively) positioned within the upper 10 m of water column during both day and night. Probably the differences observed between our results and this later study are result of the depth of Tolo Harbour (<20 m).

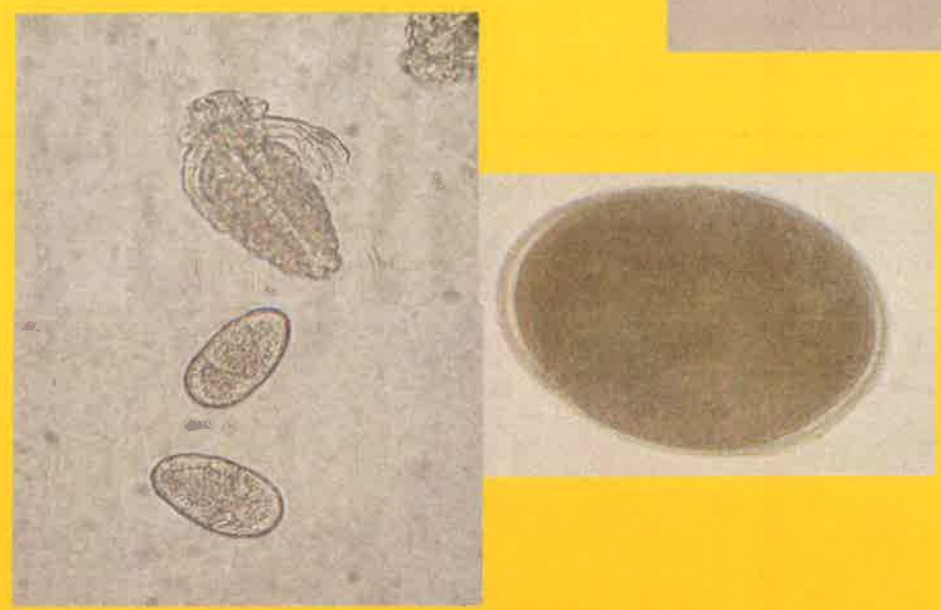
*Evadne* spp. appeared mainly in the upper 20 m of the water column. These is in good agreement with the data reported by Alcaraz (1970), Checkley et al. (1992), but differed from Onbé & Ikeda (1995), who reported that the population density of *Evadne* spp. peaked at 30 m.

Zooplankton tend to inhabit deeper water during daylight and migrate upwards at night. The views on the factors inducing and modifying the migrations are variable including avoidance of predators, metabolic advantages and changes in the nutritional value of algae and/or in the amount of available food. The hypothesis most widely supported is that zooplankton migrates in order to reduce mortality caused by visually orienteering predators. Cases of reversed migration have also been reported and they have been usually connected to predation pressure by invertebrate predators. The amplitude of vertical migrations tends to increase with zooplankton size (Horppila 1997).

Neither of the marine cladocerans species showed a clear distinct diel vertical migration pattern as also evidenced in other studies (Checkley et al. 1992, Mullin & Onbé 1992). However, vertical reverse migrations of low amplitude have been reported by other authors (Onbé 1974, Onbé 1977, Checkley et al. 1992, Mullin & Onbé 1992, Kim et al. 1994, Onbé & Ikeda 1995, Saito & Hattori 2000). At least for

podonids, the possible explanation for this reverse migration behavior is related to feeding. *Evadne* spp. is a group known as raptorial feeders, which need the light to find their prey (Mullin & Onbé 1992, Onbé & Ikeda 1995). In the case of *P. avirostris*, a strict filter feeder, the explanation seems to rely on its vulnerability to invertebrate predation (e.g chaetognaths and euphausiids) (Lampert 1993, Ohman et al. 1983, Nip et al. 2003, Barz & Hirche 2005).

In summary, *Penilia avirostris* was mainly distributed in the upper 60 m of the water column, whereas *Evadne* spp. was more superficial, appearing in the upper 40 m. Maximal abundance of *P. avirostris* occurred between 20 and 30 m of depth, while *Evadne* spp. maximal abundance was located between 10 and 20 m. The vertical distribution of both species seems to be related to the presence of a well establish thermocline.



# CHAPTER II. LIFE HISTORY AND POPULATION DYNAMICS OF THE MARINE CLADOCERAN *PENILIA AVIROSTRIS* (BRANCHIOPODA: CLADOCERA) IN THE CATALAN SEA (NW MEDITERRANEAN)

Released as a published article.
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Journal of Plankton Research (submitted)

# CHAPTER II. LIFE HISTORY AND POPULATION DYNAMICS OF THE MARINE CLADOCERAN *PENILIA AVIROSTRIS* (BRANCHIOPODA: CLADOCERA) IN THE CATALAN SEA (NW MEDITERRANEAN)

## INTRODUCTION

Cladocerans are important contributors to the fauna and energy dynamics of freshwater ecosystems (Richman 1958, Lampert 1987). However, in marine ecosystems these animals have been little studied (Egloff et al. 1997). *Penilia avirostris* is a seasonally abundant and widely distributed cladoceran in neritic waters of tropical and subtropical waters, expanding its distribution towards northern temperate latitudes since the mid 20$^{th}$ century (Lochhead 1954, Della Croce 1964, Della Croce & Venugopal 1973, Ærtebjerg et al. 2003, Jonhs et al. 2005). When abundant, *P. avirostris* may play an important role in marine organic matter production, by concentrating organic energy of nano- and microplankton and making it available to consumers of higher trophic levels (Paffenhöfer & Orcutt 1986, Turner et al. 1988, Atienza et al. 2006a, b).

Information about the population dynamics of *Penilia avirostris* is mostly limited to their abundance and seasonal distribution (Della Croce 1964, Onbé & Ikeda 1995, Tang et al. 1995, Marazzo & Valentin 2003a, Valentin & Marazzo 2003, Wong et al. 2004). These studies revealed that the temporal distribution of marine cladocerans is irregular: at certain times of the year, they occur in the plankton at high densities. Following the peaks, populations decline rapidly, and the cladocerans eventually disappear from the plankton. Until now, the factors controlling this pattern remain unclear. Some authors suggest that temperature may play an important role in the population dynamics of *P. avirostris* (Onbé & Ikeda 1995); however, other factors have to be considered such as food availability, chemical composition of seston, and photoperiod (Egloff et al. 1997).

Although, the general trend of its population cycle is known, the difficulty of rearing these organisms in laboratory has complicated the estimation of most of their reproductive parameters (Della Croce 1964, Tang et al. 1995, Marazzo & Valentin 2003b, Wong et al. 2004). The life cycle of *Penilia avirostris* is characterized by an alternation between gamogenesis and parthenogenesis. Their populations are initiated by the hatching of resting embryos, followed by peaks of high abundance when parthenogenetic females reproduce (Onbé 1973, 1978). In marine populations gamogenetic individuals usually appear immediately after population maxima, coinciding with decreasing parthenogenetic reproduction. Factors triggering the

transition from parthenogenesis to gamogenesis remain unclear (Onbé 1974, Fofonoff 1994). In freshwater cladocerans it is known that crowding, short day-length, temperature and food availability act as stimulus to induce gamogenesis on parthenogenetic females (Stross & Hill 1968, Carvalho & Hughes 1983, Stross 1987, Fitzsimmons & Innes 2006). Stross (1965) suggested that at least two stimulus are necessary to induce gametogenic reproduction. Besides, other authors suggest that this transition between both types of reproduction is related to the presence of *Wolbachia* or hormones (Stouthamer et al. 1999, Minelli & Fusco 2006). Gamogenic reproduction produces resting eggs, which sink and remain in the sea bottom during the parental population seasonal disappearance from the water column (Onbé 1985, Egloff et al. 1997).

The general trend of population dynamics of *Penilia avirostris* briefly described here, resemble that of many other freshwater cladocerans (Threlkeld 1987). However, opposite to them, we lack deep knowledge about the reproductive characteristics of *P. avirostris* at each of the different phases of its seasonal cycle. Consequently, our objective was to study the seasonal distribution of the population of *P. avirostris* in the Catalan Sea (NW Mediterranean), taking special care at describing in detail the reproductive condition of the females along the seasonal cycle. We believe that certain aspects of *P. avirostris* life cycle should contribute to explain the explosive growth and sudden disappearance of this species from the water column. We also discuss here the influence that some biological and physical factors could have on the temporal variation of this species.

## METHODOLOGY

*Penilia avirostris* population size and dynamics were studied from June 2003 to December 2004 at a nearshore station (1 mile from coast) located off Barcelona (Catalan Sea, NW Mediterranean), characterized by shallow waters in open coast. The samples were collected by vertical net tows (without flowmeter) pulled by hand from the bottom (max. 38 m depth) to the surface with a microplankton net (53 µm mesh, 25 cm mouth diameter). The content of the cod end was preserved in borax-buffered formaldehyde at 4% final concentration.

In the laboratory, the total abundance of *Penilia avirostris* was determined by stereomicroscope counts of two 5-ml aliquots from each sample (sample volume: 250-ml), resulting in at least 300 individuals of *P. avirostris* counted per sample. In addition, for each sample 50 individuals of *P. avirostris* were randomly sorted out, sized and staged. Organism were classified as juveniles (<500 µm), non-reproducing females, parthonegenetic female (with embryos), gametogenetic female (with resting eggs), and males. Body length (BL, from the tip of the head to the base of the caudal setae; Uye 1982) was converted into dry weight (DW, µgC) using the length-weight

relationship log (DW) = 2.66 log BL – 7.369 (Atienza et al. 2006a), and assuming that carbon content was 50% of dry weight (Uye 1982).

For each of the 50-individual sorted groups, all reproducing females (i.e. carrying embryos or resting eggs) were dissected carefully with fine needles under the stereomicroscope, and the embryos or eggs in the brood pouch were removed, counted and examined. Resting eggs were easily identified because they are ovoid, flattened, with a thick chitinous membrane and opaque (Figure 2.1E), occupying the entire brood pouch. Parthenogenetic embryonic development was divided into four different stages based on the external morphology of the embryo, following the detailed description by Della Croce & Bettanin (1965) and the simplified approach of Wong et al. (2004). Stage 1 corresponds to the parthenogenetic egg, ellipsoidal (although the posterior border of the head can be distinguished in advanced phase), surrounded by an elastic membrane and almost completely filled with yolk granules (Figure 2.1A). In stage 2 the cover membrane disappears, the second antenna starts to develop and the thorax region is differentiated (Figure 2.1B). During stage 3 the embryo elongates, the second antenna is fully formed, and the first antenna starts to elongate (Figure 2.1C). At this stage all the thoracic segments are visible, and sometimes rudiments of thoracic appendages are evident. Stage 4 embryos are similar to adults, with all the thoracic appendages completely formed, and the carapace and the eye fully developed (Figure 2.1D).

The population growth rates ($r$, $d^{-1}$) were calculated from successive pairs of population abundance between two sampling dates (respectively $N_1$ and $N_2$) as

$$r = 1/t * \ln (N_2/N_1) \qquad \text{Equation (1)}$$

where t is the corresponding time interval between samplings (in days).

Recruitment from parthenogenetical eggs was estimated using the egg ratio method (Edmonson 1968, Paloheimo 1974), which yields an estimate of the instantaneous per capita birth rate ($b$, $d^{-1}$),

$$b = \ln ((E/N) + 1)/D \qquad \text{Equation (2)}$$

where E is the number of parthenogenetic eggs and embryos recorded, N is the total population size, and D is the egg development time (in days). D was estimated from surface temperature in the water column using the equation of Bottrell (1975) for freshwater cladocerans,

$$\log D = 0.847 (\log T)2 - 3.609 \log T + 3.796 \qquad \text{Equation (3)}$$

where T is temperature in Celsius degrees. The use of Bottrell's equation is warranted by the study of Valentin & Marazzo (2004), who obtained field estimates of embryonic development time for *Penilia avirostris* (2-3 d) similar to the estimates by

Bottrell's equation (2.4-2.7 d) at the respective temperatures (26.0 and 24.7 °C, respectively).

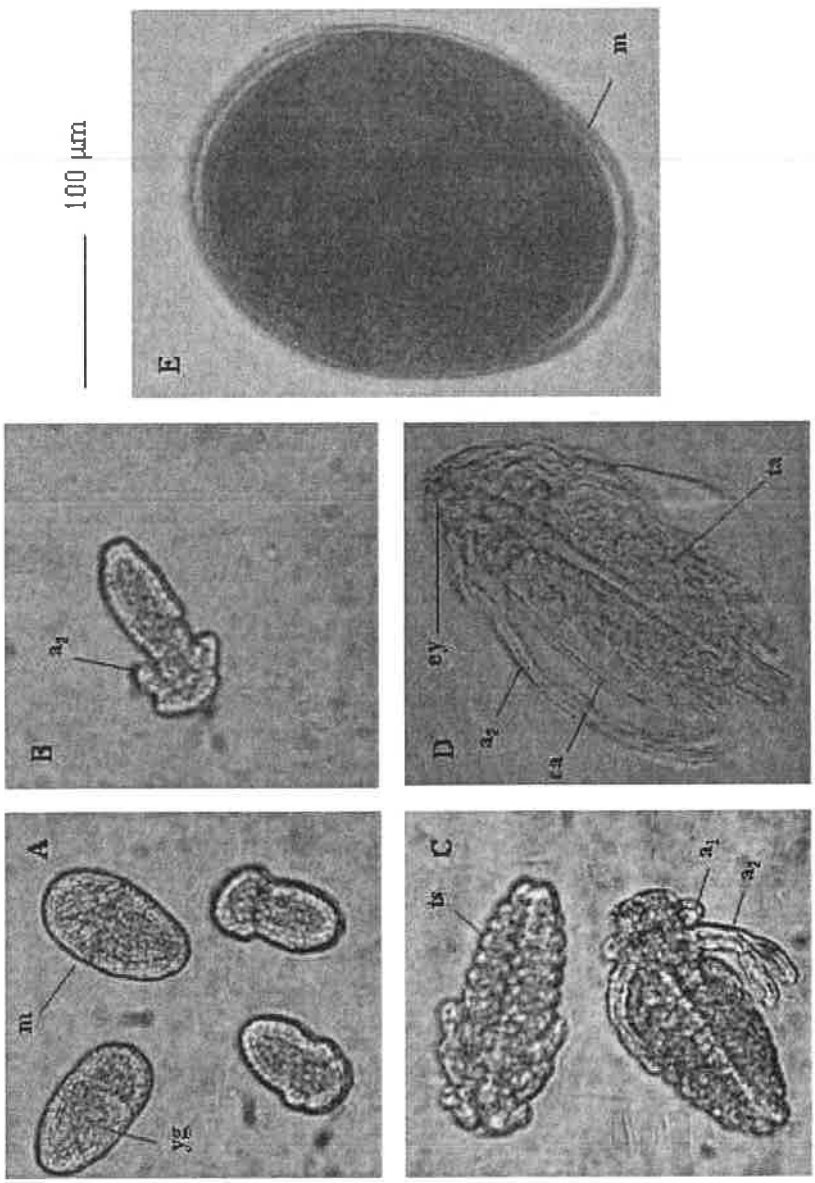



Figure 2.1. Developmental stages of *Penilia avirostris* defined for this study. A) Stage 1; B) Stage 2; C) Stage 3; D) Stage 4; E) Resting egg. $a_1$: first antenna; $a_2$: second antenna; ca: carapace; ey: eye; h: head; m: membrane; ta: thoracic appendices; ts: thoracic segments; yg: yolk granules. Magnification 200X. Scale bar denotes 100 µm.

## RESULTS

Figure 2.2 shows the temporal variation of surface temperature, chlorophyll *a* concentration, and *Penilia avirostris* densities during the period of study. Temperature ranged from ca. 12 °C in winter up to ca. 28 °C in summer, and chlorophyll *a* concentration showed peaks in winter, spring and early summer, depicting the typical pattern for the northwestern Mediterranean (Lipej et al. 1997, Calbet et al. 2001). *P. avirostris* populations showed a clear seasonal pattern of abundance through the sampling period (Figure 2.2C), characterized by abrupt increase and decline of the yearly population standing stocks. During most of the year *P. avirostris* were absent from the water column, first individuals starting to appear at the beginning of July, and reaching peak values by the end of the month (ca. 2500-3000 ind $m^{-3}$, Figure 2.2C). This population of *P. avirostris* maintained until the end of August (2003)-September (2004), when the population suddenly reduced its presence in the water column to almost complete absence (followed in 2003 for sporadic low peaks, <500 $ind^{-1}$ $m^{-3}$, until December).

The temporal variation of *Penilia avirostris* population composition is shown in Figure 2.3. During the growing phases and the peaks of high abundance the populations were evenly dominated by juveniles, non-reproducing females and parthenogenetic females (i.e. with embryos). When the populations were in the waning phase (middle September in both years), the composition of the community changed, and males appeared in the community, followed by gametogenic females (i.e. with resting eggs). Afterwards, during 2003 *P. avirostris* kept its representation in plankton under very low levels, mostly as females, until they completely disappeared. In the autumn 2003 *P. avirostris* peaks, juveniles represented a much lower fraction of the population compared to other periods, being the relative contribution of non-reproducing females higher, which indicates that recruitment during those peaks failed.

Table 2.1 shows the body sizes of *Penilia avirostris* adults. Gamogenic females were significantly larger that males and the other 3 female types (Two-way ANOVA,

Table 2.1. *Penilia avirostris* comparative size of the different reproductive stages between years. Avg: average; SE: standard error; n: sample size; ** significant at 0.01.

| | 2003 | | | 2004 | | | |
|---|---|---|---|---|---|---|---|
| Reproductive stage | Avg | SE | n | Avg | SE | n | |
| Females | 632.3 | 5.01 | 353 | 606.03 | 5.31 | 188 | ** |
| Females with embryos | 673.4 | 4.99 | 298 | 641.46 | 4.56 | 228 | ** |
| Males | 651.1 | 8.93 | 31 | 629.05 | 16.71 | 23 | |
| Females with resting eggs | 799.8 | 16.66 | 8 | 721.92 | 11.41 | 19 | ** |

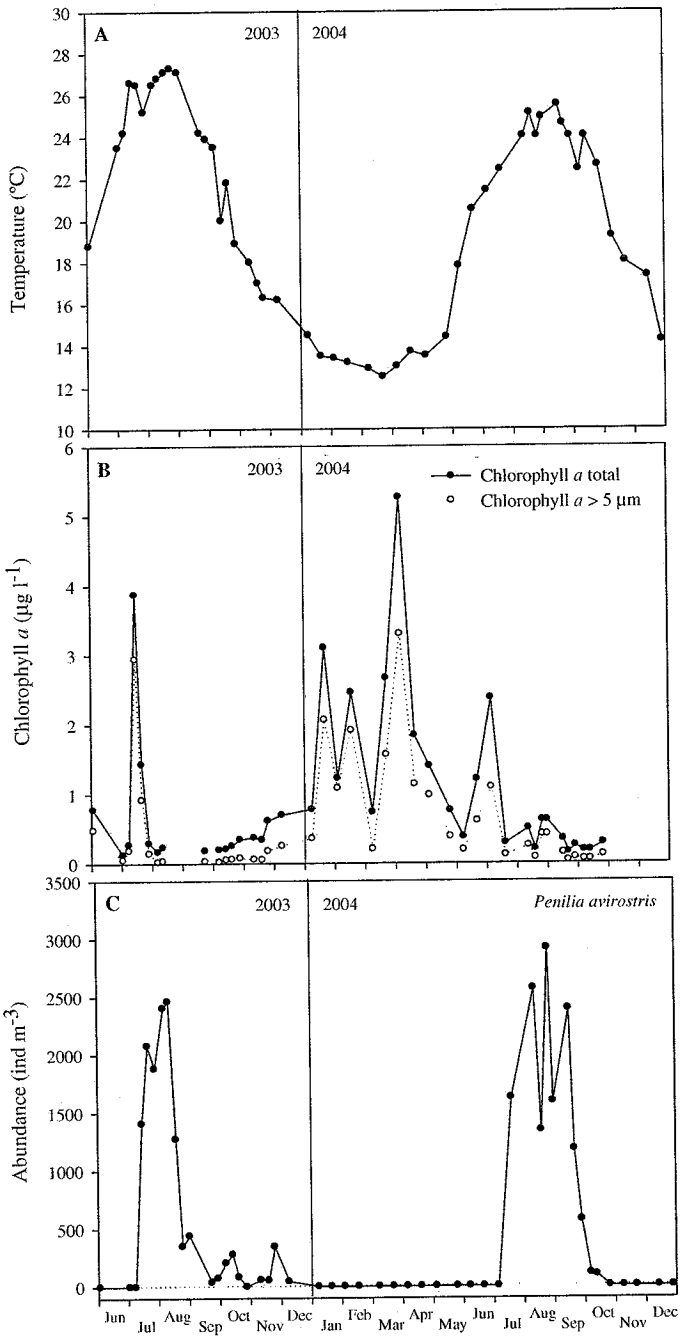



Figure 2.2. Seasonal variation of surface water temperature (A), chlorophyll *a* concentration (B), and *Penilia avirostris* abundance (C) in the coastal Catalan Sea during the study.

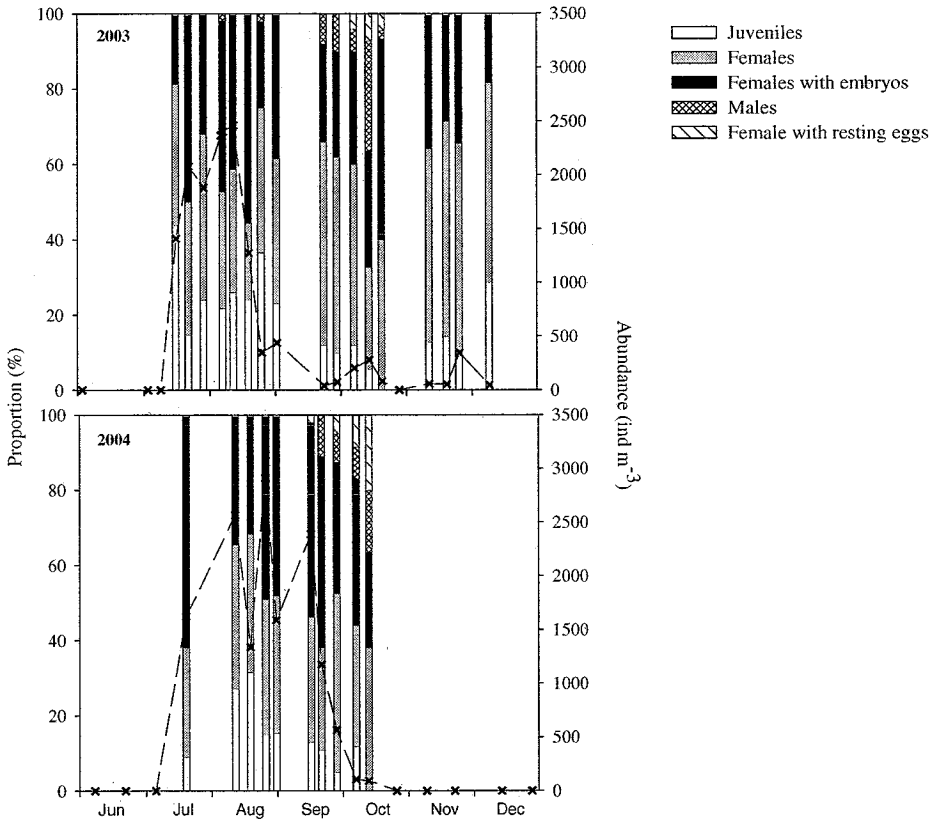



Figure 2.3. Population composition (as %) of *Penilia avirostris* during summer 2003 (upper panel) and 2004 (lower panel). Abundance of *P. avirostris* from Figure 2.2 is overlaid (dashed line) for the sake of the comparison.

2-tailed, $p < 0.05$; Tukey's HSD post-hoc test). Mean sizes for year 2003 were significantly larger (Two-way ANOVA, 2-tailed, $p < 0.01$), partly as a consequence of the fact that the late autumn females in 2003 (absent in 2004) inhabited colder waters and were significantly larger than the summer ones (Figure 2.4, Table 2.2).

Brood size ranged from 1 to 8 embryos per female, and was positively related to female body size (2003: $r = 0.79$, $p < 0.001$; 2004: $r = 0.86$, $p < 0.001$; Figure 2.5). Most females typically carried 2-4 embryos (Figure 2.6), and the ANOSIM test confirms that there is no significant differences in mean brood size between 2003 and 2004 (89.2%). There was no clear trend of variation in brood size through the population development (Figure 2.7). Interestingly, during the phases of decline of the population of *Penilia avirostris* and even during the late peaks in 2003, parthenogenic females carried a significant number of embryos. Regarding females

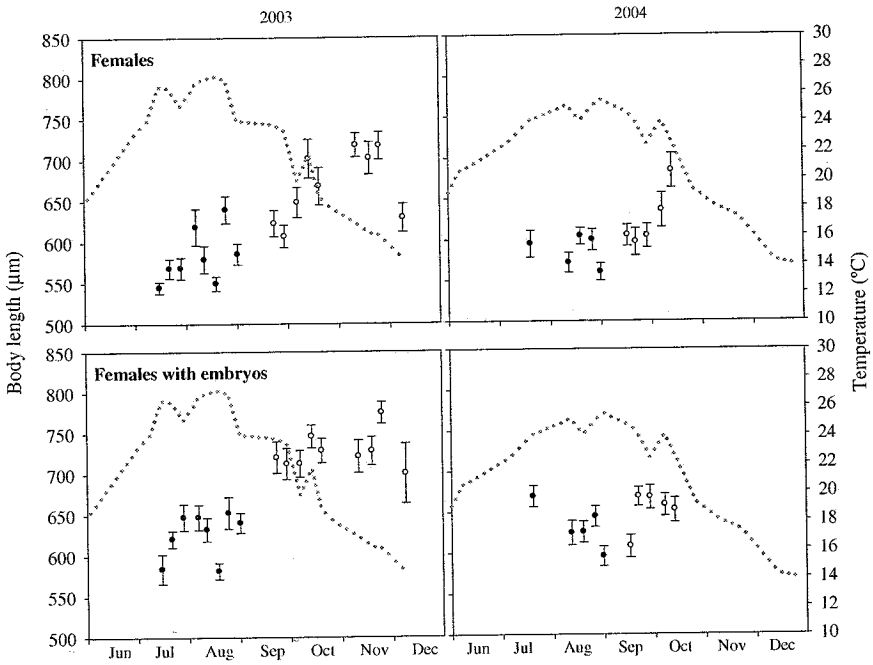



Figure 2.4. Changes in body size of parthenogenetic females (non-reproducing and embryo-carrying) through the seasonal presence of *Penilia avirostris* in the Catalan Sea. Filled circles: summer samples (July-August); Open circles: autumn samples (September-October-November). Dotted line is surface water temperature (from Figure 2.2).

Table 2.2. Comparative size of *Penilia avirostris* parthenogenetic females (females with and without embryos) between different periods in the same year. Avg: average; SE: standard error; n: sample size; ** significant at 0.01.

| **2003** | Summer | | | Autumn | | | |
|---|---|---|---|---|---|---|---|
| Reproductive stage | Avg | SE | n | Avg | SE | n | |
| Females | 583.4 | 5.68 | 142 | 666.1 | 6.56 | 211 | ** |
| Females with embryos | 624.7 | 5.16 | 161 | 730.6 | 6.08 | 137 | ** |

| **2004** | Summer | | | Autumn | | | |
|---|---|---|---|---|---|---|---|
| Reproductive stage | Avg | SE | n | Avg | SE | n | |
| Females | 585.5 | 5.69 | 96 | 627.5 | 8.57 | 92 | ** |
| Females with embryos | 635.6 | 6.33 | 122 | 648.2 | 6.55 | 106 | |

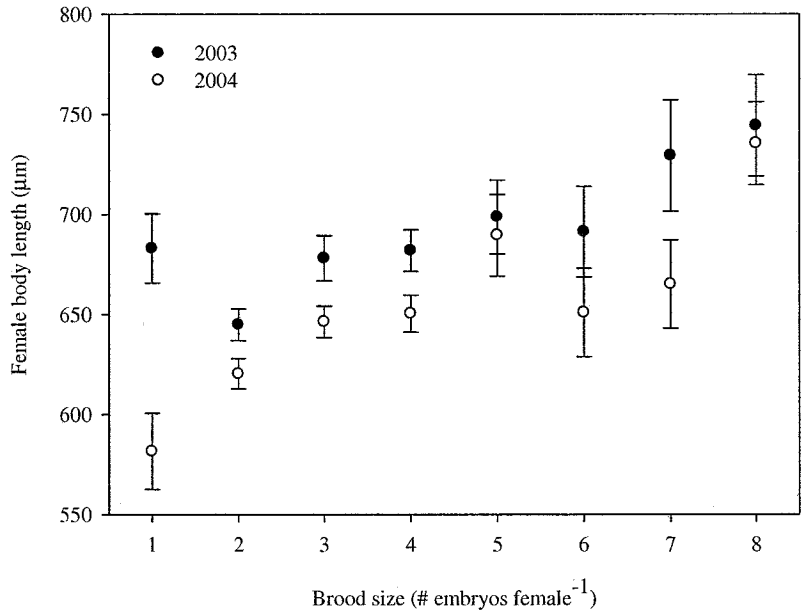



Figure 2.5. Scatterplot of brood size and body size of female *Penilia avirostris*.

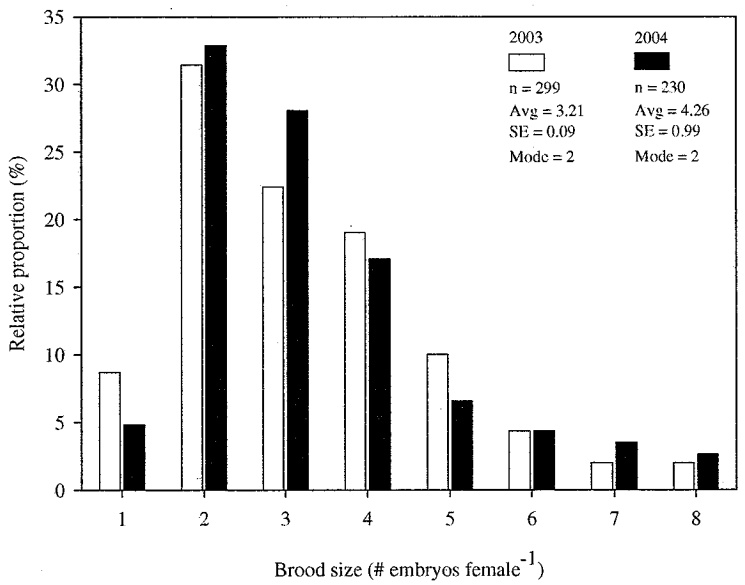



Figure 2.6. Frequency distribution of brood size in *Penilia avirostris* population in the Catalan Sea. Arithmetic mean, standard error and mode of the distribution are also given. Avg: average; SE: standard error; n:

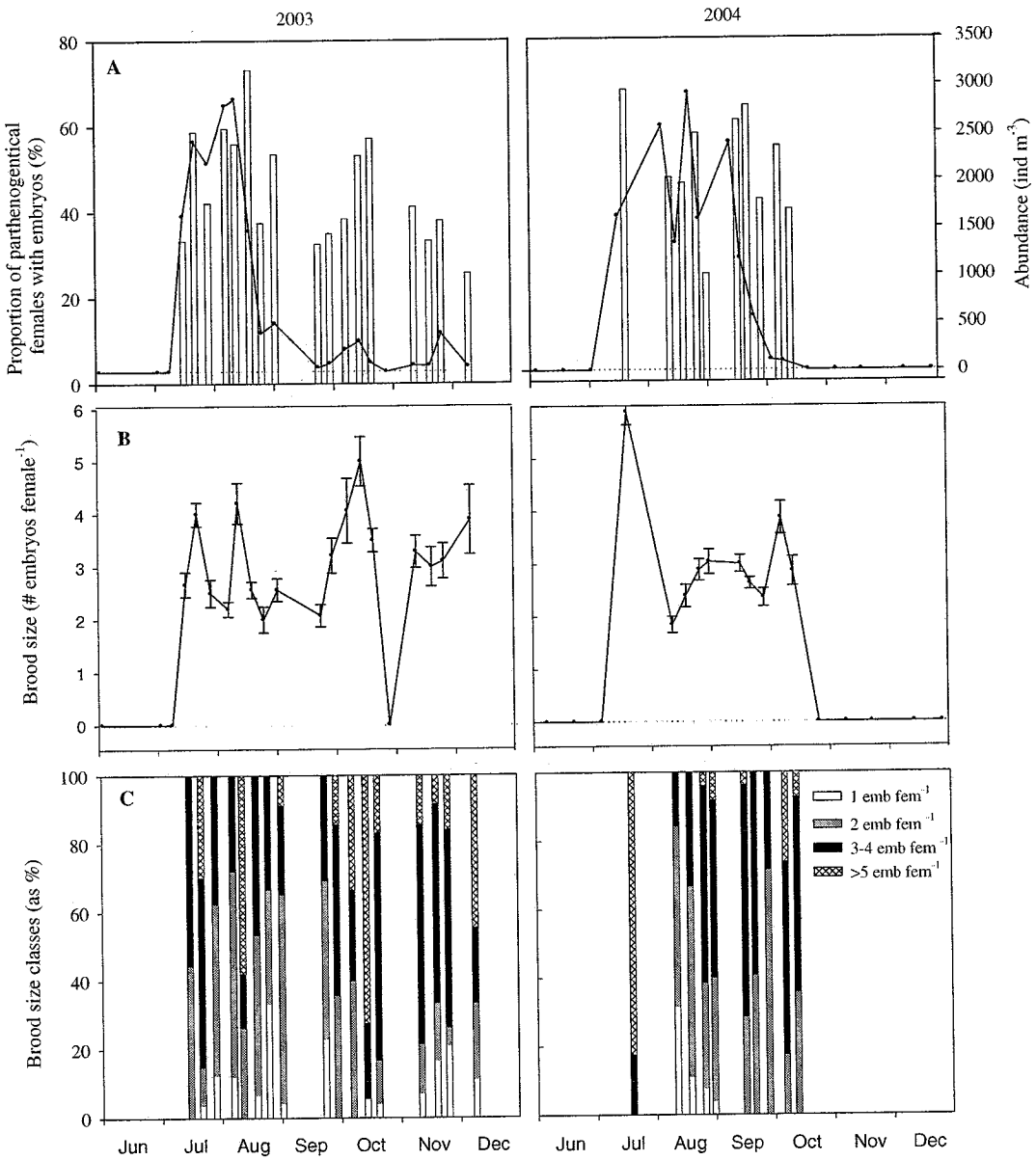



Figure 2.7. (A) Proportion of parthenogenetic females carrying embryos during the seasonal presence of *Penilia avirostris* in the Catalan Sea. Abundance of *Penilia avirostris* from Figure 2.2 is overlaid (continuous line) for the sake of the comparison. (B) Variation of brood size (average ± 1SE). (C) Brood size class distribution (as %).

with resting eggs, they typically carried only one egg, except for two females out of 16 in 2003, which carried two. Moreover, is interesting to point out that the proportion of parthenogenetic females with embryos (in relation to the total parthenogenic females) was higher during the increasing periods of the population, and lower when the population was decreasing (Figure 2.7A).

Table 2.3. Size of the different embryo stages of *Penilia avirostris*. Avg: average; SE: standard error; n: sample size. No significant differences were found.

| | 2003 | | | 2004 | | |
|---|---|---|---|---|---|---|
| Embryos stage | Avg | SE | n | Avg | SE | n |
| Stage 1 | 86.5 | 4.61 | 88 | 85.5 | 4.22 | 68 |
| Stage 2 | 179.8 | 3.68 | 57 | 179.4 | 3.31 | 50 |
| Stage 3 | 221.4 | 2.75 | 103 | 219.9 | 3.69 | 87 |
| Stage 4 | 315.4 | 6.88 | 51 | 305.0 | 8.63 | 23 |
| Resting eggs | 282.3 | 14.97 | 8 | 277.2 | 2.99 | 1 |

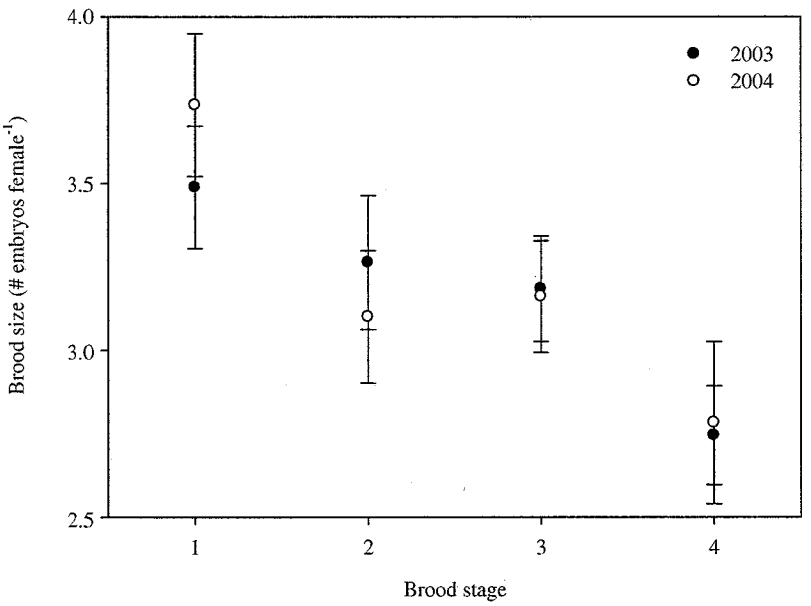



Figure 2.8. Scatterplot of embryo stage and brood size of *Penilia avirostris*.

Embryo length of *Penilia avirostris* differed according to developmental stage (Table 2.3) ranging from 86 to 315 μm. The latter stage set the smaller body size for the free living *P. avirostris* in the water column. No significant differences were found in embryo length between both years (Two-way ANOVA, 2-tailed, $p < 0.05$). There was a negative correlation between embryo stage and brood size (2003: $r = -0.95$, $p < 0.001$; 2004: $r = -0.90$, $p < 0.001$; Figure 2.8).

### Population growth, birth rates, and mortality rates of *Penilia avirostris* population

The estimated birth rates were very variable, with values up to ca. 0.8-1 $d^{-1}$ in some periods (Figure 2.9B). This variability reflected mainly the changes in abundance of embryo-carrying females and brood size, whereas the embryonic developmental time, which depended on the changes in surface water temperature during the seasonal presence of *Penilia avirostris*, varied between 1.45 to 2.36 days. Birth rates were maximum during the initial blooming period, thereafter showing diverse peaks not associated with changes in *P. avirostris* density (Figures 2.9A and 2.9B). Actually, during declining phases of *P. avirostris* population birth rates were rather high.

The instantaneous population rate of change (Figure 2.9C) ranged from -0.55 to 1.04 $d^{-1}$. Positive high population growth rates, equivalent to ca. 1 doubling per day, were recorded at mid July for both years, coinciding with the growth of the *Penilia avirostris* populations. During the period of population stability, population growth rates were variable but close to zero. The greatest decreases in population occurred in October.

The discrepancy between the observed population growth rates and the calculated birth rates provides an estimate of population mortality or loss rates (data not shown).

## DISCUSSION

The order Cladocera has not been very successful in colonizing the marine environment in comparison to freshwaters, and it has required the evolution of adaptation mechanisms at the morphological, physiological and behavioural level (Critescu & Hebert, 2002). The acquisition of a closed brood pouch to keep the embryos in a suitable nourishing environment, the presence of a resting egg provided with a thick wall instead of a ephippium, and the predatory grasping mode instead of filter-feeding seem to be essential features for this colonization (Lochhead 1954, Aladin & Potts 1995, Critescu & Hebert 2002). The seasonal distribution of *Penilia avirostris* populations in the Catalan Sea (NW Mediterranean) was studied in relation to a wide variety of reproductive and life history parameters. We attempted to relate

all these parameters to find the possible reasons for its explosive population growth and the sudden disappearance from the water column. *P. avirostris* showed a noticeable seasonality, being abundant between July and October, when higher abundance during August.

The seasonal pattern shown by *Penilia avirostris* populations in the Catalan Sea begins with a sudden appearance of *P. avirostris* in the water column at late spring- early summer, with rapid population growth period until a dense population is established and maintained during the rest of the season, and a population decline by the end of summer. Whereas *P. avirostris* can exhibit continuous presence in the zooplankton community in tropical and subtropical latitudes (e.g. Agulhas Bank, Algiers, Sierra Leone; Della Croce & Venugopal 1973), the unimodal seasonal pattern reported here for this species is the typical one exhibited in temperate ecosystems, as previously described in the Mediterranean (Alcaraz 1970, Siokou-Frangou 1996, Lipej et al. 1997, Calbet et al. 2001, Fernández de Puelles et al. 2003, Ribera D'Alcalà et al. 2004) and in many other parts of the world ocean (Della Croce & Venugopal 1973, Alcaraz 1981, Rocha 1982, Onbé & Ikeda 1995, Tang et al. 1995).

The abrupt appearance and vanishment of the populations is very characteristic for this marine cladoceran and reflects a typical opportunistic life history linked to the production of resting eggs to overcome unfavourable seasons and to parthenogenetic reproduction for fast population growing (Kim et al. 1994, Egloff et al. 1997, Valentin & Marazzo 2003a). In this regard, the life cycle of *Penilia avirostris* follows the general pattern exhibited by other marine and freshwater cladocerans, being characterized by two modes of reproduction, parthenogenesis and gametogenesis (Smith 1963, Sanders & Wickham 1993, Boersma & Vilverberg 1996). Related to both types of reproduction, also two kinds of eggs are produced by this marine cladoceran, a thin-walled parthenogenetic egg and a thick-walled resting egg. Resting eggs are larger (279 μm), fewer in number, and full of yolky cells if they are compared to parthenogenetic eggs (86 μm). The production of more than one brood of resting eggs by a gamogenic female has not been established. In general, gamogenic females had one resting eggs, although some specimens could have two resting eggs (Egloff et al. 1997). In podonids when two resting eggs are present, one or both may be abort (Egloff et al. 1997). In our study females always carried one resting eggs, with two exceptions during 2003 where two resting eggs were present in the brood pouch. In *P. avirostris* the outer membrane of resting eggs is calcified thus increasing the weight of the egg and ensuring that it sinks after release from the female and perhaps providing protection from predators in the sediments (Egloff et al. 1997). Some authors (Onbé 1985) have speculated that resting eggs may survive passage through the digestive systems of planktivores and detritivores. In addition, resting embryos that sink may be an important adaptation to avoid transport by strong currents to distant and unfavourable habits. The distribution and abundance of resting eggs has been supposed to be a critical factor influencing the overall

distribution, seasonal population dynamics, and long-term variations in the abundance of cladocerans

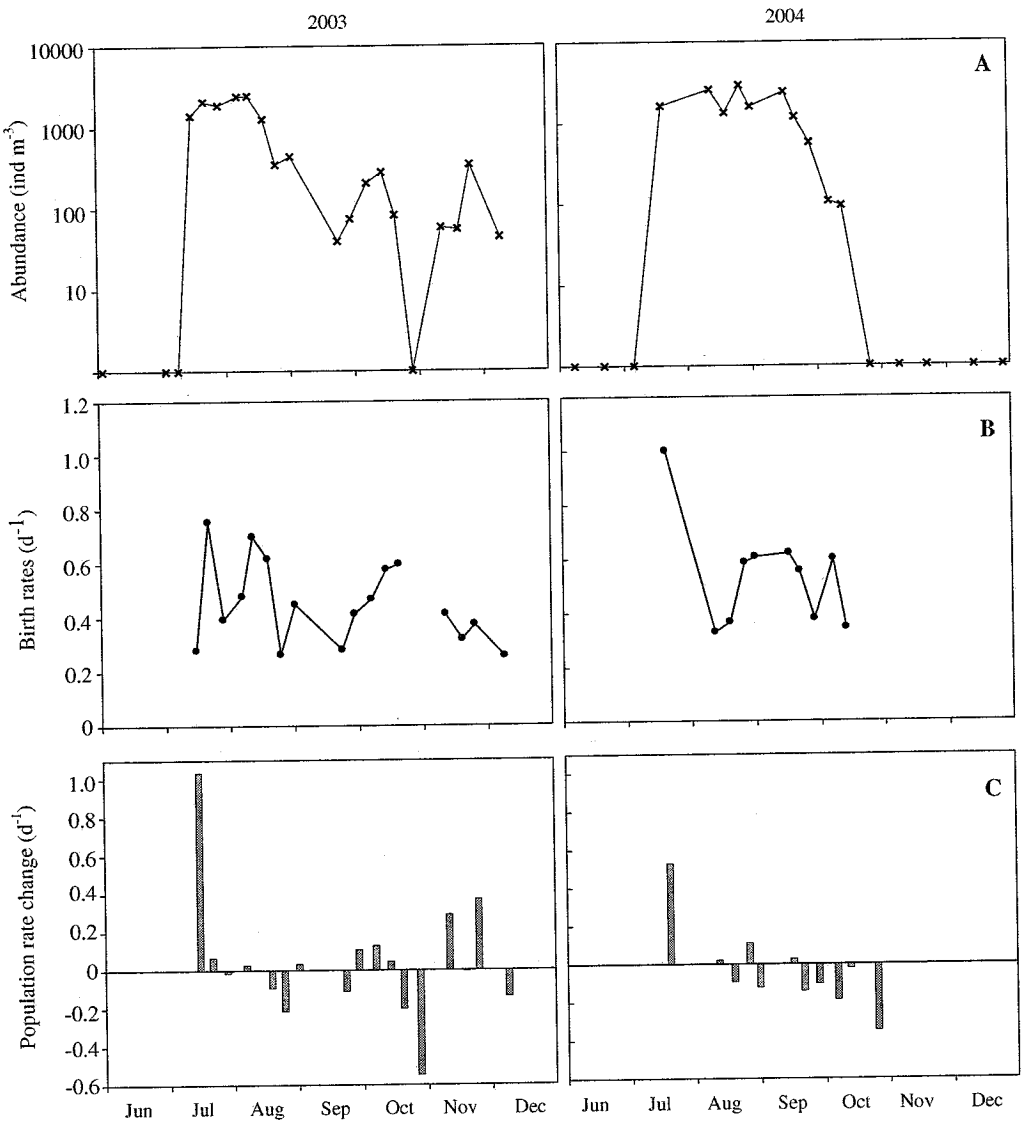



Figure 2.9. (A) Abundance of *Penilia avirostris*. (B) Estimated birth rates. (C) population rate of change. Notice that abundance data are the same that is shown in Figure 2.2, but here they are in log scale for the comparison with the population change rate data.

(Viitasalo & Katajisto 1994). Some studies shown the presence of cladoceran' resting eggs in the Mediterranean (Moscatello & Belmonte 2004, Sioko-Frangou et al. 2005), however, the temporal variation of resting eggs were little studied. Only Sioko-Frangou et al. (2005) reported that resting eggs of the cladocerans *P. avirostris*, *Evadne* spp., and *Podon* spp. were common in the sediments of NW Aegean Sea (Greece), and also that *P. avirostris* resting eggs were more abundant on September and October, ranging between 63 to 76 egg $m^{-2}$. In other regions, such as Inland Sea of Japan, the seasonal fluctuations in the abundance of resting eggs of this marine cladoceran are described in detail (Onbé 1985).

Besides, birth and growth rates are achieved by a combination of brood size and developmental times, and constitute useful parameters in the population dynamics of this marine cladoceran. Estimated birth and growth rates for *Penilia avirostris* (*b,* 0.7-1.1 $d^{-1}$; maximum *r*, 1.0 $d^{-1}$) are much higher than those estimated for freshwater cladocerans (b, 0.01-0.40 $d^{-1}$, maximum *r* 0.20 $d^{-1}$ for *Daphnia*; De Mott 1983). As a consequence of brood and short generation time at the initial phases of population appearance growth could be spectacular. High values for *r* (0.8-1.4 $d^{-1}$) were found for *Evadne nordmanni* and *Podon leuckarti* populations in the Kiel Fjord (Poggensee & Lenz 1981), which could be a consequence of pædogenesis (Egloff et al. 1997). It is important to point out that our estimates of birth rates were calculated by Paloheimo´s (1974) method which is influence by the estimation done of developmental time, and they are also influence by temperature.

Our data on brood size are similar to previous reports for this species (Della Croce & Venugopal 1973, Angelino & Della Croce 1975, Tang et el. 1993, Valentin & Marazzo 2003a, 2003b), although we did not observe values up to 13 embryos per female as recorded by Angelino & Della Croce (1975) in the Agulhas Bank and Knysna Lake (South Africa). In general, brood sizes of marine cladocerans are higher during the initial phases of population growth, and decrease rapidly as population increases (Onbé 1974, Platt & Yamamura 1986, Mullin & Onbé 1992, Fofonoff 1994). We did not find any clear pattern, although higher brood sizes were detected at the beginning of the 2004 populations. A simple calculation considering an average initial brood size of 4 (which corresponds with our mean value of brood size at the begging of the population), 2 days embryonic developmental time (Mullin & Onbé 1992; Atienza et al. submitted, chapter V), and 1 day of pre-reproductive animals (Atienza et al. submitted, chapter V) renders that every single female produces near 100 young individuals in 8 days. Therefore, parthenogenetic reproduction helps us to understand the rapid appearance and dominance of this marine cladoceran in the water column. Also, we found a positive correlation between temperature and the brood size (data non shown), when populations start to grow the temperature is also increasing, and brood size was also increasing, enabling the population to increase at high rates. Alcaraz (1970) also found that temperature was positively correlated with the reproductive potential (brood size) for *Evadne spinifera.*

Once the embryos were deposited on the brood chamber, development is also a crucial parameter to understand population growth. There are only three estimations of embryo developmental time using natural data, Mullin & Onbé (1992), Marazzo & Valentin (2004), and Atienza et al. (submitted) (chapter V), which reported ≈2 days as the developmental time for *Penilia avirostris'* embryos in natural conditions. We made an estimation using Botrell´s equation (1975), which was developed for freshwater cladocerans, and as we said in the methodology, the accuracy of this equation was previously validated by Marazzo & Valentin (2004). These authors reported that direct estimations of developmental time of *P. avirostris* agree with the values obtained by the equation. Our estimations is only a preliminary approach and laboratory experiments are required to determine the natural duration of overall embryonic development and to estimate the real effect of temperature on this variable. To achieve this short generation times the oligolecithical nature of the eggs of marine cladocerans is important and the nourishment by the "placental organ" is determinant in this development (Egloff et al. 1997). The rapid growth during development is a consequence of the volume increase fourfold between the egg (stage 1) and the neonate (stage 4). Neonates are one-half to two-thirds to their eventual length as adults, which agrees with the typical proportion observed by other authors (Egloff et al. 1997).

This shift from parthenogenetic to gamogenetic reproduction is an interesting phenomenon when studying cladoceran dynamics and which is not fully understand. In general, population maxima are accompanied by the onset of gamogenic reproduction and a sharp reduction in parthenogenetic reproduction. In natural populations, only a proportion of parthenogenetic females turn into gamogenic. This trend was also observed in our study, were the maximum recorded proportion of gamogenic individuals ranged from 4 % to less than 8 % in 2003 and from 1 % to 20 % during summer 2004, which corresponds to a small fraction of the total population. Marazzo & Valentin (2003b, 2004) reported the only previous estimations for this marine cladoceran, (between 11% and 24%) which agree with our results. Also, our estimates are similar to 8% - 25% for *Evadne nordmanni* and *E. spinifera*, and 5% - 10% *Pseudoevadne tergestina* (Onbé & Ikeda 1995). Our results are, however in the lower range reported for other marine cladocerans (10-46 % *Pleopis polyphemoides*, Fofonoff 1994; 20-23 % *Pseudoevadne tergestina,* Onbé 1978; 50-80 % *Podon leuckarti*, Egloff et al. 1997; and 25-60 % *Evadne nordmanni*, Fofonoff 1994). After sexual reproduction, resting eggs are produced and there are capable of undergoing on a prolonged diapause and carrying the populations through overwintering periods (Egloff et al. 1997, Korovchinsky & Boikova 1996).

As previously shown, the explosive growth of *Penilia avirostris* populations could be explained by parthenogenetic reproduction, short developmental time, and higher birth rates. In contrast, the possible causes for their suddenly disappearance form the water column are not well understood. Different parameters, such as

decrease of temperature, photoperiod, food availability, turbulence, crowding, and predation have been suggested (Stross & Hill 1968, 1969, Gyllström & Hansson 2004). We will try to address how these parameters could explain the abrupt decrease in *P. avirostris* populations in the Catalan Sea.

While the causes of this shift remain unknown, there is a constant pattern in the appearance of gamogenic animals: in populations of *Penilia avirostris* and other marine cladocerans species, gamogenic females and males invariably appear when the population density is maxima (Egloff et al. 1997), which agrees with our observations. Although the mechanisms of sex determination in marine cladocerans remain unidentified (Egloff et al. 1997), it is known they are linked to environmental conditions, at least for freshwater species (Frey 1982, Fofonoff 1994): when resources are favourable for population growth, parthenogenetic reproduction predominates; when resources are less favourable, gamogenetic reproduction predominates. Also, some authors suggest that sex determination is linked to the presence of the intracellular bacteria of the genus *Wolbachia* (Stouthamer et al. 1999).

Therefore, to fully understand the shift between these two types of reproduction it is necessary to know why males start to appear in the population. Temperature, day length, food concentration, and population density are suggested as the main factors that induce the production of males and in the switch to sexual reproduction in freshwater species (Stross 1969, 1987, Kleiven et al. 1992). Previous studies show that several stimuli are necessary to initiate the induction of males, and in *Daphnia* these are well known, including photoperiod, population density, water temperature and food availability (Berg & Pálsson 2001, Olmstead & LeBlanc 2001). Although the exact combination of signals required seems to vary between taxa. We did not find any correlation between temperature (or any other parameters) and the apparition of males or gametogenetic females, which point to multiple stimuli having a synergic effect on the apparition of males.

An interesting aspect about our seasonal pattern is that on 2003, *Penilia avirostris* disappeared from the water column in late October, but in middle November started to appear again, although the population was dominated by females without embryos or resting eggs. This similar event was reported by Alcaraz (1970) in Castellon waters, and by Lipej et al. (1997) in the Gulf of Trieste. Those authors found that *P. avirostris* populations started to decline after September, but in early October populations increased again, being in the plankton at low numbers until December. Lipej et al. (1997) described that large gametogenic females were the dominant organisms from September to December, which is opposite to our findings. Although the same pattern was previously reported, we suggest that the disappearance of *P. avirostris* in late October was only a local event and was not extended in all the Catalan Sea. In chapter I it is shown that during September 2003 the disappearance of this marine cladoceran occurred only in the central shelf of the Catalan Sea (where the sampling station was located), associated to the presence of high saline waters. Therefore, the

appearance of organisms in the middle of November could be the result of the disappearance of those saline waters, and the extension of the presence of *P. avirostris* from the other regions of the Catalan Sea (north and south).

Other possible reason for the decreasing of *Penilia avirostris* populations is the temperature, which has been proposed as the main physical factor that control *P. avirostris* populations (Gieskes 1971a, Onbé & Ikeda 1995). In general, populations of *P. avirostris* are often associated with warmer water in the northern (Lipej et al. 1997, Calbet et al. 2001) and southern hemisphere (Ramírez & Pérez Seijas 1985, Resgalla & Montú 1993, Onbé 1999). According to this, the marked seasonality in the occurrence of *P. avirostris* in the plankton seems highly modulated by water temperature. We found a positive correlation between the abundance of this marine cladoceran and temperature. However, the influence of temperature on the population cycles of *P. avirostris* is not fully understood, and we suggest that probably this influence is related with an optimum range where *P. avirostris* could reproduce and grow successfully. Some authors pointed out that the distribution of *P. avirostris* is mostly restricted to waters above 18°C but can range between 12°C and 30°C (Kim et al. 1994, Kim & Onbé 1995), and it is known that this species had an optimum temperature around 25°C (China: Tang et al. 1995, Japan: Onbé et al. 1996). Recently, Johns et al. (2005) showed that this marine cladoceran is increasing its prevalence in the North Sea, where the sea surface temperature have been increasing over the last decade (more than 3°C, and reaching temperatures higher than 19°C). This is a clear example of how temperature can modulate the population dynamic and the occurrence of this species. Moreover, temperature influences birth and population growth rates. Birth rates seem to be more affected by temperature because the direct effect of this factor on the development time. We observed a dramatic declines in *b* in early fall that probably reflected a slower development at lower temperatures, declining in clutch size, and high proportions of females developing resting eggs.

Additionally, physical transport and turbulence may cause the disappearance of this marine cladoceran. Valentin & Marazzo (2003) produced a model to simulate the peaks of *Penilia avirostris* abundance, and the seasonal fluctuations of its population. They used rainfall, salinity and wind data to force their model, and they found that rainy days, which are associated with the passage of cold fronts and the consequently alteration of the hydrological structure of the water column, in conjunction with strong southwest winds are sufficient to simulate the variability during population maxima. We tried to establish correlations or at least to found any qualitative relation between the variation on *P. avirostris* abundance and the wind velocity or rainfall. We could not found any relation to the wind velocity, but we found that rainy days were related to declines in the abundance of *P. avirostris* (especially during 2003) (Figure 2.10).

Food availability was suggested to affect the seasonal dynamic of *Penilia avirostris*. Lipej et al. (1997) and Calbet et al. (2001) indicated that during periods of warm waters (summer), the water column was stratified and the concentrations of nutrients and chlorophyll above the pycnocline layer are rather low, whereas pico- and nanoplanktonic autotrophs are abundant (Vaqué et al. 1997). *P. avirostris* is a filter feeder that ingested preferentially nanoflagellates, and the higher abundance of these organisms results in a higher availability of food that is rapidly exploit by this marine cladoceran (Chapter V). We explored any relationship between the observed seasonal pattern and the concentrations of chlorophyll *a*, but we did not identify any correspondence.

Finally, the last explanation that we consider for the disappearance of *Penilia avirostris* populations could be predation pressure due to invertebrates and vertebrates. Predatory zooplankters have been shown in some instances to have a significant impact on prey populations (Verity & Smetacek 1996). The literature on freshwater zooplankton is replete with examples of predators' effect on prey morphology, size, composition, and abundance (Gorokhova 2004, Thys & Hoffmann 2005). In marine waters, examples are fewer, but it is cleat that predators, such as ctenophores, chaetognaths and fishes can decimate marine cladoceran populations (Nip et al. 2003, Barz & Hirche 2005). In particular, chaetognaths predators (Canino & Grant 1985, Duró & Saiz 2000) can make up a large proportion of zooplankton wet weight, and are potentially one of the main sources of predation pressure on the zooplankton community.

In summary, in the Catalan Sea (NW Mediterranean), the abundance of *Penilia avirostris* varies widely over time, being absent for the water column during most of the year and achieving dense population on summer. Parthenogenetic reproduction and embryonic developmental time achieved in higher birth and population growth rates, which are the main reasons for the explosive growth of *P. avirostris* populations. The declining periods of *Penilia avirostris* populations are characterized by the shift from parthenogenic to gametogenic reproduction.

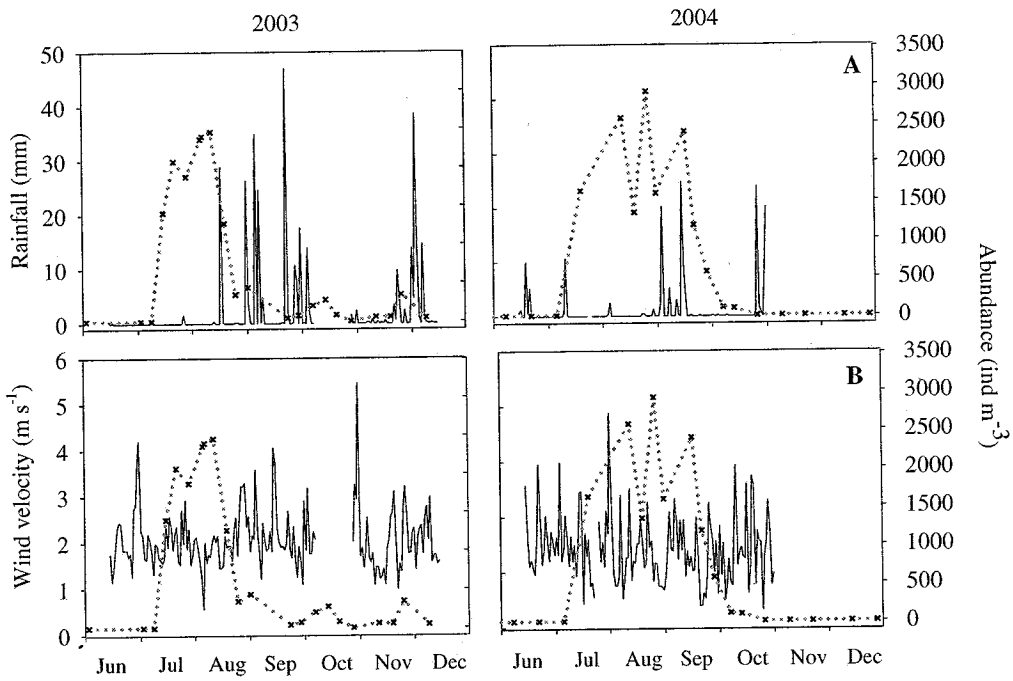
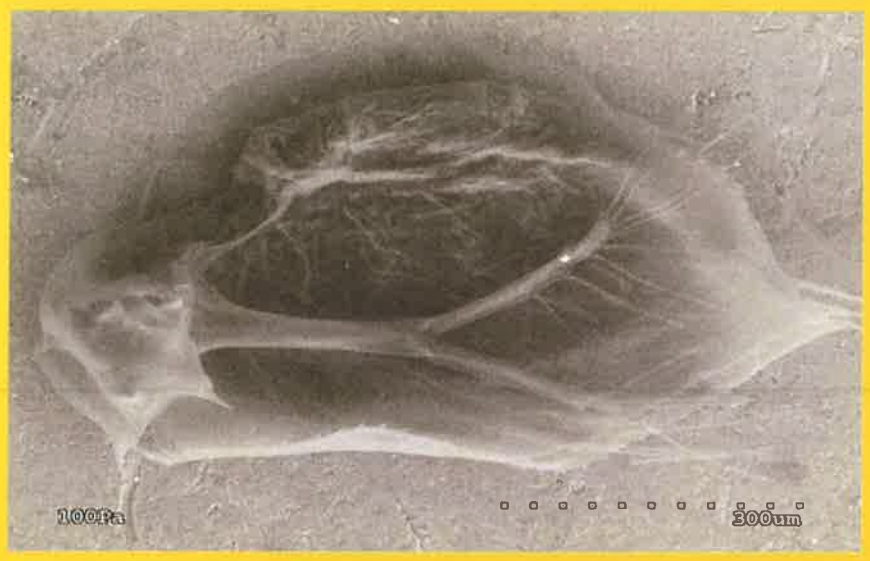
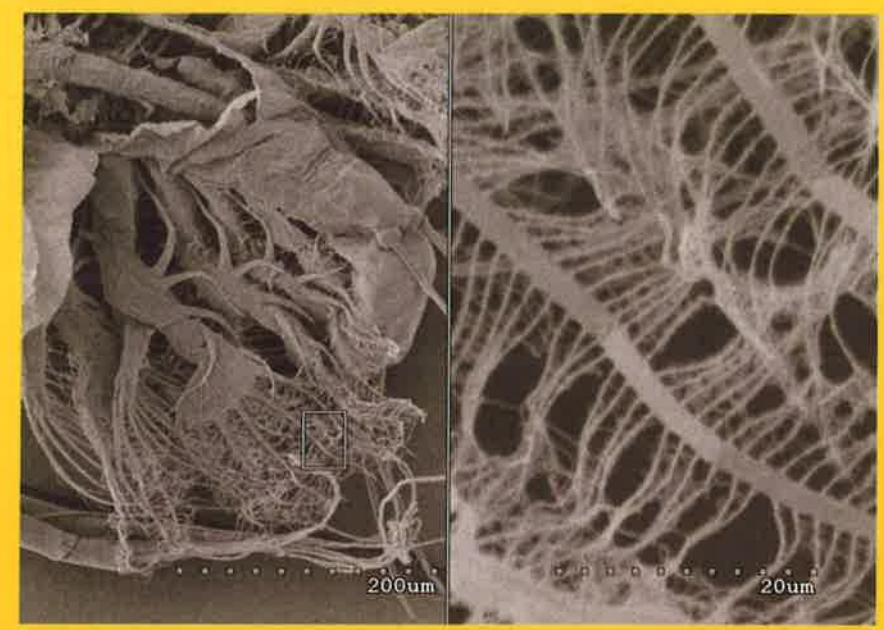



Figure 2.10. Rainfall (A) and wind velocity (B) at a fixed station on the Catalan Sea (NW Mediterranean). Dark line: environmental variable; Dash line: abundance of *Penilia avirostris*.




# CHAPTER III. FEEDING ECOLOGY OF THE MARINE CLADOCERAN *PENILIA AVIROSTRIS*: NATURAL DIET, PREY SELECTIVITY AND DAILY RATION

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# CHAPTER III. FEEDING ECOLOGY OF THE MARINE CLADOCERAN *PENILIA AVIROSTRIS*: NATURAL DIET, PREY SELECTIVITY AND DAILY RATION

## INTRODUCTION

The ecological role of any organism is determined by its position and significance in food webs. Decisive characteristics are body size, food spectrum and feeding strategies. Feeding is one of the most important processes for zooplankton because it supplies the needs to maintain its production and activity. Also, it is the main route for the transfer of energy and matter from lower to higher trophic levels (Valiela 1995, Båmstedt et al. 2000). Therefore, in order to understand the dynamics of pelagic food webs is important to know how consumers select their food and what the rates of prey ingestion are.

Classically, most of the studies on feeding rates and the factors controlling them in marine planktonic systems have concentrated on copepods, the major component of mesozooplankton. However, in recent decades other groups like appendicularians, ctenophores or medusae have received more attention because of their relevance to food web dynamics (Lopez-Urrutia et al. 2003, Gonzalez et al. 2004, Maar et al. 2004). In the present study we will focus on one of such other groups, the marine cladocerans.

Contrarily to freshwaters, where cladocerans are the dominant mesozooplanktonic group, in the marine environment cladocerans have not been ecologically successful (Egloff et al. 1997). According to this low diversity and the overall low abundance found in the oceans, especially in cold and high latitude ecosystems, cladocerans have been commonly neglected in marine autoecological studies. However, marine cladocerans can in fact build up a large fraction of the zooplankton standing stock in many coastal and even oceanic environments on a seasonal basis and, probably, in these occasions, they play a major role in the dynamics of planktonic food webs (Turner et al. 1988, Kim et al. 1989). Of all marine cladocerans, only one species has been described as truly filter feeder, the sidid *Penilia avirostris*, which inhabits near-shore waters of tropical and warm temperate areas all over the world (Della Croce 1964, Onbé 1985, Onbé & Ikeda 1995, Calbet et al. 2001, Marazzo & Valentin 2001).

Very few studies have reported on the feeding of *Penilia avirostris* (e.g. Paffenhöffer & Orcutt 1986, Turner et al. 1988, 1998, Katechakis & Stibor 2004). Among those, to our knowledge, no studies encompassing the whole spectrum of naturally occurring autotrophic and heterotrophic prey types have been conducted to

characterise the feeding ecology of this cladoceran. This lack of information on the feeding habits of *P. avirostris* has made difficult to establish its relevance in marine planktonic communities. Here, as part of a series of studies focused on the ecological role of *P. avirostris* in marine food webs, we investigated the feeding rates of *P. avirostris* under a broad range of natural diets in their seasonal peak of abundance, and determined their prey size spectrum. Our aim was to describe the diet composition and prey selectivity patterns of this cladoceran on the different components of the microbial community, and to establish the variation in their food uptake as a function of prey availability.

## METHODOLOGY

Eight grazing experiments were conducted in either of two stations located in coastal waters (ca. 1 mile from coast) off Masnou and Barcelona (Spain) during the summer periods of 2002-2003. Both sampling sites have similar characteristics and constitute shallow waters in open coast. Further details on hydrographic characteristics and zooplankton composition are found in Cebrián et al. (1996) and Calbet et al. (2001).

Water for experiments was collected at 1 m depth with a transparent hydrographic bottle and transported to the laboratory within 1 hour. The water was gently poured into a 50-l bucket and reverse-flow filtered by gently submerging a 30 cm diameter polyvinyl chloride cylinder fitted with a 100-μm mesh bottom. Once the prey suspension was ready, it was amended with a nutrient mixture (15μM $NH_4Cl$ and 1μM $Na_2HPO_4$) to compensate for nutrient enrichment due to zooplankton excretion.

*Penilia avirostris* were collected by short oblique net tows with a Juday-Bogorov net (200-μm mesh, 40-cm diameter) fitted with a 5-l plastic bag as cod end. Once onboard, the bag mouth was tied with a string to prevent *P. avirostris* from sticking to the air-water interface. The samples were transported to the laboratory in an isothermic container within 1 hour of collection.

The experiments consisted of incubations in Pyrex bottles (625-ml for experiments during 2002, and 1200-ml for experiments during 2003), filled with the natural microbial community (<100 μm) and added grazers (*Penilia avirostris*) for the experimental treatments. Groups of *P. avirostris* were sorted with a wide-mouthed pipette under the stereomicroscope, and placed in the experimental bottles (33 ind $l^{-1}$). In total, 4 experimental bottles and 4 control bottles (without grazers) were incubated for 24 h on a plankton wheel (0.2 rpm) in a temperature-controlled room at *in situ* temperature and photoperiod (Table 3.1). Three additional "initial" bottles were used to determine the initial prey concentrations. After the incubation, the

water was gently poured through a 135 μm sieve to collect the grazers, which were checked for activity and preserved with formaline (4% final concentration).

Table 3.1. Experimental conditions and carbon content (per individual; mean ± SE) of the cladocerans incubated in the experiments conducted.

| | Exp 1 | Exp 2 | Exp 3 | Exp 4 | Exp 5 | Exp 6 | Exp 7 |
|---|---|---|---|---|---|---|---|
| Date | 23/7/02 | 14/8/02 | 04/9/02 | 26/9/02 | 22/7/03 | 29/7/03 | 07/8/03 |
| Temperature *in situ* (°C) | 24.2 | 23.8 | 23.1 | 22.0 | 26.5 | 25.5 | 26.1 |
| Temperature Incubation (°C) | 23.8 | 23.0 | 23.3 | 22.0 | 25.8 | 25.5 | 26.8 |
| Body mass (μg C $ind^{-1}$) | 0.57 (0.06) | 0.70 (0.07) | 0.60 (0.01) | 0.84 (0.03) | 0.67 (0.05) | 0.49 (0.02) | 0.50 (0.01) |

The microbial components analyzed in the grazing experiments were heterotrophic bacteria, *Prochlorococcus*, *Synechococcus*, pico- and nanoflagellates (<2 μm, 2-5 μm, and >5 μm), dinoflagellates, diatoms (single and in chains) and ciliates.

Samples (2 ml) for heterotrophic bacteria, *Prochlorococcus* and *Synechococcus* were preserved in paraformaldehyde + glutaraldehyde (1% + 0.05% final concentrations, respectively) and stored at – 80°C until analysis. Flow cytometry analysis was conducted with a FACScalibur flow cytometer following the procedures in Gasol & del Giorgio (2000). Heterotrophic bacteria biomass was estimated from volume determinations (V) using the relationship $\mu gC = 0.12\ V^{0.7}$ (Norland 1993). *Prochlorococcus* and *Synechococcus* biomasses were determined after assuming a carbon content of 0.123 pgC $\mu m^{-3}$ and an equivalent spherical diameter (ESD) of, respectively, 0.60 and 1.0 μm (Waterbury et al. 1986).

For the assessment of pico and nanoflagellate abundance, samples were preserved with glutaraldehyde (1% final concentration) for 3 to 6 h (4°C in dark), filtered onto 0.8-μm black polycarbonate membrane filters, and stained with DAPI (5 μg $ml^{-1}$ final concentration) (Porter & Feig 1980). At least 300 cells were counted and classified according to size (<2, 2-5, and >5 μm) using epifluorescence microscopy (Olympus BX40). For the <2 μm fraction, a nominal size of 2 μm was assumed; for the other two categories, forty cells were measured in each case. The carbon per cell was estimated using a factor of 0.22 pgC $\mu m^{-3}$ (Børsheim & Bratbak 1987).

For the determination of dinoflagellate, diatom and ciliate concentrations 200 ml samples were preserved with 1% acidic Lugol's solution. In 2003 we realized of a

potential source of ciliate losses due to the sieving of water to collect *Penilia avirostris* at the end of the experiments prior to withdrawing the samples. We conducted an additional experiment to investigate the effects of such standard procedure by comparing it with gently direct siphoning into the preservation bottles. Significant differences were found between treatments (aloricated ciliates: unsieved = 1.20 ± 0.01 SE, sieved = 0.79 ± 0.01 SE; loricated ciliates: unsieved = 2.92 ± 0.01 SE, sieved = 2.02 ± 0.07 SE; one-way ANOVA, $p<0.05$), the sieving process reducing the ciliate abundance by ca. 30%. Because of this result, samples were gently siphoned directly to bottles containing the required dose of acidic Lugol's. Data belonging to previous experiments (2002) were corrected accordingly. Ciliate abundance (in all experiments) was further increased by a factor of 30% to correct for losses due to Lugol's preservation (Broglio et al. 2004, Calbet and Saiz 2005). 100 ml aliquotes were allowed to settle in Utermöhl chambers and counted in their entirety with an inverted microscope. Digital pictures of at least 60 cells of each type were taken and sized. Cell volumes were converted into carbon content using a factor of 0.19 pg C $\mu m^{-3}$ for ciliates (Putt & Stoecker 1989), the equation log (pgC $cell^{-1}$) = 0.811 (log V) - 0.541 for diatoms, and log (pgC $cell^{-1}$) = 0.819 (log V) - 0.119 for dinoflagellates (Menden-Deuer & Lessard 2000).

The relation between body length (L) and dry weight (DW) of *Penilia avirostris* was also determined. Groups (10-15) of similar size individuals were measured from the tip of the head to the base of the caudal setae (Uye 1982), placed on pre-combusted and pre-weighed aluminum caps and dried at 55-60°C for 24 hours. Dry weight measurements (DW, µg) were made on an ATI CAHN C-35 Microbalance. Figure 3.1 shows the relationship between size and biomass for *Penilia avirostris*. The fitted equation was used to estimate the biomass of cladocerans in the grazing experiments. Carbon content was assumed to be 50% of dry weight (Uye 1982).

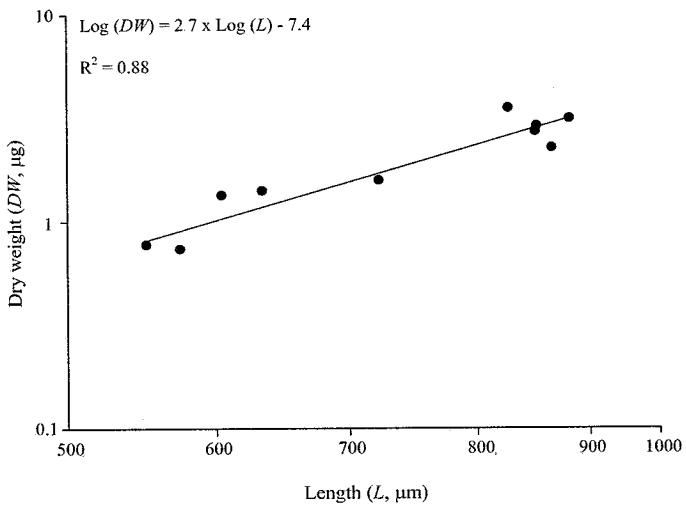


Figure 3.1. *Penilia avirostris* length-dry weight relationship.

Clearance and ingestion rates were calculated for each prey type according to Frost (1972) after verification that prey growth rates in grazing bottles were significantly different and lower than in the controls (one-way ANOVA test, two-tailed $p < 0.05$). Bacteria growth rates were never decreased by the presence *Penilia avirostris*, in fact in occasions they were enhanced.

Clearance rates on diatoms were computed separately for single cells and those in chains. Diatom chains were not very long, ranging between 2-3 cells up to 6 cells per chain. To take into account potential changes in chain length after grazing activity, clearance rates were computed based on the number of cell per chains at the start and end of the incubations. This procedure warranted the computation of actual ingestion rates.

Selectivity patterns of *Penilia avirostris* were determined through the normalized selectivity coefficient $W'$ (Vanderploeg 1994). This coefficient was calculated from clearance rates ($F_i$) on the different groups of $i$ prey as $W_i' = F_i / F_{pref}$ where $F_{pref}$ is the clearance rate on the preferred prey, (i.e. maximum clearance rate observed in the incubation). By definition, preferred prey have a selectivity coefficient of $W'_{pref} = 1$, whereas non-eaten prey have $W_i' = O$. This selectivity index is independent of the number or prey included in the analysis (Vanderploeg 1994). The selectivity coefficients were computed within each experiment based on the average clearances (between replicate bottles) for each considered prey. In order to determine the $F_{pref}$ in an experiment, initially we conducted a Hsu's test for best (significance level 0.05, after previous ANOVA test, Hsu 1981) to find maximum rates among the clearance rates exhibited by *Penilia avirostris* for each prey type in that experiment. According to the results of the Hsu's tests, all prey items, which were cleared at maximal rates, were allocated a selectivity coefficient of 1.

## RESULTS

Tables 3.2 and 3.3 show the initial microbial community of each experiment. In terms of biomass, heterotrophic bacteria dominated the microbial community, with concentrations ranging between 17.6 and 122.2 $\mu gC\ l^{-1}$. Medium (2-5 $\mu m$) and large (>5 $\mu m$) sized flagellates were the following more important contributors (respectively, 3.6 – 15.5 and 4.3 – 29.1 $\mu gC\ l^{-1}$). Dinoflagellates, diatoms and ciliates were the lowest contributors to the bulk of plankton, with some exceptions (Exp 5 and Exp 6, chain forming diatoms). Total community biomass ranged between 49.2 and 208.4 $\mu gC\ l^{-1}$. Clearance rates of *Penilia avirostris* were highly variable and covered a broad prey size spectrum, ranging from <2 $\mu m$ up to 30 $\mu m$ ESD (Table 3.2). Autotrophic and heterotrophic bacteria were not grazed upon in none of the experiments (in some incubations were in fact significantly enhanced). Regarding the

Table 3.2. Initial size (ESD, μm) of the different components of the microbial community. HetBact: Heterotrophic bacteria; Proch: *Prochlorococcus*; Synec: *Synechococcus*; Flag: Flagellates; Dinoflag: Dinoflagellates; Single-Diat: Single diatoms; Chain-Diat: Chain-forming diatoms. Avg: mean values (± SE).

| | Exp 1 | Exp 2 | Exp 3 | Exp 4 | Exp 5 | Exp 6 | Exp 7 | Exp 8 | **Avg** |
|---|---|---|---|---|---|---|---|---|---|
| HetBact | | | | | | | | | |
| ESD | 0.51 | 0.51 | 0.52 | 0.54 | 0.54 | 0.50 | 0.51 | 0.52 | 0.52 (0.01) |
| Proch | | | | | | | | | |
| ESD | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 [1] |
| Synec | | | | | | | | | |
| ESD | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 [1] |
| < 2 μm Flag | | | | | | | | | |
| ESD | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.00 [1] |
| 2-5 μm Flag | | | | | | | | | |
| ESD | 4.1 | 3.7 | 3.8 | 3.9 | 3.9 | 3.8 | 3.6 | 3.7 | 3.82 (0.02) |
| > 5 μm Flag | | | | | | | | | |
| ESD | 8.2 | 8.0 | 7.7 | 8.7 | 8.0 | 7.6 | 7.9 | 7.5 | 7.94 (0.06) |
| Ciliates | | | | | | | | | |
| ESD | 21.6 | 25.4 | 18.8 | 23.1 | 14.8 | 15.0 | 17.3 | 19.6 | 19.51 (0.62) |
| Dinoflag | | | | | | | | | |
| ESD | 17.3 | 30.5 | 27.9 | 18.8 | 14.2 | 14.4 | 20.4 | 20.0 | 17.02 (1.38) |
| Single-Diat | | | | | | | | | |
| ESD | 8.0 | 15.2 | 13.8 | 8.0 | 8.3 | 10.9 | 9.3 | 7.3 | 11.34 (0.60) |
| Chain-Diat | | | | | | | | | |
| ESD | 12.6 | 19.7 | 20.6 | 1.4 | 13.3 | 17.4 | 11.7 | 9.1 | 16.43 (0.66) |

[1] Assumed values (see Methodology)

other components of the microbial community, flagellates (the three size classes) were regular components of the diet, with clearance rates ranging between 7 to 45 ml $\mu gC^{-1}$ $d^{-1}$. Dinoflagellates and diatoms, although frequently constituted important contributors to diet, occasionally were not grazed significantly by *P. avirostris*. Ciliates were rarely consumed by *P. avirostris* (only in three out of eight experiments).

In order to relate feeding rates of *Penilia avirostris* to food availability, the inclusion of bacteria on food availability bulk estimates might provide a wrong picture since they were not grazed on. From here on, we excluded bacteria from calculations and defined *edible food* as that microbial biomass prone to be ingested by the cladoceran. Figure 3.2 shows the composition of the edible food for *P. avirostris* in

the experiments conducted. The initial edible microbial community was dominated in terms of biomass by flagellates, which comprised >50% of carbon in most of the experiments, with secondary contributions of dinoflagellates (Exp 2), diatoms (Exp 3, Exp 5 and Exp 6), or ciliates (Exp 4).

Table 3.3. Initial abundance (Abund.; cells ml $^{-1}$), and biomass (µgC l$^{-1}$) of the different components of the microbial community. Abundance of Het-Bact x $10^6$. Total biomass: Accumulated microbial biomass for each experiment (µgC l$^{-1}$). HetBact: Heterotrophic bacteria; Proch: *Prochlorococcus*; Synec: *Synechococcus*; Flag: Flagellates; Dinoflag: Dinoflagellates; Single-Diat: Single diatoms; Chain-Diat: Chain-forming diatoms. Avg: mean values (± SE).

| | Exp 1 | Exp 2 | Exp 3 | Exp 4 | Exp 5 | Exp 6 | Exp 7 | Exp 8 | **Avg** |
|---|---|---|---|---|---|---|---|---|---|
| HetBact | | | | | | | | | |
| Abund. | 1.00 | 0.87 | 1.73 | 0.72 | 2.33 | 0.67 | 0.41 | 0.25 | 0.99 (0.24) |
| Biomass | 41.2 | 17.6 | 24.7 | 22.9 | 122.2 | 42.1 | 21.6 | 20.7 | 39.1 (12.32) |
| Proch | | | | | | | | | |
| Abund. | 7533 | 2577 | 10937 | 8983 | 188 | 514 | 8051 | 1123 | 4988 (1530) |
| Biomass | 0.10 | 0.04 | 0.15 | 0.12 | 0.003 | 0.01 | 0.11 | 0.02 | 0.1 (0.02) |
| Synec | | | | | | | | | |
| Abund. | 95983 | 70000 | 39139 | 43252 | 17238 | 30535 | 29442 | 35928 | 45190 (9029) |
| Biomass | 6.2 | 4.5 | 2.5 | 2.8 | 1.1 | 2.0 | 1.9 | 2.3 | 2.9 (0.58) |
| < 2 µm Flag | | | | | | | | | |
| Abund. | 3706 | 3850 | 1955 | 4770 | 6809 | 4710 | 3764 | 3644 | 4151 (487) |
| Biomass | 3.4 | 3.5 | 1.8 | 4.4 | 6.3 | 4.3 | 3.5 | 3.4 | 3.8 (0.45) |
| 2-5 µm Flag | | | | | | | | | |
| Abund. | 808 | 2298 | 553 | 1106 | 2190 | 2408 | 1906 | 1896 | 1646 (254) |
| Biomass | 6.2 | 13.2 | 3.6 | 7.4 | 14.8 | 15.5 | 10.3 | 11.5 | 10.3 (1.51) |
| > 5 µm Flag | | | | | | | | | |
| Abund. | 193 | 321 | 80 | 384 | 291 | 195 | 191 | 159 | 227 (35) |
| Biomass | 12.1 | 19.2 | 4.3 | 29.1 | 16.9 | 9.7 | 10.7 | 7.6 | 13.7 (2.78) |
| Ciliates | | | | | | | | | |
| Abund. | 0.8 | 0.6 | 1.9 | 9.1 | 4.4 | 3.4 | 3.9 | 3.0 | 3.4 (0.9) |
| Biomass | 0.8 | 1.0 | 1.2 | 11.0 | 1.4 | 1.1 | 2.0 | 2.3 | 2.6 (1.22) |
| Dinoflag | | | | | | | | | |
| Abund. | 4.7 | 4.7 | 1.4 | 2.0 | 3.0 | 3.2 | 2.0 | 4.5 | 3.2 (0.5) |
| Biomass | 2.3 | 9.3 | 2.2 | 1.2 | 0.9 | 1.0 | 1.5 | 3.1 | 2.7 (0.99) |
| Single-Diat | | | | | | | | | |
| Abund. | 55 | 12 | 19 | 20 | 64 | 33 | 3 | 2 | 26 (8) |
| Biomass | 1.5 | 1.5 | 2.0 | 0.5 | 1.8 | 1.9 | 0.1 | 0.05 | 1.2 (0.29) |
| Chain-Diat | | | | | | | | | |
| Abund. | 27 | 2 | 25 | 18 | 463 | 254 | 0.1 | 2 | 99 (60) |
| Biomass | 2.2 | 0.6 | 6.7 | 1.1 | 43.0 | 45.0 | 0.01 | 0.1 | 12.3 (6.95) |
| Total Biomass | 76.0 | 70.4 | 49.2 | 80.5 | 208.4 | 122.6 | 51.7 | 51.0 | |

Ingestion rates in terms of daily rations ranged between 26% to 157% body carbon d-1 and were positively related to total edible prey biomass (Figure 3.3). No evidence of saturation was observed over the range of edible food biomass found during the studied period.

Table 3.4 *Penilia avirostris* weight-specific clearance rates (ml $\mu gC^{-1}$ $d^{-1}$; mean ± SE). *Growth*: values in experimental bottles higher than controls; 0: no significant ingestion. HetBact: Heterotrophic bacteria; Proch: *Prochlorococcus*; Synec: *Synechococcus*; Flag: Flagellates; Dinoflag: Dinoflagellates; Single-Diat: Single diatoms; Chain-Diat: Chain-forming diatoms.

| | Exp 1 | Exp 2 | Exp 3 | Exp 4 | Exp 5 | Exp 6 | Exp 7 | Exp 8 |
|---|---|---|---|---|---|---|---|---|
| HetBact | 0 | 0 | 0 | 0 | *growth* | *growth* | 0 | *growth* |
| Proch | *growth* | *growth* | *growth* | 0 | *growth* | *growth* | *growth* | 0 |
| Synec | *growth* | 0 | 0 | 0 | *growth* | 0 | 0 | 0 |
| < 2 µm Flag | 41 (4.4) | 10 (0.8) | 15 (0.2) | 13 (0.5) | 19 (1.2) | 32 (1.9) | 34 (1.7) | 17 (1.0) |
| 2-5 µm Flag | 35 (4.3) | 24 (0.9 | 10 (0.7) | 45 (2.4) | 19 (2.1) | 33 (1.8) | 40 (2.4) | 39 (3.5) |
| > 5 µm Flag | 23 (2.5) | 39 (3.9) | 7 (0.3) | 19 (0.9) | 17 (1.8) | 43 (2.3) | 30 (1.7) | 33 (1.8) |
| Ciliates | 0 | 0 | 0 | 0 | 10 (0.5) | 31 (4.3) | 0 | 8 (0.8) |
| Dinoflag | 31 (3.3) | 38 (3.4) | 0 | 25 (1.9) | 25 (1.3) | 44 (3.5) | 24 (3.1) | 31 (1.2) |
| Single-Diat | 19 (2.0) | 0 | 0 | 19 (3.2) | 2 (0.44) | 54 (5.4) | 45 (9.8) | 0 |
| Chain-Diat | 0 | 0 | 41 (7.3) | 22 (4.4) | 5 (0.5) | 16 (1.0) | 100 (25.0) | 23 (3.0) |

Regarding selectivity, we found significant differences among clearance rates for the different prey types within each experiment (one-way ANOVA, $p < 0.05$; Table 3.4), which is an indication of selection. Figure 3.4 shows the selectivity coefficients *W'* as a function of the relative edible prey availability. No clear recurrent pattern was identified. A linear positive relationship is suggested in Exp 2, Exp 3, and Exp 8, whereas a negative relationship seems to appear in Exp 6. In other experiments no trend was observed (Exp 1, Exp 4, Exp 5, and Exp 7). A more interesting and intriguing result of this analysis, however, is that *Penilia avirostris*, in spite of being typically considered a passive filter feeder, displayed variability in prey preference

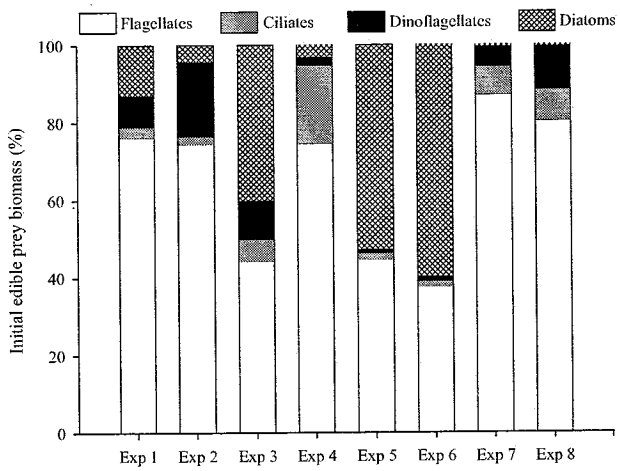


Figure 3.2. Biomass contribution (as %) of the different microbial groups considered (excluding the bacterioplankton) at the beginning of the experiments.

among experiments independently of prey type, and also independently of prey contribution to available biomass. For instance, if we consider a particular prey type like the 2-5 μm flagellates, in 3 out of 8 experiments they constitute the preferred prey ($W' = 1$), whereas in the rest of experiments they show medium ($W'$ between 0.6 and 0.8) or very low preference ($W'$ between 0.2 and 0.4), independently of their relative contribution to food availability (Figure 3.4). There seems to be no dependence on the initial food composition neither, as can be seen by just comparing the patterns in $W'$ in the selectivity graphs for Exp 1, Exp 3, Exp 7 and Exp 8 (Figure 3.4), all conducted at a similar initial food concentration.

## DISCUSSION

### Lower prey size threshold in *Penilia avirostris*

This study is one of the few that illustrates the feeding ecology of the marine cladoceran *Penilia avirostris* under natural diet, thus estimating the grazing of *P. avirostris* under a wide spectrum of natural microbial prey. The clearance rates from the present study are in the same range of values as in previous reports of *P. avirostris* feeding on heterotrophic flagellates (34- 96 ml $ind^{-1}$ $d^{-1}$, Turner et al. 1988), diatoms (14-40 ml $ind^{-1}$ $d^{-1}$, Turner et al. 1988), the haptophyciae *Isochrysis galbana* (4 - 24 ml $ind^{-1}$ $d^{-1}$, Paffenhöfer & Orcutt 1986), ciliates (4-33 ml $ind^{-1}$ $d^{-1}$, Broglio et al. 2004), and on different components of the microbial community (20-30 ml $ind^{-1}$ $d^{-1}$, Katechakis et al. 2004).

One of the intriguing and ecologically relevant questions about the feeding of *Penilia avirostris* is whether or not they are capable to graze on bacterial and picoflagellate communities and therefore impact directly on the microbial loop, similarly to its freshwater relative *Daphnia*. Daphnids are important consumers of natural bacteria and small flagellates (Jürgens 1994), their ability to feed on small particles being determined by the morphology of the filtering apparatus (filter mesh-size; Lampert 1987, Gerritsen et al. 1988). Impacts on picoplanktonic communities about 50% due to Daphnids are frequent in freshwater planktonic food webs (Degans et al. 2002, Zollnet et al. 2003).

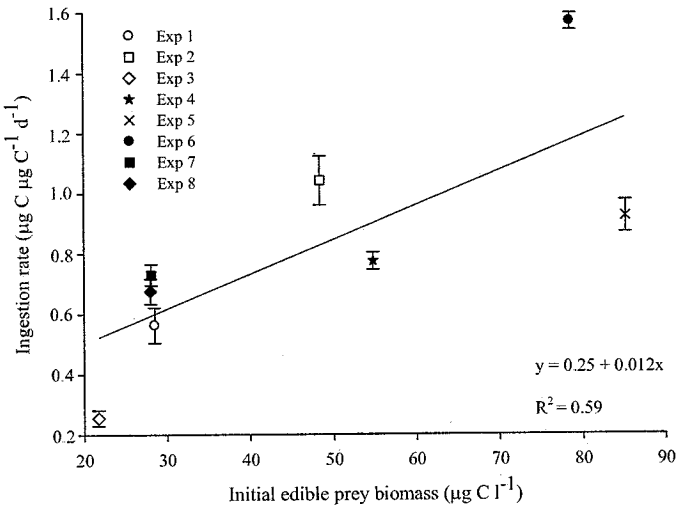


Figure 3.3. *Penilia avirostris* relationship between ingestion rates and initial edible prey biomass. Error bars represent ±1 SE.

In the case of *Penilia avirostris*, there has been controversy about its capability to feed on picoplankton. Some studies have suggested that bacteria could be an important source of carbon for this cladoceran (Pavlova 1967, Paffenhöfer & Orcutt 1986, Lipej et al. 1997). However, Turner et al. (1988) concluded that this organism could ingest relatively large or clumped bacteria, but not natural bacterioplankton (of smaller size). This latter conclusion agreed with Gore's (1980) observation who reported for *P. avirostris* preferential ingestion on plastic beads >1 µm. Our results confirm the observations made by Turner et al. (1988), showing that natural heterotrophic and phototrophic bacteria cannot be grazed by *P. avirostris*. However, our study also evidences that the gap for the lower prey size threshold is very narrow, because <2 µm flagellates were significantly consumed in all the experiments (although at overall lower clearance rates). Hence, the threshold for minimum prey size for *P. avirostris* seemed to fall between *Synechococcus* size (1 µm) and the <2 µm flagellates. The disagreement of our results with the recent work of Katechakis &

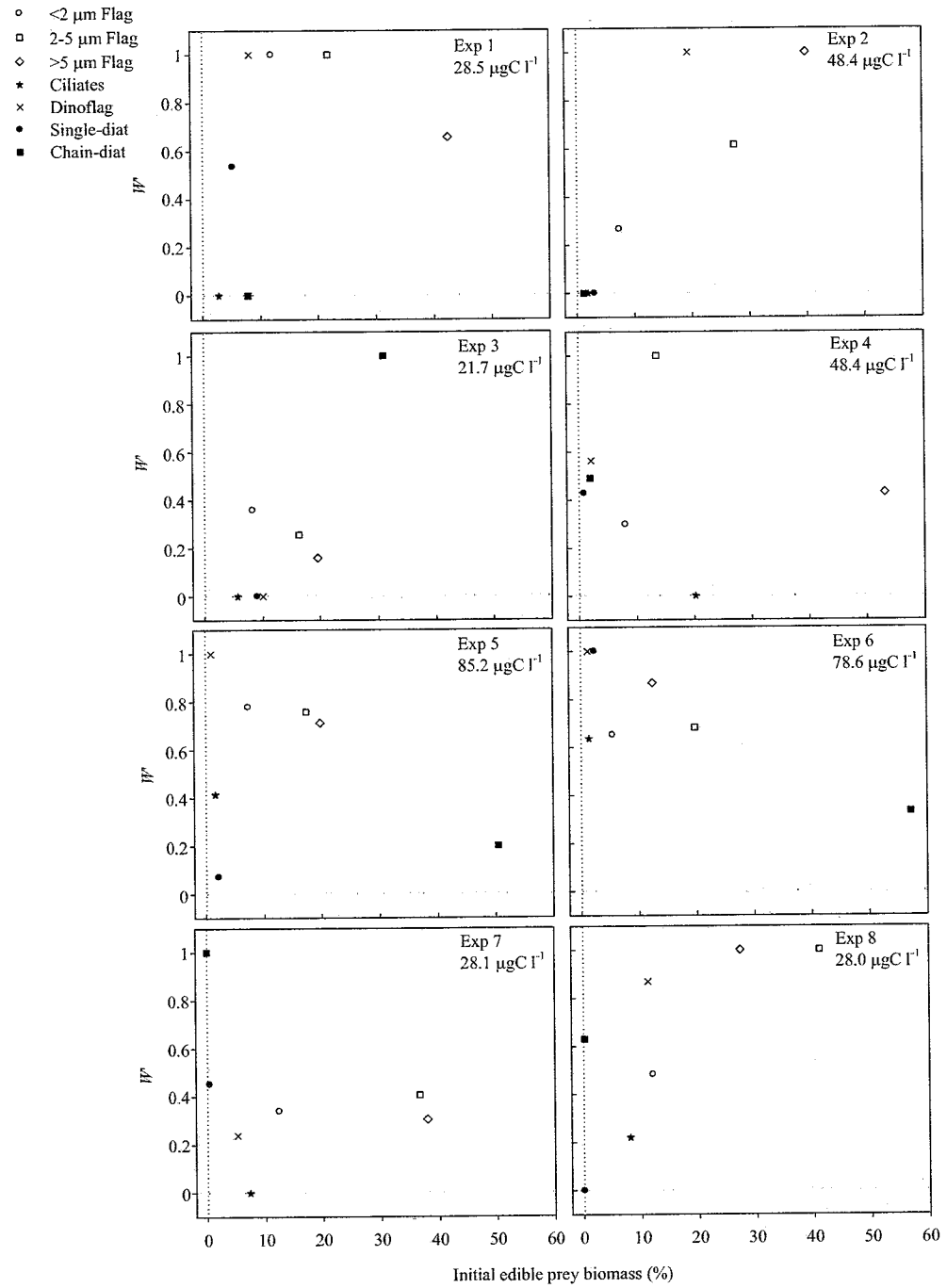


Figure 3.4. *Penilia avirostris* relationship between selectivity coefficient W´ and prey contribution to initial edible biomass. Flag: Flagellates; Dinoflag: Dinoflagellates; Single-Diat: Single diatoms; and Chain-Diat: Chain-forming diatoms.

Stibor (2004), where no grazing on <2.5 μm prey was observed, is probably due to the methodology used by those authors to preserve and quantify bacteria and small flagellate samples. Our conclusions are further confirmed by the observations of intersetular distances (1-2 μm) under light microscopy for our Mediterranean Sea specimens, which are also in agreement with the ≤2 μm values under scanning electron microscopy reported by Turner et al. (1988) for the West Atlantic *P. avirostris*.

One may expect, however, that there might be genetic and ontogenetic differences in the intersetular gap, which could explain some of the variability observed and the discrepancies among studies reported. The fact that the intersetular distance increases with body size in Daphnids (Brendelberger & Geller 1985) suggests that perhaps younger (smaller) *Penilia avirostris* than the individuals used in our experiments might extend their prey size spectrum to smaller items than the ones reported here. Other factors could also affect the lower prey size thresholds reported here. For example, changes in the hydrophobicity or electrostatic charges of the cell membrane (see discussion below) could affect the retention efficiency of the phyllopods. Due to the narrow gap for the lower prey size threshold observed, slight variations in the average size of the picoplanktonic fraction would result in variability in the clearance rates on <2 μm flagellates displayed by *P. avirostris*.

From an ecological point of view, the fact that *Penilia avirostris*, opposite to *Daphnia*, cannot graze on heterotrophic and autotrophic bacteria indicates that this marine cladoceran cannot exert a direct control on bacterial production. But the capability of *P. avirostris* to feed on <2 μm flagellates allows for a trophic shortcut to the microbial loop from very close to its base, resulting in a more efficient transfer of energy towards upper consumers compared to other marine zooplankters like copepods. In addition, *P. avirostris* may also have indirect effects on bacterial production by grazing on the first order bacterivorous grazers (<2 μm flagellates).

### Prey selectivity by *Penilia avirostris*

*Penilia avirostris* is a typical filter feeder that, similarly to Daphnids, is expected to largely exhibit passive and mechanical selection, wherein phyllopods form a mesh that retains particles (Paffenhöfer & Orcutt 1986, Turner et al. 1988). Our observations seem to contradict, or at least not fully confirm, such theory.

In the present study we found relatively high clearance rates on flagellates, diatoms and dinoflagellates, whereas other prey were either not grazed upon because of their size (i.e. bacteria) or grazed at low rates likely because of their ability to escape from feeding currents (ciliates; Jakobsen 2001, 2002). Our results partially contrast with those by Katechakis & Stibor (2004) and Katechakis et al. (2004) who reported maximum ingestion rates by *Penilia avirostris* in the prey range between 15-70

μm (equivalent to 6-28 μm ESD) corresponding to diatoms and dinoflagellates. However, the comparison is difficult because their investigation was conducted under very enriched conditions (up to 575 μgC $l^{-1}$), not representative of the natural microbial community where *P. avirostris* inhabits in the Mediterranean.

The three major diet components in our study (flagellates, dinoflagellates and diatoms) alternated their role as preferred prey from experiment to experiment. Flagellates were preferred prey in 6 occasions, and dinoflagellates and diatoms constituted preferred prey, respectively, 4 and 3 times. It is also important to notice that often there was more than one preferred prey for a given experiment. These variations in *Penilia avirostris* preferences are puzzling and do not seem to be related neither to prey size (for instance in Exp 1) nor to prey abundance (either in relative or absolute terms). One may think that part of this preference for certain prey may be artifactual. For instance, the feeding activity of *P. avirostris* may break apart diatom chains, resulting in a higher selection for diatom chains in front of single cells (diatom chains disappear from incubation bottles, whereas single diatom cells increase their abundance). There could be also trophic cascade effects masking real patterns. Feeding on both <2 and 2-5 μm flagellates by *P. avirostris* may result in a release of grazing pressure on <2 μm flagellates by heterotrophic and mixotrophic 2-5 μm flagellates, therefore masking the feeding of *P. avirostris* on those smallest flagellates. This hypothesis does not seem the hold, however, because in 5 out of the 8 experiments *W'* was the same for <2 and 2-5 μm flagellates (Figure 3.4). There is also the possibility that variations on *P. avirostris* body size among experiments could explain the variations on *W'* patterns. However, we explored this possibility by measuring the intersetular distance of 2 animals from each experiment and found no difference. Changes in surface properties such as hydrophobicity or electrostatic charge of cell membrane of both prey and the cladoceran phyllopods have also been considered as plausible explanations for such changes in selectivity (Lampert 1987, Vanderploeg 1994).

Besides these hypothetical explanations for our results, there are evidences in Daphnids that prey selectivity patterns can be modified to some extent by the individual as a function of size and quality. Vanderploeg (1994) reviewed such particle selection mechanisms in freshwater cladocerans. Very large particles (e.g. filamentous algae) can be rejected by the abdominal claw or just by decreasing the carapace gap. Particles can be rejected even when contained in boluses. Further evidences are given by Lampert & Brendelberger (1996), who reported that *Daphnia* can adjust the area of the filter screens and the appendage beat rate as a function of food concentration. The application of these behaviorally-driven mechanisms of prey selection in the closely-related *P. avirostris* seems plausible. In fact, Turner et al. (1988) observed that *P. avirostris* mostly ingested small heterotrophic flagellates (2-5 μm) and diatoms (4-12 μm), but was unable to graze upon the intermediate sized

*Pseudoisochrysis paradoxa* (5-6 μm). Paradoxically, Paffenhöfer & Orcutt (1986) reported ingestion of the similarly-sized *Isochrysis galbana*. Therefore, we feel that the variability in prey preference observed in our experiments reflect true changes in selectivity by *P. avirostris*, although the mechanisms underneath are not fully clear.

## Feeding performance of *Penilia avirostris*

In the present study, *Penilia avirostris* maximal daily food rations (157% body carbon $d^{-1}$) were higher than those reported previously (Pavlova 1967, Paffenhöfer & Orcutt 1986), but similar to the maximal value (151%) reported by Broglio et al. (2004) at food concentrations (phytoplankton and ciliate) of 125 μg C $l^{-1}$. Our results also agree with estimates for the freshwater cladoceran *Daphnia* (52-115% body carbon $d^{-1}$ Burns & Schallenberg 2001, 100-157% body carbon $d^{-1}$ DeMott et al. 1998, and 148-312% body carbon $d^{-1}$ Sterner et al. 1993). More noticeable is the efficient performance of *P. avirostris* at the low food concentrations that characterize its natural habitat, with daily rations up to 73% at concentrations <30 μgC $l^{-1}$. This high efficiency of *P. avirostris* under oligotrophic conditions may explain its fast blooming and dominance in coastal zooplankton during the stratification period, where oligotrophic conditions prevail. However, it poses the question why *P. avirostris* does not spread to the oligotrophic open ocean waters. Very likely the dependence on resting embryos in the sediment for the development of a new seasonal cohort next year restricts its habitat to shelf waters. This geographic confinement also has the consequence of constraining *P. avirostris* on a seasonal basis to the stratification period, because the richer conditions of shelf waters during the rest of the year may have deleterious effects (clogging) on their filter-feeding appendages (Paffenhofer & Orcutt 1986). At similar low food concentrations daily food rations of copepods are <30% body carbon $d^{-1}$ (Table 3.5). Copepods are less efficient feeding on small prey such (<5 μm, Bergreen et al. 1988), which are the major contributors to seston biomass under such oligotrophic conditions (Agawin et al. 2000). We can compare *P. avirostris* to another important filter feeder in coastal waters as well. The appendicularians perform similarly well at equivalent food concentrations (Table 3.5), with the peculiarity that they can do also well in richer waters and ingest bacterioplankton (Scheinberg et al. 2005). Having a similar or even better feeding performance than *P. avirostris*, it is not clear why appendicularians do not dominate summer coastal waters in the NW Mediterranean (Calbet et al. 2001).

In this study we have characterized the feeding ecology of *Penilia avirostris*, the only filter-feeding cladoceran in the marine environment. *P. avirostris* has a broad spectrum of prey types, their diet being constituted mainly by flagellates, dinoflagellates and diatoms. In contrast to a typical, passive filter feeder, *P. avirostris* shows behaviourally driven plasticity in their prey selection by mechanisms not fully understood. The species seem to be optimally adapted to oligotrophic environments,

exhibiting relatively high daily rations compared to the most common zooplankters in the oceans (the copepods).

Table 3.5. Comparison of daily food rations (DR, % body carbon $d^{-1}$) at low food availability (C, µgC $l^{-1}$) of *Penilia avirostris* and other zooplankters.

| *Penilia avirostris*[1] | | *Oikopleura dioica*[2] | | *Oikopleura dioica*[3] | | Calanoid copepods[4] | |
|---|---|---|---|---|---|---|---|
| C | DR | C | DR | C | DR | C | DR |
| 22 | 26 | 80 | 131 | 40 | 87 | 20-40 | 17 |
| 28 | 73 | 320 | 214 | 80 | 198 | 40-70 | 24 |
| 28 | 68 | 481 | 281 | 150 | 347 | 70-100 | 27 |
| 28 | 56 | 641 | 88 | | | | |
| 48 | 104 | 1602 | 97 | | | | |
| 55 | 78 | | | | | | |
| 79 | 157 | | | | | | |
| 85 | 92 | | | | | | |

[1] This study; field data
[2] Acuña & Kiefer 2000; laboratory experiments
[3] Selander & Tiselius 2003; laboratory experiments
[4] Saiz & Calbet (unpublished.); field data obtained from a review of literature.

# Chapter IV. Trophic impact, metabolism, and biogeochemical role of the marine cladoceran *Penilia avirostris* and the co-dominant copepod *Oithona nana* on NW Mediterranean coastal waters

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# CHAPTER IV. TROPHIC IMPACT, METABOLISM, AND BIOGEOCHEMICAL ROLE OF THE MARINE CLADOCERAN *PENILIA AVIROSTRIS* AND THE CO-DOMINANT COPEPOD *OITHONA NANA* ON NW MEDITERRANEAN COASTAL WATERS

## INTRODUCTION

Within marine zooplankton, copepods have been traditionally considered as the main link between primary producers and the icthyoplankton, as well as, between microbial and classic food webs (Verity & Smetacek 1996). However, this dominance of copepods does not always hold, especially in coastal waters where other groups of holo and meroplankton may built up a major fraction of zooplankton standing stock. For instance, in temperate and tropical ecosystems, marine cladocerans are the dominant zooplankters of summer communities in coastal waters, when the water stability increases and prokaryotic picoplankton comprise much of the primary producer's biomass (Paffenhöfer 1983, Onbé 1985, Calbet et al. 2001). In the coastal NW Mediterranean, during the summer season, the cladoceran *Penilia avirostris* shares their preponderance on the zooplanktonic community with the cyclopoid copepod *Oithona nana* (Calbet et al. 2001). Both groups of organisms are cosmopolitan and occur commonly in nearshore tropical, subtropical and temperate waters (Onbé 1985, Wong et al. 1992, Calbet et al. 2001).

The association of these two species in stratified, unproductive ecosystem like the NW Mediterranean, suggests that they must be exploiting different trophic resources. *Penilia avirostris* ingests particles of small size (Gore 1980, Paffenhöfer & Orcutt 1986, Turner et al. 1988), whereas *Oithona* spp. seems to prey upon larger prey (e.g. net phytoplankton, ciliates, detritus, nauplii, and fecal pellets) as a consequence of its raptorial feeding behavior (Lampitt & Gamble 1982, González & Smetacek 1994, Broglio et al. 2004). Thus, it is expected that both species are not direct competitors, because they may feed upon different trophic components.

Understanding the fate of ingested material by consumers is crucial to predict how changes in food webs affect the biogeochemical cycling of nutrients. Ingested food is respired, egested in feces, excreted as inorganic or organic compounds, or incorporated into the food web (Anderson et al. 2005). Excretion is important for nutrient recycling, as it produces readily assimilable inorganic or organic nutrients for primary producers or osmotrophs (Frangoulis et al. 2005). Among them, nitrogen and phosphorus are considered as the most important macronutrients for primary producers in aquatic systems.

The differences between cladocerans and copepods do not solely rely on their feeding strategies, but also on their contribution to the nutrient recycling. Zooplanktonic groups with different body N:P ratios are expected to differ in their relative rate of nutrient recycling (Sterner 1990). Cladocerans have lower N:P ratios than copepods. This difference between cladocerans and copepods in elemental composition suggests (according to the stoichiometric model) that the recycling of P should be higher when copepods dominate the community. On the other hand, data from freshwater systems (Elser et al. 1988, Sterner et al. 1992) suggest that the recycling of nitrogen might be more significant in communities where cladocerans are important contributors to the bulk of zooplankton.

Finally, part of the organic matter produced in the surface waters is respired. Respiration is also an index of energy demands and represents the minimum requirements in terms of carbon. Therefore, in order to complete our understanding of the global carbon cycle and the role played by zooplankton it is essential to determine their respiration rates (Hernández-León & Ikeda 2005).

The aim of our study is, therefore, to determine the ecological impact and the biogeochemical role of the two most abundant species of the summertime zooplankton community of the NW Mediterranean coast, the cladoceran *Penilia avirostris* and the cyclopoid copepod *Oithona nana*. The study will contemplate the trophic impact (upon the different components of the microbial food web), the possible partition of the ecological niche, and their relevance in the recycling of inorganic nutrients and $CO_2$ production.

## METHODOLOGY

### Sample collection

The experiments were conducted with samples collected in a near shore station (1 mile from coast) located off Barcelona and Masnou (20 kilometers north of Barcelona, NW Mediterranean) during the summer periods of years 2002, 2003 and 2004. Grazing experiments were conducted in 2002 and 2003, and metabolic experiments, to assess respiration and excretion rates, were conducted in 2003 and 2004. To facilitate comparison with other studies from the same project we used the code assigned to the different experiments that we did. The abbreviation Gra corresponds to grazing experiments and the abbreviation Met corresponds to metabolic experiments.

Zooplankton samples for community composition and abundance were collected by vertical net tows from the bottom (10 to 38 m depth) to the surface with a microplankton net (53 μm mesh, 25 cm diameter). Samples were preserved in borax-buffered formaldehyde at 4% final concentration. Zooplankton abundance and species composition were estimated by counting and identifying under

stereomicroscope at least 500 individuals per sample. To estimate their biomass, pictures of 30 *Penilia avirostris* and 30 *Oithona nana* were taken and digitalized with a Power Macintosh computer provided with a frame grabber, and subsequently analyzed with NIH Image software. Dry weights were estimated from size measurements using the length-weight relationship log DW = 2.66 log L – 7.369 and log DW = 3.16 log L – 8.18 for respectively *P. avirostris* (Atienza et al. 2006a) and *O. nana* (Hopcroft et al. 1998), where "DW" is dry weight in μg and "L" is body length in μm (total length for *P. avirostris*; prosome length for *O. nana*). Dry weights were converted into carbon units (μgC) assuming that carbon content was 50% (Uye 1982) and 40% DW for *P. avirostris* and *O. nana*, respectively.

The organisms for grazing and metabolic experiments were collected by short vertical and oblique net tows with a Juday-Bogorov net (200-μm mesh, 40-cm diameter). In order to reduce the organism damage, a 5-l plastic bag was used instead of a closed cod end. Once onboard the plastic bags were transferred to an isothermic container, and transported to the laboratory within 1 hour of collection. The bag was tied with a string to eliminate the air inside and to prevent *Penilia avirostris* from sticking to the air-water interface.

### Grazing rates of *Penilia avirostris* and *Oithona nana*

Water for grazing experiments was collected at 1 m depth with a transparent hydrographic bottle, gently transferred to carboys and transported to the laboratory within 1 hour. The water was gently poured into a 50-l bucket and reverse-flow filtered by submerging a 30 cm diameter polyvinyl chloride (PVC) cylinder with a bottom of 100 μm pore-size mesh. Once the suspension was ready, it was amended with a nutrient mixture (15μM $NH_4Cl$ and 1μM $Na_2HPO_4$) to compensate for nutrient enrichment due to zooplankton excretion.

Experimental bottles (Pyrex 625-ml for 2002 experiments, and Pyrex 1200-ml for 2003 incubations) were filled with the natural suspension (<100 μm) and the experimental organisms were added to a concentration of 30-50 *Penilia avirostris* $l^{-1}$ and 60-200 *Oithona nana* $l^{-1}$. The experiment consisted of 4 replicate treatment bottles with each grazer and 4 additional control bottles without grazers. Bottles were incubated for 24 h on a plankton wheel (0.2 rpm) in a temperature-controlled room at *in situ* temperature and photoperiod (Table 4.1).

Subsamples of each treatment and from 3 additional initial bottles were taken to determine the chlorophyll concentration as estimator of phytoplankton biomass. Other samples were taken to quantify the different components of the microbial community. After the incubation, the water was gently poured through a 135 μm sieve to collect grazers, which were checked for activity and preserved with formaline (4% final concentration). The biomass of copepods and cladocerans was estimated

from pictures of the animals taken under a stereomicroscope. The images were digitized and the body length was measured (for details see above).

Table 4.1. List of grazing experiments (Gra). Water temperature (°C), and mean individual biomass (µgC ind$^{-1}$) of *Penilia avirostris* and *Oithona nana* on the incubation bottles. Average values (± SE).

| | Gra-1 | Gra-2 | Gra-3 | Gra-4 | Gra-6 | Gra-7 | Gra-8 |
|---|---|---|---|---|---|---|---|
| Date | 23/7/02 | 14/8/02 | 04/9/02 | 26/9/03 | 29/7/03 | 07/8/03 | 12/8/03 |
| Temperature | 24.2 | 23.8 | 23.1 | 22.0 | 25.5 | 26.1 | 27.1 |
| Biomass *P. avirostris* (µgC ind$^{-1}$) | 0.57 (0.06) | 0.70 (0.07) | 0.60 (0.01) | 0.84 (0.03) | 0.49 (0.02) | 0.50 (0.01) | 0.50 (0.02) |
| Biomass *O. nana* (µgC ind$^{-1}$) | 0.32 (0.002) | 0.35 (0.001) | 0.32 (0.009) | 0.24 (0.004) | 0.33 (0.002) | 0.34 (0.003) | 0.33 (0.001) |

Chlorophyll *a* (Chl *a*) was determined by filtering 75-ml and 150-ml onto GF/F Whatman and 5 µm pore-size polycarbonate Nucleopore™ filters, respectively. The filters were analyzed fluorometrically according to Parson et al. (1984) after acetone extraction.

The microbial components considered in the grazing experiments included heterotrophic bacteria, *Prochlorococcus*, *Synechococcus*, nanoflagellates (<2 µm, 2-5 µm and >5 µm), dinoflagellates, diatoms (single and chain-forming) and ciliates.

Bacterial community was determined by flow cytometry analysis (FACScalibur Becton and Dickinson) on samples preserved with paraformaldehyde + gluteraldehyde (1% + 0.05% final concentration, respectively) and stored at – 80°C until analysis. For further details on the analytical protocol see Atienza et al. (2006a). Bacterial biomass was determined using the relationship µgC = 0.12 $V^{0.7}$ (Norland 1993). *Prochlorococcus* and *Synechococcus* biomasses were obtained assuming a carbon content of 0.123 pgC µm$^{-3}$ (Waterbury et al. 1986).

Flagellate abundance was estimated by epifluorescense microscopy. 50-ml samples were preserved in gluteraldehyde (1% final concentration). One 20-ml subsample were filtered onto 0.8-µm pore-size black polycarbonate membrane filter and stained with DAPI (5 µg ml$^{-1}$ final concentration) for 5 min (Porter & Feig 1980). At least 300 cells were counted and classified into three categories: <2 µm, 2-5 µm, and >5 µm. Forty cells of each of the two last categories were sized. For the <2 µm flagellate fraction, a nominal size of 2 µm was assumed. Mean cell diameters of

flagellates were converted into cell carbon by using a factor of 0.22 pgC $\mu m^{-3}$ (Børsheim & Bratbak 1987). In 2003 experiments, >5 μm flagellates were counted from 80-ml subsample filtered onto 2 μm pore-size black polycarbonate membrane filters.

To determine the concentration of dinoflagellates, diatoms and ciliates, 200-ml subsamples were preserved with 1% acidic Lugol's solution, and let to settle for 48 h in 100 ml Utermöhl chambers. The whole chamber was counted under an inverted microscope (Nikon DIAPHOT 200) at 200X magnification. A correction loss-factor of 30% was applied to the ciliate abundance data to compensate for losses due to fixation (Broglio et al. 2004). Additionally, another 30% correction factor was applied to samples from 2002 to account for losses during handling. For further details on the justification of these correction factors, as well as, for the biomass determination procedure, see Atienza et al. (2006a).

Clearance and ingestion rates were calculated for each prey type according to Frost (1972) equations. Grazing significance was calculated by the comparison of prey growth rates between grazing and control bottles (one-way ANOVA test, two-tailed $p < 0.05$); when these differences were not significant, nil feeding rates were assigned.

The trophic impact on the standing stock of each prey type exerted by the whole copepod and cladoceran community was determined from their biomass and weight-specific clearance rates assuming a homogenous prey and predator distribution in the water column.

### Metabolic activity of *Penilia avirostris* and *Oithona nana*

Seawater for the metabolic experiments was collected in the same way that water for grazing experiments. Once in the laboratory, water was filtered-through a 0.2 μm Nucleopore filter with a peristaltic pump and aerated near to saturation. Acid-washed experimental bottles (72-ml Falcon for excretion experiments, and 125 ml BOD bottles for respiration activity assessment) were filled with the filtered seawater and the experimental animals were added at a concentration of 50-100 *Penilia avirostris* $bottle^{-1}$ and 60-100 *Oithona nana* $bottle^{-1}$. The experiment consisted of 5 replicate treatment bottles for each species, and 2 additionally initial and 5 control bottles without grazers. Bottles were incubated for 24 h in a temperature-controlled room at *in situ* temperature and in the dark (Table 4.2).

After incubation, oxygen bottles were fixed and analyzed by Winkler titration method (Ikeda et al. 2001) using a Mettler Toledo DL50 Graphix Titrator. Nutrient concentration (ammonia and inorganic phosphate) was determined in aliquots of 5-ml, siphoned from the experimental bottle to an acid-clean culture tube. Ammonia

($NH_4$-N) and phosphate ($PO_4$-P) concentrations were determined by the reactions of Bertheld and molibdate, respectively (Hansen & Koroleff 1999), using a Uvikon 923 Double Beam UV/VIS Spectophotometer.

Table 4.2. List of metabolic experiments (Met). Water temperature (°C), and mean individual biomass (µgC ind$^{-1}$) of *Penilia avirostris* and *Oithona nana* on the incubation bottles. Temp: temperature. Average values (± SE).

| | Met-1 | Met-2 | Met-3 | Met-4 | Met-5 | Met-6 | Met-7 | Met-8 |
|---|---|---|---|---|---|---|---|---|
| Date | 09/7/03 | 04/8/03 | 20/7/04 | 27/7/04 | 03/8/04 | 12/8/04 | 16/8/04 | 19/8/04 |
| Temp | 23.5 | 23.5 | 23.1 | 25.1 | 23.1 | 24.3 | 25.1 | 24.8 |
| ***Excretion*** | | | | | | | | |
| Biomass *P. avirostris* (µgC ind$^{-1}$) | 0.53 (0.07) | 0.60 (0.08) | 0.54 (0.06) | | | | | |
| Biomass *O. nana* (µgC ind$^{-1}$) | | | | | | 0.23 (0.02) | | 0.24 (0.01) |
| ***Respiration*** | | | | | | | | |
| Biomass *P. avirostris* (µgC ind$^{-1}$) | | | 0.70 (0.06) | 0.71 (0.02) | 0.72 (0.04) | | | |
| Biomass *O. nana* (µgC ind$^{-1}$) | | | | | | 0.25 (0.00) | 0.27 (0.01) | 0.25 (0.01) |

Once the oxygen and nutrient samples were taken, the remaining water was sieved through a 100 µm mesh and the experimental organisms were collected and preserved with formaline (4% final concentration) for biomass determination, as for the feeding experiments (see details above).

We verified that element concentration (N, P or $O_2$) in experimental bottles was significantly different than in the control bottles (one-way ANOVA test, two-tailed $p < 0.05$); when these differences were not significant, no respiration or excretion rates were assigned. For the respiration incubations, the differences between control and experimental bottles were always <10% for *Penilia avirostris* and <5% for *Oithona nana*. For comparative purposes all the metabolic parameters were

calculated in terms of weight specific excretion or respiration. We calculated the quotient between ammonia excretion and phosphate excretion to determine the stoichiometric composition of excretion products.

Respiration rates were converted to carbon demands using a coefficient RQ of 0.97 (Båmstedt et al. 2000). The ratio between oxygen consumption and $NH_4$ excretion was calculated to determine the catabolism type (lipids-proteins).

## RESULTS

### Planktonic community composition

The abundance of the most representative groups of the zooplankton and the initial biomass of *Penilia avirostris* and *Oithona nana* at the time of the experiments are shown in Table 4.3. The zooplankton community was numerically dominated by *O. nana*, followed by *P. avirostris* both species combined accounting for more than 50% of the total zooplankton in most of the experiments. In terms of biomass *P. avirostris* was the highest contributor to zooplankton bulk in all the experiments.

Chl *a* concentrations were low during the study, ranging from 0.16 μg $l^{-1}$ in Gra-7 to 1.12 μg $l^{-1}$ in Gra-1 (Table 4.4). In Gra-1 the >5 μm fraction represented most of the bulk of Chl *a*. On the other hand, the phytoplanktonic communities of Gra-7 and Gra-8 were clearly dominated by <5 μm cells.

The different components of microbial community during the experiments are also shown in Table 4.4. Heterotrophic bacteria abundance ranged between $0.25x10^6$ cells $ml^{-1}$ to $1.73x10^6$ cells $ml^{-1}$. Cyanobacteria were less abundant, with concentrations around $10^3$ cells $ml^{-1}$ for *Prochlorococcus* and $10^4$ cells $ml^{-1}$ for *Synechococcus*.

Other important contributors to the planktonic community were flagellates. The abundance and proportion of the different size-fractions of the flagellate community were quite stable along the study, with concentrations around 4000 cells $ml^{-1}$ for <2 μm flagellates. A clear exception was Gra-3, in which the abundance of <2 μm flagellates was half of the usually found in the other experiments. The abundance of 2-5 μm flagellates ranged by a factor of 4 in the different experiments, from 553 cells $ml^{-1}$ in Gra-3, to 2408 cells $ml^{-1}$ in Gra-6. The abundance of the largest size-fraction of flagellates (>5 μm) were less variable and quite low, ranging from 80 cells $ml^{-1}$ (Gra-3) to 195 cells $ml^{-1}$ (Gra-6).

Another component of the nanoplanktonic community, at times important, was diatoms. Small-size chain-forming diatoms (*Skeletonema* spp.) were remarkably abundant during Gra-6. For the rest of experiments diatoms were, in general, poorly represented in plankton, especially in Gra-7 and Gra-8.

Table 4.3. Community composition (ind $m^{-3}$) of the most relevant groups of zooplankton, including biomass (µgC $ind^{-1}$) of *Penilia avirostris* and *Oithona nana*. Gra: grazing experiments; Met: metabolism experiments.

| **Abundance** | Gra-1 | Gra-2 | Gra-3 | Gra-4 | Gra-6 | Gra-7 | Gra-8 |
|---|---|---|---|---|---|---|---|
| *Penilia avirostris* | 8302 | 7922 | 12587 | 4861 | 4950 | 5250 | 5500 |
| *Oithona nana* | 7538 | 14373 | 27575 | 4653 | 9575 | 8700 | 9900 |
| *Evadne* spp. | 611 | 509 | 2910 | 116 | 675 | 500 | 600 |
| Apendicularians | 968 | 2490 | 4074 | 1366 | 1125 | 300 | 200 |
| Other copepods[1] | 5500 | 10412 | 11496 | 13033 | 7575 | 6325 | 5050 |
| **Biomass** | | | | | | | |
| *P. avirostris* | 5313 | 4753 | 4657 | 3354 | 2465 | 3035 | 3179 |
| *O. nana* | 1206 | 2443 | 4136 | 558 | 2566 | 1322 | 1643 |

| **Abundance** | Met-1 | Met-2 | Met-3 | Met-4 | Met-5 | Met-6 | Met-7 | Met-8 |
|---|---|---|---|---|---|---|---|---|
| *Penilia avirostris* | 3753 | 4220 | 3438 | 4074 | 4717 | 5897 | 4604 | 2506 |
| *Oithona nana* | 4637 | 5064 | 5348 | 6757 | 7905 | 7854 | 7619 | 4202 |
| *Evadne* spp. | 80 | 291 | 789 | 577 | 554 | 643 | 448 | 107 |
| Apendicularians | 268 | 175 | 1502 | 815 | 864 | 429 | 244 | 201 |
| Other copepods[1] | 7505 | 3681 | 5297 | 3412 | 6222 | 5415 | 4808 | 4222 |
| **Biomass** | | | | | | | | |
| *P. avirostris* | 2214 | 2659 | 2544 | 2332 | 2510 | 4492 | 2692 | 2534 |
| *O. nana* | 1135 | 1256 | 1236 | 1525 | 1998 | 1896 | 1982 | 992 |

[1] Without including nauplii

Regarding to the other microplankton groups, dinoflagellates greatly contributed to the bulk of the microbial community in Gra-1, Gra-2 and Gra-8, with abundance of c.a. 5 cells $ml^{-1}$. Gra-4 was conducted during a peak of abundance of ciliates (9 cells $ml^{-1}$), whereas in Gra-3, Gra-6 and Gra-8 the initial abundance of both groups was quite similar, with values ranging from 1 to 4 cells $ml^{-1}$.

Table 4.4. Initial abundance of the different groups of the microbial community in the grazing experiments. Average values, chlorophyll *a* in μg $l^{-1}$ and rest of the groups in cells $ml^{-1}$. Chl *a*: Chlorophyll *a*; HetBact: Heterotrophic bacteria; Proch: *Prochlorococcus*; Synec: *Synechococcus*; Flag: Flagellates; Dinoflag: Dinoflagellates; Single-Diat: Single diatoms; and Chain-Diat: Chain-forming diatoms.

| | Gra-1 | Gra-2 | Gra-3 | Gra-4 | Gra-6 | Gra-7 | Gra-8 |
|---|---|---|---|---|---|---|---|
| Chl *a* total | 1.12 | 0.32 | 0.30 | 0.62 | 0.29 | 0.16 | 0.23 |
| Chl *a* >5μm | 0.86 | 0.12 | 0.16 | 0.23 | 0.14 | 0.002 | 0.03 |
| HetBact | $1.00x10^6$ | $0.87x10^6$ | $1.73x10^6$ | $0.72x10^6$ | $0.67x10^6$ | $0.41x10^6$ | $0.25x10^6$ |
| Proch | 7533 | 2577 | 10937 | 8983 | 514 | 8051 | 1123 |
| Synec | 95983 | 70000 | 39139 | 43252 | 30535 | 29442 | 35928 |
| <2 μm Flag | 3706 | 3850 | 1955 | 4770 | 4710 | 3764 | 3644 |
| 2-5 μm Flag | 808 | 2298 | 553 | 1106 | 2408 | 1906 | 1896 |
| >5 μm Flag | 193 | 321 | 80 | 384 | 195 | 191 | 159 |
| Ciliates | 0.8 | 0.6 | 1.9 | 9.1 | 3.4 | 3.9 | 3.0 |
| Dinoflag | 4.7 | 4.7 | 1.4 | 2.0 | 3.2 | 2.0 | 4.5 |
| Single-diat | 55 | 12 | 19 | 20 | 33 | 3 | 2 |
| Chain-diat | 27 | 2 | 25 | 18 | 254 | 0 [a] | 2 |

## Grazing rates of *Penilia avirostris* and *Oithona nana*

The clearance rates of *Penilia avirostris* on the different groups of the microbial community are shown in Table 4.5. One remarkable result obtained from these experiments was that *P. avirostris* cleared all microbial components considered, with the exception of heterotrophic bacteria or cyanobacteria. For the rest of the components of the microbial community, clearance rates ranged between 4 ml $ind^{-1}$ $d^{-1}$ to 50 ml $ind^{-1}$ $d^{-1}$ (mean 16.4 ml $ind^{-1}$ $d^{-1}$ ± 0.63 SE). *P. avirostris* consumed Chl *a* and nanoflagellates of all sizes at variable rates during the study, overall ranging from 4 ml $ind^{-1}$ $d^{-1}$ to 27 ml $ind^{-1}$ $d^{-1}$ but with a peak (38 ml $ind^{-1}$ $d^{-1}$) in Gra-4 where the clearance rate of the 2–5 μm flagellates was higher than in the rest of the groups and experiments. While some prey types were eaten in all experiments, others were eaten occasionally. Thus, ciliates were ingested only in two of the experiments and at low rates (4 ml $ind^{-1}$ $d^{-1}$ in Gra-8, and 15 ml $ind^{-1}$ $d^{-1}$ in Gra-6). Dinoflagellates were consumed in almost all experiments at rates ranging from 12 to 26 ml $ind^{-1}$ $d^{-1}$.

Finally, chain-forming (*Skeletonema* spp.) and single diatom cells, were ingested on 4 and 5 experiments (respectively), their clearance rates presenting the highest variability, between 11 ml $ind^{-1}$ $d^{-1}$ and 27 ml $ind^{-1}$ $d^{-1}$ for single-cell diatoms, and between 8 ml $ind^{-1}$ $d^{-1}$ to 50 ml $ind^{-1}$ $d^{-1}$ for chain-forming diatoms.

Table 4.5. Clearance rates of *Penilia avirostris* (ml ind $d^{-1}$; average ± SE). *Growth*: values in experimental bottles higher than controls; 0: no ingestion. Chl *a*: Chlorophyll *a*; HetBact: Heterotrophic bacteria; Proch: *Prochlorococcus*; Synec: *Synechococcus*; Flag: Flagellates; Dinoflag: Dinoflagellates; Single-Diat: Single diatoms; Chain-Diat: Chain-forming diatoms.

| | Gra-1 | Gra-2 | Gra-3 | Gra-4 | Gra-6 | Gra-7 | Gra-8 |
|---|---|---|---|---|---|---|---|
| Chl *a* total | 10.1 (0.58) | 19.1 (2.15) | 5.9 (0.87) | 16.6 (0.54) | 13.2 (1.97) | 15.1 (1.73) | 24.9 (1.10) |
| Chl *a* >5µm | 7.3 (0.32) | 22.6 (0.90) | 4.4 (0.92) | 9.9 (0.49) | 13.9 (2.46) | 17.1 (2.56) | 27.4 (3.48) |
| HetBact | 0 | 0 | 0 | 0 | *growth* | 0 | *growth* |
| Proch | *growth* | *growth* | *growth* | *growth* | *growth* | *growth* | 0 |
| Synec | *growth* | 0 | 0 | 0 | 0 | 0 | 0 |
| <2 µm Flag | 22.1 (0.41) | 7.1 (0.43) | 8.8 (0.16) | 11.3 (0.73) | 15.7 (0.54) | 17.0 (0.60) | 8.6 (0.28) |
| 2-5 µm Flag | 18.6 (0.41) | 16.6 (1.50) | 6.3 (0.47) | 37.7 (2.56) | 16.4 (0.36) | 20.3 (0.92) | 19.1 (1.06) |
| >5 µm Flag | 12.7 (0.65) | 26.7 (1.51) | 3.9 (0.25) | 16.2 (1.04) | 20.9 (0.44) | 15.2 (0.71) | 16.4 (0.32) |
| Ciliates | 0 | 0 | 0 | 0 | 15.2 (1.80) | 0 | 4.0 (0.27) |
| Dinoflag | 17.0 (1.93) | 25.9 (1.52) | 0 | 21.2 (1.66) | 21.6 (1.58) | 11.9 (1.48) | 15.5 (0.15) |
| Single-diat | 10.7 (1.61) | 0 | 0 | 16.2 (2.85) | 26.8 (3.15) | 22.6 (4.77) | 0 |
| Chain-diat | 0 | 0 | 24.4 (4.41) | 18.4 (3.50) | 8.1 (0.68) | 49.9 (12.21) | 11.1 (1.04) |

*Oithona nana* clearance rates are shown in Table 4.6. No ingestion was detected on Chl *a*, heterotrophic bacteria, cyanobacteria and flagellates <2 µm. Clearance rates varied between 1 ml $ind^{-1}$ $d^{-1}$ (on 2-5 µm flagellates Gra-7) to 36 ml $ind^{-1}$ $d^{-1}$ (on ciliates Gra-3) (mean 9.8 ml $ind^{-1}$ $d^{-1}$ ± 1.25 SE). Flagellates >2 µm were ingested by

*O. nana* only in two of the experiments, clearance rates ranging from 1 ml $ind^{-1}$ $d^{-1}$ to 9 ml $ind^{-1}$ $d^{-1}$ for 2-5 μm flagellates and 1 ml $ind^{-1}$ $d^{-1}$ to 2 ml $ind^{-1}$ $d^{-1}$ for >5 μm flagellates. Ciliates were the preferred prey in most of the experiments, clearance rates ranging from 4 to 36 ml $ind^{-1}$ $d^{-1}$. Dinoflagellates were ingested in 3 experiments with a constant clearance rate (6-7 ml $ind^{-1}$ $d^{-1}$). Finally, single cell diatoms were ingested in 3 experiments at a uniform clearance rate, from 4 to 6 ml $ind^{-1}$ $d^{-1}$, and chain-forming diatoms were ingested by *O. nana* just in one occasion (Gra-3) at 22 ml $ind^{-1}$ $d^{-1}$.

Table 4.6. *Oithona nana*, clearance rates (ml ind $d^{-1}$; average ± SE). *Growth*: values in experimental bottles higher than controls; 0: no ingestion. Chl *a*: Chlorophyll *a*; HetBact: Heterotrophic bacteria; *Proch*: *Prochlorococcus*; *Synec*: *Synechococcus*; Flag: Flagellates; Dinoflag: Dinoflagellates; Single-Diat: Single diatoms; Chain-Diat: Chain-forming diatoms.

| | Gra-1 | Gra-2 | Gra-3 | Gra-4 | Gra-6 | Gra-7 | Gra-8 |
|---|---|---|---|---|---|---|---|
| Chl *a* total | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chl *a* >5μm | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| HetBact | *growth* | *growth* | *growth* | 0 | *growth* | 0 | *growth* |
| Proch | *growth* | *growth* | *growth* | 0 | *growth* | *growth* | *growth* |
| Synec | *growth* | 0 | 0 | *growth* | *growth* | 0 | 0 |
| <2 μm Flag | 0 | 0 | *growth* | 0 | 0 | 0 | 0 |
| 2-5 μm Flag | 0 | *growth* | *growth* | 9.3 (1.0) | 0 | 0.5 (0.1) | 0 |
| >5 μm Flag | 0 | 2.0 (0.2) | *growth* | *growth* | 0 | 0 | 1.1 (0.2) |
| Ciliates | 24.1 (4.41) | 0 | 35.7 (3.45) | 17.2 (2.13) | 5.4 (1.27) | 4.3 (1.14) | 7.0 (0.15) |
| Dinoflag | 0 | 0 | 0 | 0 | 6.0 (1.9) | 6.6 (1.8) | 6.7 (0.0) |
| Single-diat | 3.5 (0.81) | 0 | 0 | 5.9 (0.98) | *growth* | 5.9 (1.53) | 0 |
| Chain-diat | 0 | 0 | 22.1 (2.61) | 0 | 0 | 0 | 0 |

The growth enhancements due to the presence of the copepods or the cladocerans are also shown in Tables 4.5 and 4.6. For *Penilia avirostris* the positive effect was always present on the picoplanktonic community (heterotrophic bacteria and cyanobacteria). *Oithona nana* had a positive effect also on the picoplanktonic

community, and occasionally on the nanoplanktonic flagellates and single-cell diatoms.

Daily rations for *Penilia avirostris* and *Oithona nana* are shown in Figure 4.1. Values for *P. avirostris* varied between 26% to 157% body carbon $d^{-1}$ and were higher than those of *O. nana* (2% to 68%). Nanoflagellates contributed to most of the diet of *Penilia avirostris*, representing always >50% of the carbon ingested (with the exception of Gra-3), followed by dinoflagellates. Chain forming diatoms represented

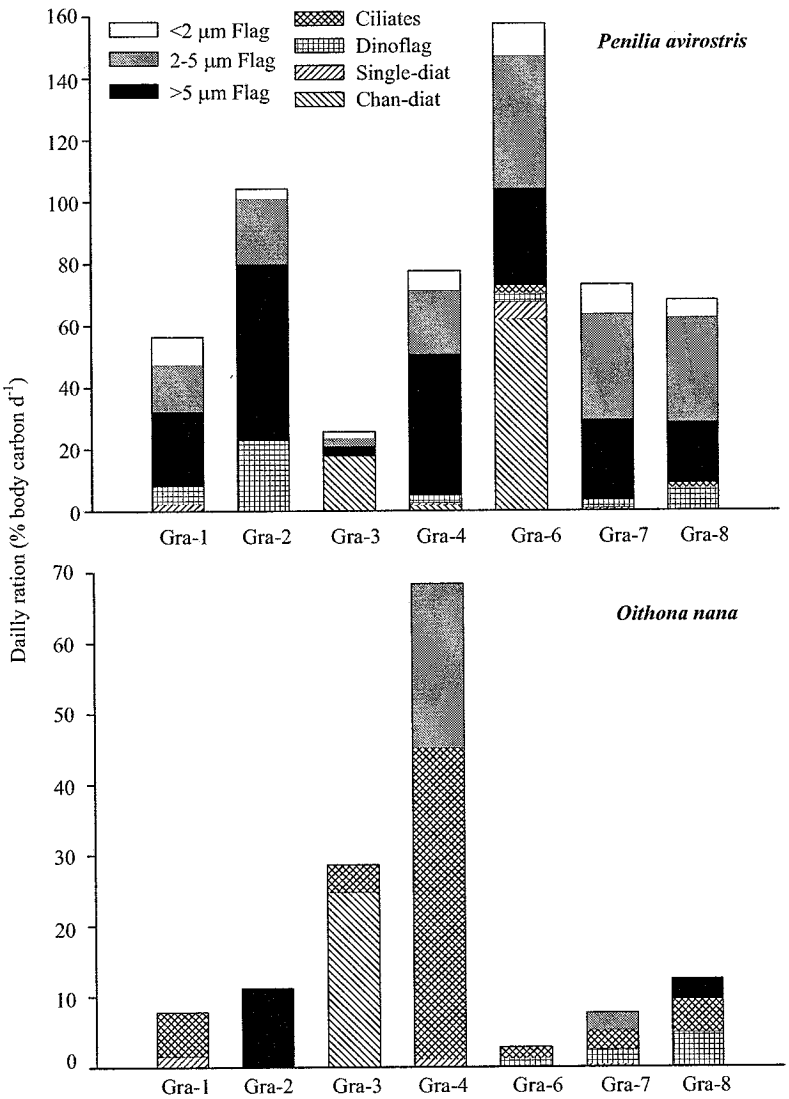



Figure 4.1. *Penilia avirostris* and *Oithona nana*. Daily rations (% body carbon $d^{-1}$) and and contribution of each prey type to carbon ingested. Flag: Flagellates; Dinoflag: Dinoflagellates; Single-Diat: Single diatoms; and Chain-Diat: Chain-forming diatoms.

a high proportion of the diet on Gra-3 and Gra-6. For *Oithona nana* ciliates comprised the majority of carbon ingested in almost all the experiments.

The trophic impact of *Penilia avirostris* and *Oithona nana* on the natural microbial communities are shown in Figures 4.2 and 4.3. The grazing impact of *P. avirostris* on the standing stock of each component of the microbial communities was always < 25% (2 to 23%) of the standing stock consumed per day, with the exception of chain-forming diatoms in Gra-7 (35%) (Figure 4.2). *P. avirostris* removed between 5-15% of the Chl *a* and flagellates in all the experiments, being their impact on the rest of prey more variable. Clear example of this variability was the impacts on ciliate standing stocks, only evident in 2 experiments (Gra-6 and Gra-8). On the other hand, *Oithona nana* grazing impact was constrained to very few groups of plankton (Figure 4.3). The grazing pressure of *O. nana* was only important in one group, the ciliates, the impact ranging between 2% and 13% of the standing stock daily. The trophic impact of these copepod on the rest of the groups were always <5%, with the exception of chain-forming diatoms in Gra-3 (15%). Heterotrophic bacteria and cyanobacteria showed a moderate increase (10-20%) of their populations (Tables 4.5 and 4.6) when *Penilia avirostris* and *Oithona nana* were present.

### Metabolic activity of *Penilia avirostris* and *Oithona nana*

*Penilia avirostris* and *Oithona nana* excretion rates are shown in Table 4.6. Weight specific ammonia excretion of *P. avirostris* ranged between 0.051 $\mu gNH_4\ \mu gC^{-1}\ d^{-1}$ and 0.078 $\mu gNH_4\ \mu gC^{-1}\ d^{-1}$. *O. nana* ammonia excretion rates were higher than that of the cladoceran, ranging between 0.11 $\mu gNH_4\ \mu gC^{-1}\ d^{-1}$ and 0.10 $\mu gNH_4\ \mu gC^{-1}\ d^{-1}$. Inorganic phosphate excretion was only detected on *O. nana* experiments (0.034 $\mu gPO_4\ \mu gC^{-1}\ d^{-1}$ in Met-8 and 0.039 $\mu gPO_4\ \mu gC^{-1}\ d^{-1}$ in Met-6). Consequently, the N:P ratio for the excretion products was only calculated for *O. nana* (6.99-7.14). To complete the metabolic activity study, the respiration rates of *P. avirostris* and *Oithona nana* are shown in Table 4.7. *P. avirostris* presented oxygen consumption rates of 0.84-1.15 $\mu l\ O_2\ ugC^{-1}\ d^{-1}$, significantly different than those of *O. nana* (0.35-0.50 $\mu l\ O_2\ \mu gC^{-1}\ d^{-1}$). The metabolic costs of respiratory activity (Table 4.7) were higher for *Penilia avirostris* (43-59 % body C $d^{-1}$) than for *Oithona nana* (18-26 % body C $d^{-1}$).

## DISCUSSION

### Grazing

The composition and abundance of the plankton community during the experiments was typical of the summer period of the Northwestern Mediterranean (Vaqué et al. 1997; Calbet et al. 2001). The microbial community was characterized

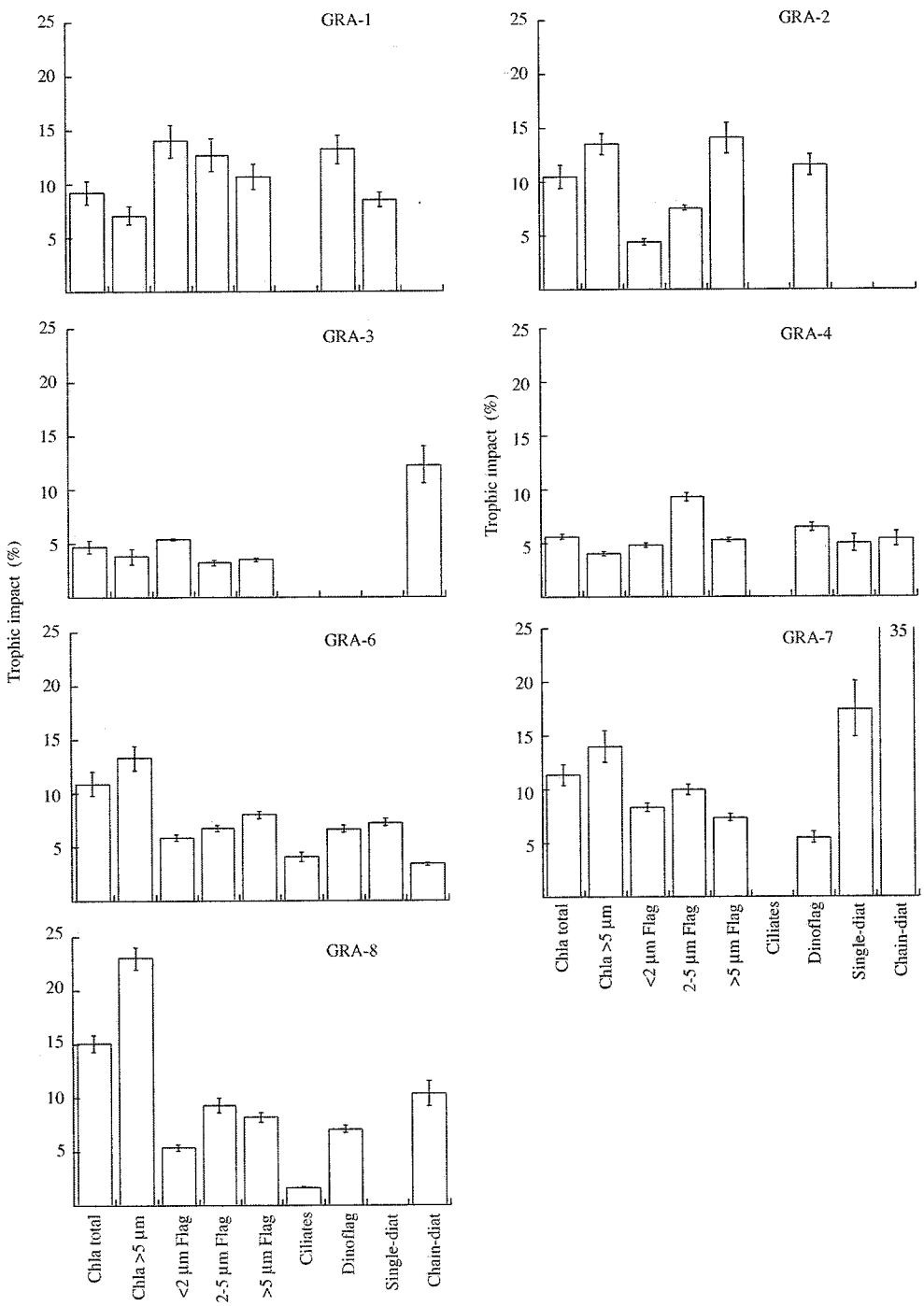



Figure 4.2. *Penilia avirostris* grazing impact (%) upon the different groups of the microbial community. Average values (% ± SE). Flag: Flagellates; Dinoflag: Dinoflagellates; Single-Diat: Single diatoms; and Chain-Diat: Chain-forming diatoms.

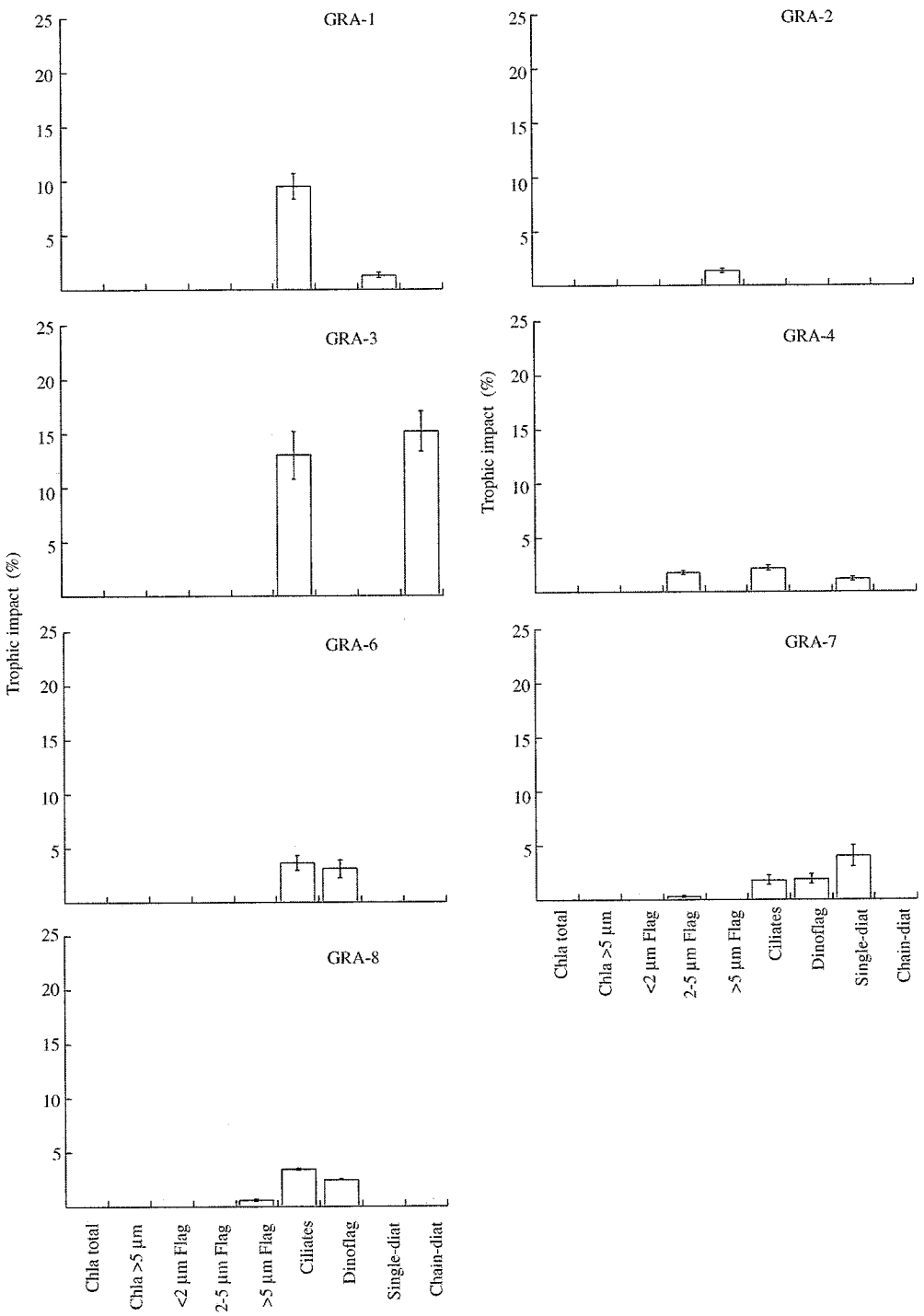



Figure 4.3. *Oithona nana* grazing impact (%) upon the different groups of the microbial community. Average values (% ± SE). Flag: Flagellates; Dinoflag: Dinoflagellates; Single-Diat: Single diatoms; and Chain-Diat: Chain-forming diatoms.

by the dominance of small cells (<5 μm) such as bacteria, cyanobacteria and small flagellates. Ciliates, dinoflagellates and chain-forming diatoms were also present sporadically at high abundance. In summer, *Oithona nana* and *Penilia avirostris* dominated the mesozooplankton community of the North Western Mediterranean.

Table 4.7. Weight specific excretion rates of *Penilia avirostris* and *Oithona nana*. Average values (± SE).

| | Met-1 | Met-2 | Met-3 | Met-6 | Met-8 |
|---|---|---|---|---|---|
| **Ammonia $NH_4$** | | | | | |
| *P. avirostris* ($\mu gNH_4\ \mu gC^{-1}\ d^{-1}$) | 0.078 (0.007) | 0.051 (0.007) | 0.069 (0.007) | | |
| *O. nana* ($\mu gNH_4\ \mu gC^{-1}\ d^{-1}$) | | | | 0.111 (0.006) | 0.100 (0.002) |
| **Phosphate $PO_4$** | | | | | |
| *P. avirostris* ($\mu gPO_4\ \mu gC^{-1}\ d^{-1}$) | N.d [1] | N.d [1] | N.d [1] | | |
| *O. nana* ($\mu gPO_4\ \mu gC^{-1}\ d^{-1}$) | | | | 0.039 (0.006) | 0.034 (0.001) |
| N:P (atomic) | *very high*[2] | *very high*[2] | *very high*[2] | 7.14 (1.14) | 6.99 (0.12) |

[1] N.d: no detected
[2] *Very high*: not calculate but high because is the result from the division by almost zero

*Penilia avirostris* feed on a wide range of suitable organisms (from <2 μm flagellates to large dinoflagellates). For a detailed study of the prey preferences of *P. avirostris* see Atienza et al. (2006a). Regarding *Oithona nana,* the diet of this copepods has been shown to be very variable, including algae and nauplii (Lampitt & Gamble 1982), nanoflagellates (Calbet et al. 2000), and even detritus or fecal pellets (González & Smetacek 1994). In our experiments, *O. nana* centered their feeding activity upon ciliates (which represents 41 % of the carbon ingested per day, on average) and dinoflagellates, seldom preying on other components of the microbial community (e.g. nanoflagellates and diatoms). Lampitt & Gamble (1982) suggested that the raptorial feeding behavior of this copepod constrains prey size to a narrower range in comparison with filter feeders, like *Penilia avirostris.*

Daily food rations for *Penilia avirostris* are similar to the values reported by Broglio et al. (2004) for the same species, but higher to those estimated by Pavlova (1967), and Paffenhöfer & Orcutt (1986). Maximum carbon intakes occurred where nanoflagellates and dinoflagellates comprised more than 70% of initial biomass (Gra-2, and Gra-4), and where diatoms were near 40% on initial carbon standing stock (Gra-6). Minimum daily rations occurred were flagellates were scarce. Daily food rations for *Oithona nana* were in most of the experiments lower than 28% of the body carbon consumed per day, with a single maximal value of 68%. Those values were slightly lower than those determined for *Penilia avirostris* and are in the same range than the daily rations reported by Castellani et al. (2005) (<17%), Londsdale et al. (2002), and Nakamura & Turner (1997) (12-27%) for *Oithona similis*. No clear pattern was identified between daily rations and prey biomass. Ciliates comprised the majority of the carbon ingested by *O. nana*.

Table 4.8. Weight specific respiration rates of *Penilia avirostris* and *Oithona nana*. Average values (± SE).

| | Met-3 | Met-4 | Met-5 | Met-6 | Met-7 | Met-8 |
|---|---|---|---|---|---|---|
| **Oxygen consumption** | | | | | | |
| *P. avirostris* ($\mu lO_2\ \mu gC^{-1}\ d^{-1}$) | 0.84 (0.12) | 1.15 (0.09) | 0.93 (0.10) | | | |
| *O. nana* ($\mu lO_2\ \mu gC^{-1}\ d^{-1}$) | | | | 0.35 (0.02) | 0.50 (0.01) | 0.48 (0.03) |
| **% carbon content** | | | | | | |
| *P. avirostris* ($\mu gC\ \mu gC^{-1}\ d^{-1}$) | 0.43 (0.06) | 0.59 (0.05) | 0.48 (0.05) | | | |
| *O. nana* ($\mu gC\ \mu gC^{-1}\ d^{-1}$) | | | | 0.18 (0.01) | 0.26 (0.00) | 0.25 (0.02) |
| O:N (atomic) | | | | | | |
| *P. avirostris* | 16.8 | | | | | |
| *O. nana* | | | | 3.9 | | 6.0 |

## Trophic impact and food-web interactions

*Penilia avirostris* exerted a moderate impact on the microbial populations of the NW Mediterranean. In all the experiments the trophic impact (reduction in the

standing stock) produced by *P. avirostris* ingestion was in general <10% for any prey considered. Only in experiments Gra-3 and Gra-6 the impact upon chain-forming diatoms was higher (≈ 25%); also in experiment Gra-7 the impact on Chl *a* > 5 μm was about 20%. The trophic impact on the standing stock of phytoplankton determined in the present study was higher than previous reports, based on gut pigments contents (≈ 1%, Wong et al. 1992; < 5% Lipej et al. 1997) or cell removal (< 1% Broglio et al. 2004). For ciliates, Broglio et al. (2004) estimated that the trophic impact of *P. avirostris* and other 2 species of marine cladocerans (*Podon* sp. and *Evadne* sp.) were < 5% of the standing stock, which is similar to the values detected in the present study. There is no other information to compare the feeding impact of this species of cladoceran on nanoflagellates, dinoflagellates or diatoms. However, it is surprising that dense populations of *P. avirostris*, with explosive reproductive strategies, exert little impact on their prey. One possible explanation may be related with the vertical distribution of zooplankton (Gabriel & Thomas 1988, Harris 1988). *P. avirostris* has been reported to be found in near surface waters, in very narrow layers (Alcaraz 1970, Alcaraz 1981, Kim et al. 1994, Onbé & Ikeda 1995). Considering that a typical *P. avirostris* patch may have c.a. 5 m diameter in the vertical scale (Alcaraz 1981, Kim et al. 1994) and taking into account the depth of the study area (10-35 m) the trophic impact calculated in Figure 4.2 should be multiplied by a factor of 2-7 if assuming a patchy distribution. If this correction is applied the impact that *P. avirostris* may have on their surrounding waters could reach values between 14% and 91% of reduction in the standing stocks of Chl *a*. Hence, detailed information on the fine scale distribution of the organisms is needed to accurately interpret the predation of zooplankton on natural communities.

*Oithona nana* was mostly impacting upon ciliate populations (up to 25% of the standing stock consumed per day in Gra-3), although the consequences of its feeding activity were variable, and barely detectable in most of the experiments. For the rest of the microbial groups, the grazing impact was always < 0% of the stock of the population reduced per day, except for the chain-forming diatoms in experiment Gra-3 where the impact reached 15% of the standing stock. In general, the removal by *O. nana*, as other oithonids, usually accounted for a moderate portion of the standing stock of their prey (Nakamura & Turner 1997, Calbet et al. 2000, Lonsdale et al. 2000, Zeldis et al. 2002, Broglio et al. 2004).

Additionally, grazing by zooplankton at higher trophic levels may be consequentially transmitted trough the food web to the lower fractions by trophic cascade interactions (Zöllner et al. 2003, Katechakis et al. 2004). *Penilia avirostris* and *Oithona nana* entered the top of the food web at different levels. When *P. avirostris* was presented in the incubations the net growth rate of nanoflagellates decreased markedly indicating that this microbial group was the most vulnerable to grazing. On the contrary, the presence of *O. nana* led sometimes to an increase in nanoflagellates (Figure 4.3), likely due to the diminution of ciliates during the incubations mediated

by copepod grazing. Both zooplankters produced an enhancing effect on the bacterial community, most likely result of the release of organic matter due to sloppy feeding and excretion, although complicated trophic cascade effects cannot be rejected. Thus, even though *P. avirostris* and *Oithona nana* affect the microbial food web by different ways, both contribute to enhance bacterial community growth.

## Metabolic activity and nutrient recycling contribution

Ecological stoichiometry constitutes an useful tool to understand the balance of energy and chemical elements in ecological interactions, especially in trophic relationships (Sterner & Elser 2002). The release and recycling of elements will be determined by the difference between ingested nutrients and those incorporated into new biomass (Andersen et al. 2004). Also, the nutrient recycling by consumers can change the proportions between recycled elements, with potentially strong effects on the autotroph community (Elser & Urabe 1999).

To our knowledge no data are available on phosphorus excretion rates of marine cladocerans, although considerable research has been conducted on freshwater species. The values reported for daphnids are variable and likely species specific, 10.85 μgP $mgDW^{-1}$ $d^{-1}$ for *Daphnia pulex* (Olsen et al. 1986), 24 μgP $mgC^{-1}$ $d^{-1}$ for *Daphnia* spp. (Perez-Martinez & Gulati 1999). Also, and see Gulati et al. (1999) for values on different species of freshwater cladocerans. The high variability presented in published P excretion rates could be the result of a methodological problem. In this line of reasoning, Olsen et al. (1986) argued that, sometimes, P release was indistinguishable from zero when incubations where done with food with high C:P ratios. Moreover, no detection of P excretion could be due to the low amount released by cladocerans, and also because bacteria on the incubations could rapidly take up the released phosphorus (Olsen et al. 1986). In the present study, P excretion of *Penilia avirostris* was below our detection limits. In order to reduce artifacts generated by bacteria contamination, filtered seawater was used to conduct the incubations.

In the case of *Oithona nana* no specific data about phosphate excretion are available. Moreover, reported values for *O. davisae* N-excretion are significantly lower (3.84-7.44 ngP $\mu gDW^{-1}$ $d^{-1}$, Hiromi & Ichihashi 1995) than the ones found in the present study. Our results, on the other hand, are similar to those reported for other copepod species (Båmstedt & Tande 1985). The release of phosphorus by zooplankton is directly coupled to its ingestion and use for growth and reproduction. Probably higher rates of P excretion in copepods are related to the lower content of P in those organisms in comparison with the high content of nucleic acids in cladocerans, especially ARN, necessary to support the high growth rates and accelerate reproductive behavior (Egloff et al. 1997, Andersen et al. 2004). Picard & Lair (2000) emphasized that omnivores require more N relative to P, and that

opportunistic r-selected species (such cladocerans) tend to have higher requirements for P relative to N, giving low N:P attributed to higher amounts of RNA required for protein synthesis and rapid growth.

The main nitrogen compound excreted by zooplankton is ammonia (Frangoulis et al. 2005; Miller & Landry 1984). Data for comparison purposes are again from freshwater systems, because of the gap on marine cladocerans. The ammonia excretion rates determined by *Penilia avirostris* in the present study are in the same range to those found for different species of daphnids (Carrillo et al. 1996, Perez-Martinez & Gulati 1999; Wiltshire & Lampert 1999). Values for *Oithona nana* were higher than those for *P. avirostris* and in the same range than those reported for oithonids and other copepods (Debs 1984, Paffenhöfer & Gardner 1984, Gaudy et al. 2000, Ikeda et al. 2001).

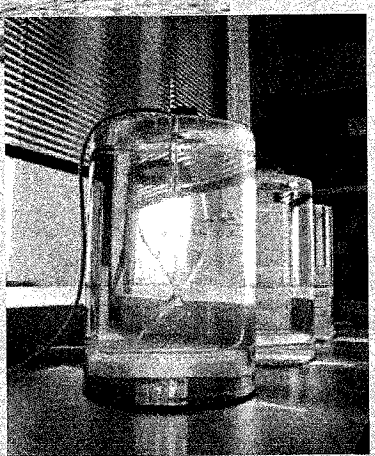
The N:P ratio of excreted products is important in terms of the ecology of the systems. N:P ratios of zooplankton excretion range from 7 to 19 (Wen & Peters 1994), which agrees with the results of the present study for *Oithona nana*. For *Penilia avirostris* no ratio was computed because P excretion was not detected. According to the data we can hypothesize that systems dominate by cladocerans will eventually present P limitation for phytoplankton growth. In contrast, in communities were copepods are the most important zooplankter the recycling of P will be faster (Carrillo et al. 1986, Walve & Larsson 1999). The same conclusion is suggested if the fluxes of nitrogen and phosphorus are estimated. Assuming requirements of N and P for primary production near the same area c.a. 30.3 mgN $m^{-2}$ $d^{-1}$ and 1.89 mgP $m^{-2}$ $d^{-1}$ (Calbet et al. 1996, Morán et al. 2002, Gasol com. per), and an average depth of 20 meters, the N-excretion by the cladoceran would account for 7.5-15.2% of the N demand. N-excretion by *O. nana* community would contribute up to 13.8% of the N requirements of phytoplankton. The P excretion by *O. nana* would supply between 38.2% and 77.0% of the P requirements of primary producers.

Regarding *Penilia avirostris* respiratory activity, there is only, to our knowledge, one study reporting oxygen consumption rates (0.26 $mgO_2$ mg-body $weight^{-1}$ $d^{-1}$ at 23-25 °C and without food, Pavlova 1967). Our values were slightly higher than those of Pavlova (1967) (0.25-0.47 $mgO_2$ mg-body $weight^{-1}$ $d^{-1}$). Other estimates for freshwater cladocerans are in the same range to those found in the present study (Macedo & Pinto-Coelho 2000). The respiration rates of *Oithona nana* were comparable to those obtained for the same specie by Lampitt & Gamble (1982), although slightly lower than the ones for other *Oithona* species (Hiromi et al. 1988, Hiromi 1994, Nakamura & Turner 1997, Mayzaud et al. 2002, Castellani et al. 2005). O:N ratios for *P. avirostris* were 17, and *O. nana* values ranged between 4 and 6, which fall in the general range reported by other authors (Gaudy & Boucher 1983) for other copepods. Ratios around 17 up to 24 are characteristics of zooplankton which catabolize equal amounts of proteins and lipids; values close to 6 imply a proteinic catabolism, which is the common situation on tropical, subtropical and temperate

seas (Gaudy & Boucher 1983, Mayzaud & Conover 1988, Christou & Moratitou-Apostolopoulou 1995).

The metabolic cost of respiration of *Oithona nana* coincide with the estimates provided by Lampitt & Gamble (1988), Hiromi et al. (1988), Nakamura & Turner (1997), and Castellani et al. (2005). The carbon required for the respiration of this specie is higher than its daily rations, which is unlike because the population was actively growing. A possible explanation was suggested by González & Smetacek (1994) who determine that *Oithona* can obtain 20-30% of the carbon requirements from detritus or fecal pellets, which were not considered in the present work (subestimation of the daily rations). Therefore, if we increase our estimates by 20%, the supplies for respiration are covered and a little amount of carbon remains available for growth and reproduction. In the case of *P. avirostris*, in all the experiments (with the exception of Gra-3) the cost of respiration was lower than the carbon ingested, and a higher proportion of carbon remains available for growth and reproduction in comparison with *O. nana*. This could help to explain the explosive population growth that marine cladocerans have during the summer season.

In summary, it is clear that *Penilia avirostris* and *Oithona nana* have separated trophic niches, and can, therefore, share habitat without competing for food resources. Even though the trophic impact estimated in this study seems to be moderate for the whole water column, both species can be strongly controlling the microbial community of their surrounding water masses. Excretion rates for N and P were different for both species, with a higher recycling of P when copepods dominated the community and a higher recycling of N when cladocerans dominated. The extend of the impact on the microbial organisms mediated by trophic cascade effects or excretion appeared variable and conditioned by the structure of the food web and condition of the organisms at the time of experiments.



# CHAPTER V. ECOLOGICAL SUCCESS OF THE CLADOCERAN *PENILIA AVIROSTRIS* IN THE MARINE ENVIRONMENT: ROLE OF FEEDING PERFORMANCE, GROSS GROWTH EFFICIENCIES AND LIFE HISTORY

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Marine Biology (submitted)

# CHAPTER V. ECOLOGICAL SUCCESS OF THE CLADOCERAN *PENILIA AVIROSTRIS* IN THE MARINE ENVIRONMENT: ROLE OF FEEDING PERFORMANCE, GROSS GROWTH EFFICIENCIES AND LIFE HISTORY

## INTRODUCTION

The marine cladoceran *Penilia avirostris* is an important component of the zooplankton community of many tropical and subtropical waters (Della Croce 1964, Tang et al. 1995, Marazzo & Valentin 2003, Rose et al. 2004). *P. avirostris* can be found from eutrophic, near shore or estuarine waters, to oligotrophic coastal waters (Onbé & Ikeda 1995, Onbé et al. 1996, Wong et al. 2004). In temperate regions this species occurs seasonally with special high abundances during summertime (Della Croce 1964, Alcaraz 1977, 1981, Paffenhöfer 1983, Onbé & Ikeda 1995, Calbet et al. 2001, Fernández de Puelles et al. 2003) and is extending its distribution to higher latitude in North Sea (Johns et al. 2005). Despite their high seasonal abundance and role in marine systems, cladocerans have been little studied compared to copepods, and the reasons for their explosive community growth are still unknown. Possible explanations could be related to life history characteristics, growth rates or gross growth efficiencies (GGE).

Life history in marine cladocerans is similar to that of their freshwater relatives (Threlkeld 1979). Populations are initiated by the hatching of resting embryos and peaks of high abundance are reached by parthenogenetic reproduction. At the end of the reproductive season, cladocerans switch to gamogenic reproduction and produce resting eggs (Egloff et al. 1997). In general, it is assumed that high abundance of cladocerans is a consequence of high rates of embryonic and post-embryonic growth, large brood sizes, and short generation times (Egloff et al. 1997, Carrillo et al. 2001). However, measures of brood size, egg production, birth and mortality rates, and developmental times, are still scarce and constitute a requirement to confirm the reasons behind such explosive community growth and blooming.

Ecological research on *Penilia avirostris* is relatively limited and mainly focused on its grazing impact and feeding behavior (Gore 1980, Paffenhöfer & Orcutt 1986, Kim et al. 1989, Lipej et al. 1997, Broglio et al. 2004, Katechakis & Stibor 2004, Katechakis et al. 2004, Atienza et al. 2006a, 2006b), and only few studies have been carried out on growth rates or developmental patterns. In relation to developmental parameters, only Della Croce (1964) with an extensive description of brood size in different regions; and Mullin & Onbé (1992), and Marazzo & Valentin (2003b) with estimations of maturation rate and developmental time, constitute the only available

literature. Regarding growth rates, Rose et al. (2004) estimated growth rates of different sizes classes of *Penilia avirostris* using preserved samples and a cohort approximation; and Paffenhöfer & Orcutt (1986) estimated growth rates and GGE of *P. avirostris* fed on *Isochrysis galbana* at different concentrations, constituting, to our knowledge, the only direct measurement of GGE available until now. However, no direct measurements of growth rates and GGE have been conducted under natural food conditions, and no direct relation was established with the explosive growth of *P. avirostris* populations.

Consequently, our aim was to study the relevant parameters for the ecological success of *Penilia avirostris,* including life history parameters, *in situ* growth and ingestion rates. Also, we provide the first estimate of GGE under *in situ* conditions. We hypothesize that besides specific life cycle strategies, *P. avirostris* should show elevated GGE to explain the ecological success of the species in oligotrophic waters. The approach will be experimental, based on incubations of *P. avirostris* populations from São Sebastião Channel (Brazil), where *P. avirostris* is one of the dominant holoplanktonic species (Resgalla and Montú 1993, Vega-Perez 1993).

## METHODOLOGY

### Study Area

The São Sebastião Channel is at approximately 23.7-23.9°S and 45.3-45.5°W between São Sebastião Island and the continent, off the northern coast of São Paulo State, Brazil. This 22-km long channel is relatively narrow (7.2 and 1.9 km wide at the northern entrance and middle sector, respectively), deep at its central basin (maximum depth of 45 m), and subjected to minor riverine input and tidal influence. As a result, local hydrodynamics is controlled mainly by the wind regime and the coastal current system (Castro et al. in press). Mean summer temperatures in the area vary between 25 and 27 °C, but relatively low temperatures may occur during this season because of bottom intrusions of the cold and nutrient-rich South Atlantic Central Water over the shelf.

### Cohort development experiments (life history)

*Penilia avirostris* cohorts were monitored in the laboratory to determine some life history parameters of this cladoceran. In addition, these cohorts provided organisms free from field collection effects to conduct additional feeding and growth incubation experiments.

Organisms for the cohorts were collected during austral summer in São Sebastião Channel by short oblique tows with a 200-μm conical net mesh (40-cm

diameter). In order to reduce organism damage a 5-l plastic bag was used instead of a closed cod end. Once onboard the plastic bag was transferred to an isothermic container, and transported to the laboratory within 1 hour of collection. The bag was tied with a string to eliminate the air inside and to prevent *Penilia avirostris* from sticking to the air-water interface.

Three different cultures were established between January 30th and March 13th 2004 (Table 5.1). From 500 to 1000 *Penilia avirostris* were randomly sorted under the stereomicroscope with a wide-mouthed pipette, and placed in 20-liter cylindrical Plexiglas tanks ($\phi$ = 30 cm; $h$ = 29.5 cm). Each tank was filled all the way up and covered with lid to avoid air-water interface. Tanks were filled with water from São Sebastião Channel collected at 1 m depth with a transparent hydrographic bottle, and reverse-flow filtered by 100 μm pore-size mesh. The water was kept in slow motion by a rotation paddle (10 rpm). Every second day 75 % of the water was renewed with new collected water, and the initial concentration of heterotrophic bacteria, *Synechococcus*, nanoflagellates (<2 μm, 2-5 μm, >5 μm), ciliates, dinoflagellates, diatoms (single cells and chain-forming), and chlorophyll *a* (total, and >5 μm) determined (see below). The tanks were kept in a temperature-controlled room under natural photoperiod and temperature conditions for 12 days.

Table 5.1. Cohort experiments of *Penilia avirostris* conducted in São Sebastião Channel. Individual initial biomass of *P. avirostris*.

| | Cohort-1 30/1/2004 | Cohort 2 13/2/2004 | Cohort 3 01/3/2004 |
|---|---|---|---|
| Temperature (°C) | 26.0 | 26.8 | 26.8 |
| Inoculation density (ind $l^{-1}$) | 25 | 50 | 50 |
| Initial biomass *P. avirostris* (μgC $ind^{-1}$) | 2.28 | 2.02 | 1.98 |

Every day, 1-2 litres of water were slowly siphoned out the tank and filtered through 100 μm to collect *Penilia avirostris* specimens for biomass and community structure assessment. The water level was maintained by adding new fresh natural seawater (filtered through 100 μm). Abundance was calculated including the dilution factor due to the daily replacement of water on the cylinders. Total abundance was accounted for the whole cylinder (20 liters). At least 60 cladocerans were examined on a stereomicroscope and classified into three different categories: juveniles (<500 μm), adult females, and adult females with embryos. The body length of the

organisms was measured from the tip of the head to the base of the caudal setae (Uye 1982) from pictures taken with a digital camera mounted on a Zeiss 300 stereomicroscope, and analyzed using a PC provided with the Zeiss AxioVert analysis software. All organisms were placed onto pre-weighed tin capsules, dried in an oven at 60 °C for 24 h, and weighed with a Mettler Toledo AG245 microbalance (± 10 μg) to determine dry weights (DW). Mean weight per individual was calculated from the number of animals per capsule. Figure 5.1 shows the length-dry weight relationship. The obtained equation was used to estimate the biomass of cladocerans in the grazing experiments from size measurements. Carbon content was assumed as

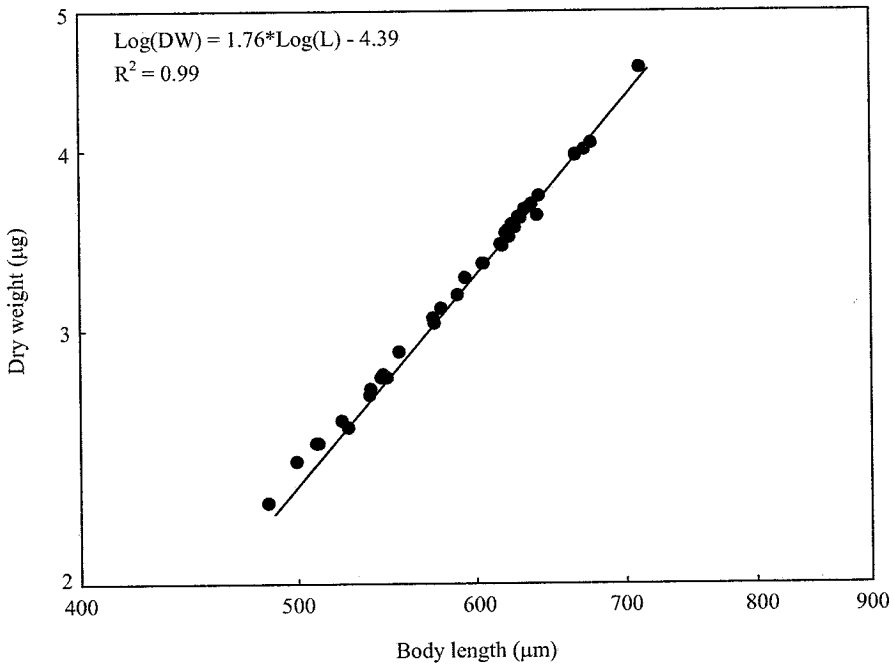


Figure 5.1. *Penilia avirostris* relationship between dry weight (DW, μg) and body length (L, μm).

50% of DW (Uye 1982). Somatic growth rate in the cohorts were estimated using the slopes of the exponential regressions of total biomass in the cylinders versus time.

## Feeding, growth and gross growth efficiencies

Eight incubation experiments were conducted with *Penilia avirostris*, 2 during cohort-1 and 3 during the other 2 cohorts monitoring (Table 5.2). Experimental organisms for the incubations were obtained from the cohorts tanks. Water for grazing experiments was from the same batch of water used to refill the cylinders. Also, it was reverse-flow filtered by 100 μm pore-size mesh and once the suspension was ready, it was amended with a nutrient mixture (15μM $NH_4Cl$ and 1μM $Na_2HPO_4$) to compensate for nutrient enrichment due to zooplankton excretion.

Experimental bottles (polycarbonate, 1200-ml Nalgene™ bottles) were filled with the natural suspension (<100 μm) of seston and 50 *Penilia avirostris* were added per bottle. The experiment consisted of 3 bottles with *P. avirostris* and 3 additional control bottles without grazers. Experimental bottles were incubated *in situ* at 2-m depth for 24 h at Segredo beach (Table 5.2). One additional bottle was used to determine the initial prey concentration. After incubation, the water was gently poured through a 100 μm sieve to collect grazers, which were checked for activity and processed for body size estimation as described above.

Table 5.2. List of incubation experiments (grazing and growth) conducted on austral summer 2004. Water temperature and individual biomass of *Penilia avirostris* on incubation bottles. Average values (± 1 SE).

| **Cohort-1** | Exp1A 03/2/2004 | Exp1B 10/2/2004 | |
|---|---|---|---|
| Temperature (°C) | 27.0 | 27.0 | |
| Biomass *P. avirostris* (μgC ind$^{-1}$) | 1.69 (0.01) | 1.54 (0.03) | |
| **Cohort-2** | Exp2A 17/2/2004 | Exp2B 20/2/2004 | Exp2C 24/2/2004 |
| Temperature (°C) | 26.8 | 25.2 | 25.8 |
| Biomass *P. avirostris* (μgC ind$^{-1}$) | 1.91 (0.01) | 1.95 (0.01) | 1.75 (0.00) |
| **Cohort-3** | Exp3A 05/3/2004 | Exp3B 08/3/2004 | Exp3C 12/3/2004 |
| Temperature (°C) | 26.2 | 26.1 | 27.3 |
| Biomass *P. avirostris* (μgC ind$^{-1}$) | 2.01 (0.01) | 1.69 (0.04) | 1.68 (0.01) |

Chlorophyll *a* (Chl *a*) concentration was determined filtering 75-ml and 150-ml onto GF/F Whatman and 5 μm pore-size polycarbonate Nucleopore™ filters,

respectively. The filters were analyzed fluorometrically according to Parsons et al. (1984) after acetone extraction.

The microbial components considered in the grazing experiments included heterotrophic bacteria, *Synechococcus*, nanoflagellates (<2 μm, 2-5 μm and >5 μm), ciliates, dinoflagellates and diatoms (single cells and chain-forming).

Heterotrophic bacteria, *Synechococcus*, and nanoflagellate abundance were estimated from water samples preserved in gluteraldehyde (1% final concentration) for 3 to 6 h (4°C in dark). Three subsamples were filtered onto 0.2, 0.8, and 2.0 μm pore-size black polycarbonate membrane filters, and stained with DAPI (5 μg $ml^{-1}$ final concentration) (Porter & Feig 1980). Filters were examined by epifluorescence microscopy (Zeiss Jenalumar). At least 1500 and 300 cells were counted for prokaryotes and nanoflagellates, respectively. Forty cells of each of the two nanoflagellate larger categories were sized; for the <2 μm flagellate fraction, a nominal size of 2 μm was assumed. Carbon cell was estimated from size by using a factor of 0.22 pgC $\mu m^{-3}$ (Børsheim & Bratbak 1987).

To determine the concentration of dinoflagellates, diatoms and ciliates, water samples were preserved with 1% acidic Lugol's solution, and let to settle in 100 ml Utermöhl chambers for 48h. The whole chamber was counted using an inverted microscope (Nikon DIAPHOT 200). A correction loss factor of 30% was applied to the ciliate abundance data to compensate for losses due to fixation (Broglio et al. 2004). At least 30 cells of each group were measured and the carbon content was estimated using a factor of 0.19 pgC $\mu m^{-3}$ for ciliates (Putt & Stoecker 1989), the equation log (pgC $cell^{-1}$) = 0.811 (log V) - 0.541 for diatoms, and log (pgC $cell^{-1}$) = 0.819 (log V) - 0.119 for dinoflagellates (Menden-Deuer & Lessard 2000).

Clearance and ingestion rates were calculated for each prey type according to Frost (1972) equations. Grazing significance was calculated by comparing prey growth rates between grazing and control bottles (one-way ANOVA test, two-tailed $p < 0.05$); when these differences were not significant, nil feeding rates were assigned.

Somatic growth rates for *Penilia avirostris* were estimated during the feeding incubations, and calculated according to the following equation:

$$G = 1/t * \ln (W_i/W_o)$$

where $t$ is duration of incubations (days) and $W_i$ and $W_o$ are the dry weight (μg) of experimental animals at the end of the incubations and in the initial sample, respectively.

Gross growth efficiency (GGE) of *Penilia avirostris* was calculated as:

$$GGE = G/I$$

where $I$ is the weight-specific ingestion rate ($d^{-1}$).

## RESULTS

### Cohort development experiments

The variation of prey biomass and composition during the cohort experiments is shown in Figure 5.2. Due to the frequent removal of water, these variations reflected changes in the *in situ* microbial community, and no changes due to confinement in the microcosms. Nanoflagellates were the main contributors to the initial microbial community biomass in all the experiments. In cohort-1 prey biomass varied from 50 µgC $l^{-1}$ to 110 µgC $l^{-1}$, and after day 4, diatoms showed an increase in biomass, becoming the dominant group by the end of the experiment (day 11). On the other hand, cohort-2 showed a slightly decrease in prey biomass, from 80 to 50 µgC $l^{-1}$ towards the end of the experiment period. Moreover, the relative composition of prey along the study followed an inverse pattern from that of cohort-1, diatoms decreasing their concentration during the experiment. Finally, cohort-3 was the most stable in terms of prey biomass (≈ 50 µgC $l^{-1}$) and composition.

Figure 5.3 shows the temporal succession of *Penilia avirostris* communities in each cohort. In all cohorts a continuous increase of *P. avirostris* abundance was observed, although the relative contribution of each stage of development was different. Cohort-1 reached 6000 ind $cylinder^{-1}$, with an initial community composed mostly of females with embryos. In this experiment the development of the cohort was evident, with a maximum of juveniles after 48h since the beginning of the experiment, a complete renovation of the population after 8-9 days, and a development time of 6-8 days. The other 2 cohort experiments, on the other hand, exhibited a more stable pattern, likely a result of the lack of synchronization of *P. avirostris* reproduction. These 2 experiments were initiated with populations more equiproportional, which confounded the different cohorts and make very difficult to

Table 5.3. *Penilia avirostris* population growth rates obtained from cohort experiments. Regressions of biomass ($W_t$, µgC $l^{-1}$) *vs* time (t, d) in the 3 cohort experiments: $\ln W_y = \ln W_o + gt$. g= estimate of growth rate; n = number of determinations.

| Cohort | $W_o$ (µgC $l^{-1}$) | g ($d^{-1}$) | $R^2$ | n |
|---|---|---|---|---|
| 1 | 1100 | 0.17 | 0.95 | 13 |
| 2 | 1700 | 0.12 | 0.95 | 13 |
| 3 | 1900 | 0.10 | 0.89 | 13 |

extract any life history parameters. Both experiments reached ≈ 4500 ind cylinder$^{-1}$, which was less than the final abundance on cohort-1. As mentioned in the methodological section, we used the slopes of the exponential regressions of total biomass versus time to estimate somatic growth rates (Table 5.3). Growth was higher in cohort.1 (0.17 $d^{-1}$) than in the other two cohort experiments, both with similar growth rates (0.12 and 0.10 $d^{-1}$ on cohort-2 and cohort-3, respectively) (one-way ANOVA test, two tailed $p < 0.05$).

The reproductive parameters of *Penilia avirostris* varied along the experiments. Figure 5.4 shows the variation of the percentage of each brood size (number of embryos per female) along the experimental cohorts. The patterns depicted in the 3 cohorts were different, brood size changed with time in cohort-1 and 3 (last cohort

Table 5.4. *Penilia avirostris* clearance rates (ml ind$^{-1}$ d$^{-1}$; average ± SE). Chl *a*: Chlorophyll *a*; HetBact: Heterotrophic bacteria; Synec: *Synechococcus*; Flag: Nanoflagellates; Dinoflag: Dinoflagellates; Single-Diat: Single diatoms; Chain-Diat: Chain-forming diatoms. *Growth*: values in the experimental bottles higher than in the controls; 0: no ingestion; Avg: average.

| | Exp1A | Exp1B | Exp2A | Exp2B | Exp2C | Exp3A | Exp3B | Exp3C |
|---|---|---|---|---|---|---|---|---|
| Chl *a* total | 3.6 (0.23) | 4.7 (0.21) | 6.5 (0.61) | 6.9 (0.15) | 7.8 (0.65) | 7.0 (0.09) | 5.8 (0.19) | 4.6 (0.10) |
| Chl *a* >5µm | 2.0 (0.11) | 3.9 (0.38) | 5.3 (0.57) | 8.8 (0.48) | 11.1 (0.73) | 6.0 (0.07) | 5.3 (0.41) | 6.3 (0.61) |
| HetBact | *growth* | *growth* | *growth* | *growth* | *growth* | *growth* | *growth* | *growth* |
| Synec | 0 | *growth* | *growth* | *growth* | *growth* | *growth* | *growth* | *growth* |
| <2 µm Flag | 14.7 (0.43) | 13.3 (0.05) | 13.2 (0.10) | 11.9 (0.08) | 12.1 (0.14) | 13.9 (0.16) | 12.6 (0.23) | 12.6 (0.46) |
| 2-5 µm Flag | 24.8 (0.55) | 24.5 (0.12) | 22.6 (0.05) | 22.3 (0.18) | 23.6 (0.19) | 23.8 (0.22) | 21.6 (0.89) | 21.7 (0.98) |
| >5 µm Flag | 10.4 (0.35) | 11.0 (0.15) | 10.5 (0.12) | 11.0 (0.13) | 10.3 (0.16) | 13.2 (0.03) | 11.4 (0.26) | 11.1 (0.22) |
| Ciliates | 8.5 (0.33) | 5.6 (0.22) | 9.7 (0.50) | 10.5 (0.39) | 9.5 (0.20) | 12.3 (0.26) | 12.9 (0.17) | 11.6 (0.14) |
| Dinoflag | 24.3 (0.63) | 7.6 (0.76) | 16.6 (1.17) | 19.4 (0.34) | 24.5 (0.51) | 23.9 (0.16) | 22.1 (0.35) | 23.0 (0.37) |
| Single-diat | 4.5 (0.28) | 13.5 (1.32) | 3.0 (0.06) | 3.3 (0.02) | 2.3 (0.54) | 3.1 (0.35) | 4.1 (0.45) | 6.6 (0.26) |
| Chain-diat | 23.9 (1.21) | 20.5 (1.60) | 4.7 (1.26) | *growth* | 13.8 (3.03) | 5.8 (0.66) | 4.2 (0.31) | 3.0 (0.40) |

showing less embryos per female in general), and was more stable in cohort-2. At the beginning of cohort-1, brood size was higher than at the end. In cohort-3 the situation was the opposite, and larger brood size was present towards the end of the culture period.

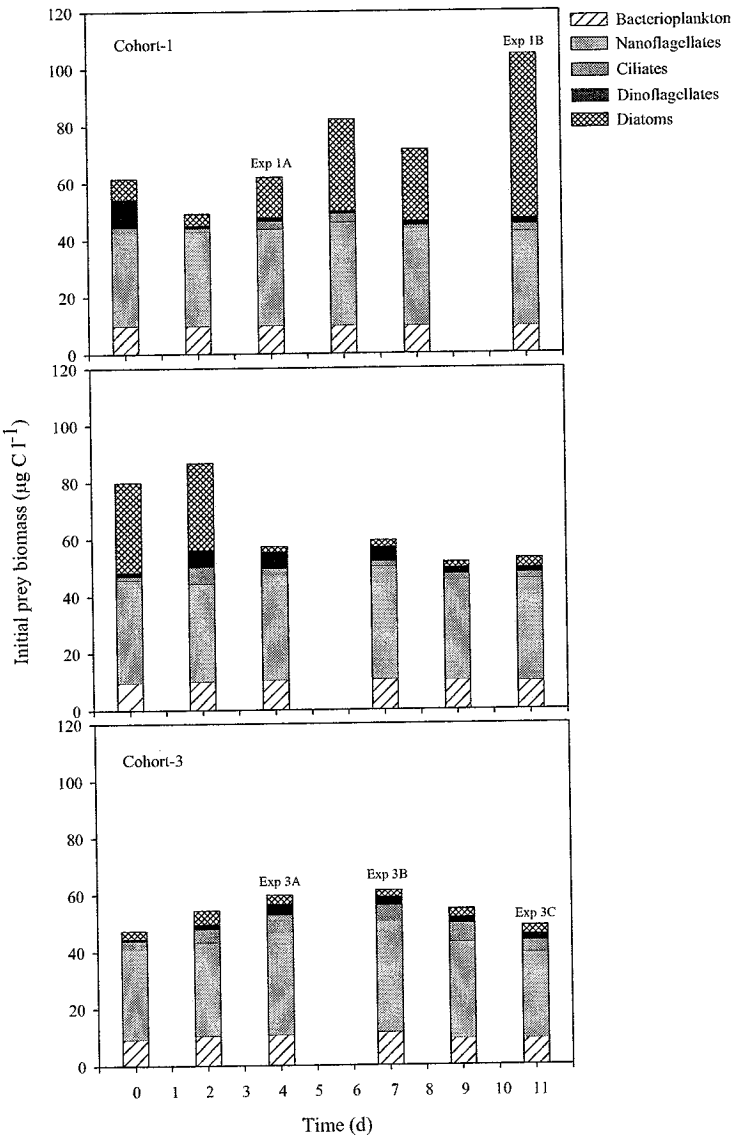


Figure 5.2. Contribution to initial prey biomass (µgC $l^{-1}$) of the different microbial groups considered during the cohort experiments. Exp = feeding incubations (see text for details).

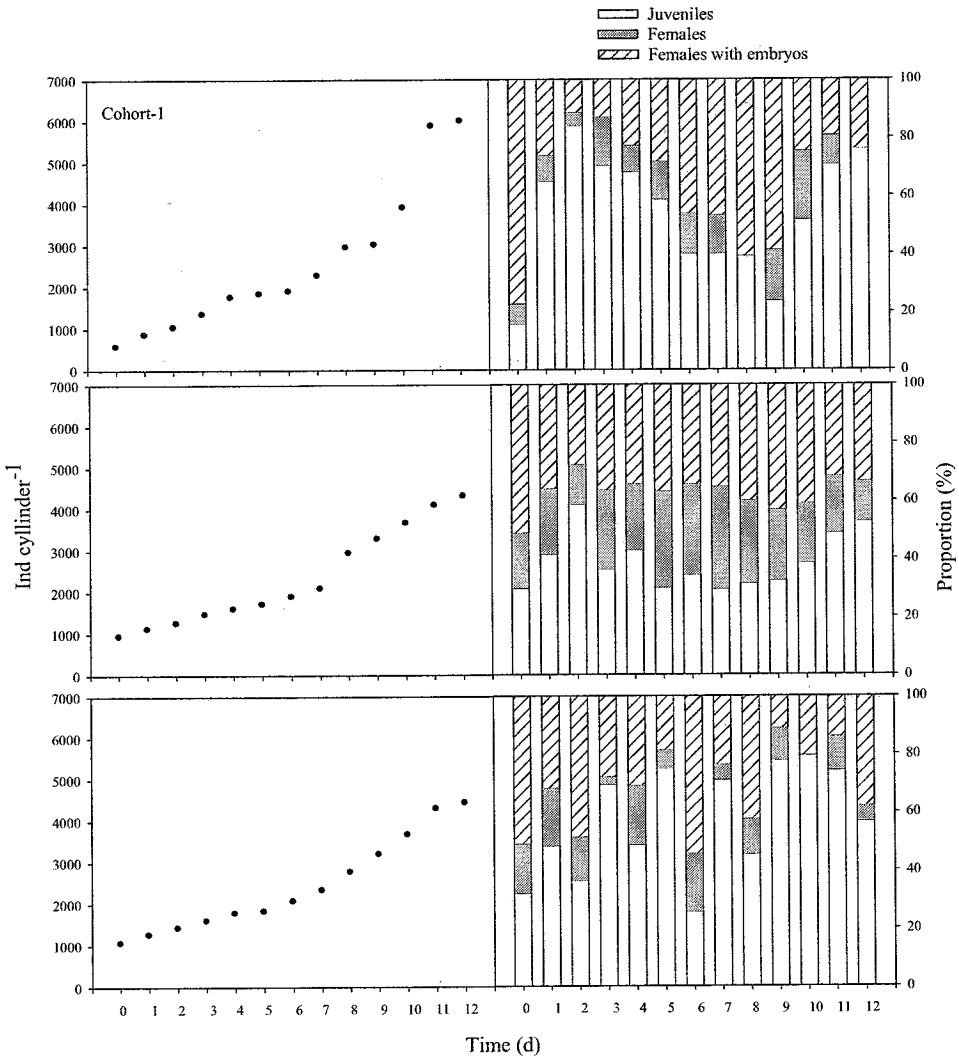


Figure 5.3. Left: Evolution of *Penilia avirostris* density (ind cylinder-1). Right: Population composition (juveniles, females and females with embryos) during cohort experiments.

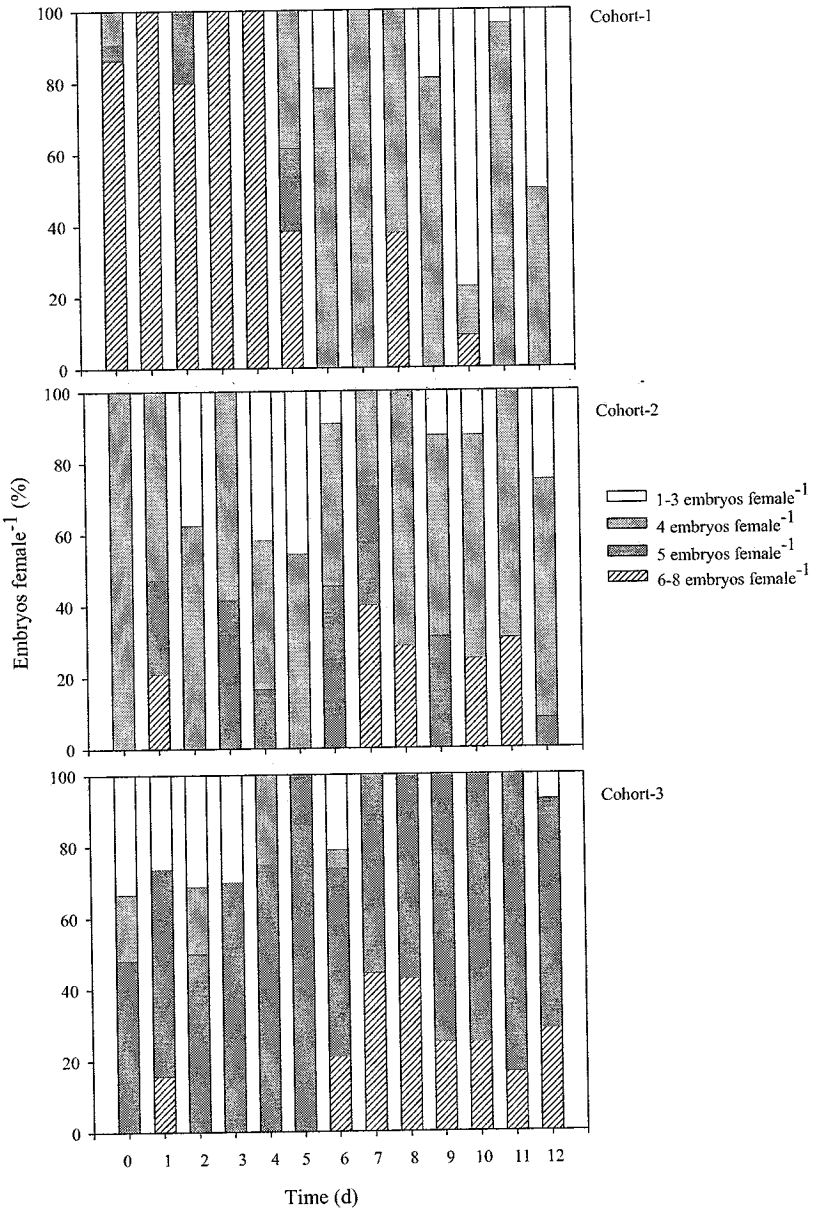


Figure 5.4. Relative distribution in brood size (embryos female$^{-1}$) during the cohort experiments.

## Feeding, growth and gross growth efficiencies

The composition of the microbial community at each incubation experiment is shown in Figure 5.5. The initial community was not exactly the same than in the cohort experiments (Figure 5.2), probably because the remaining water on the cylinders during the refill process, the addition of nutrients in the water for feeding incubations, or that the samples were not taken exactly at the same time.

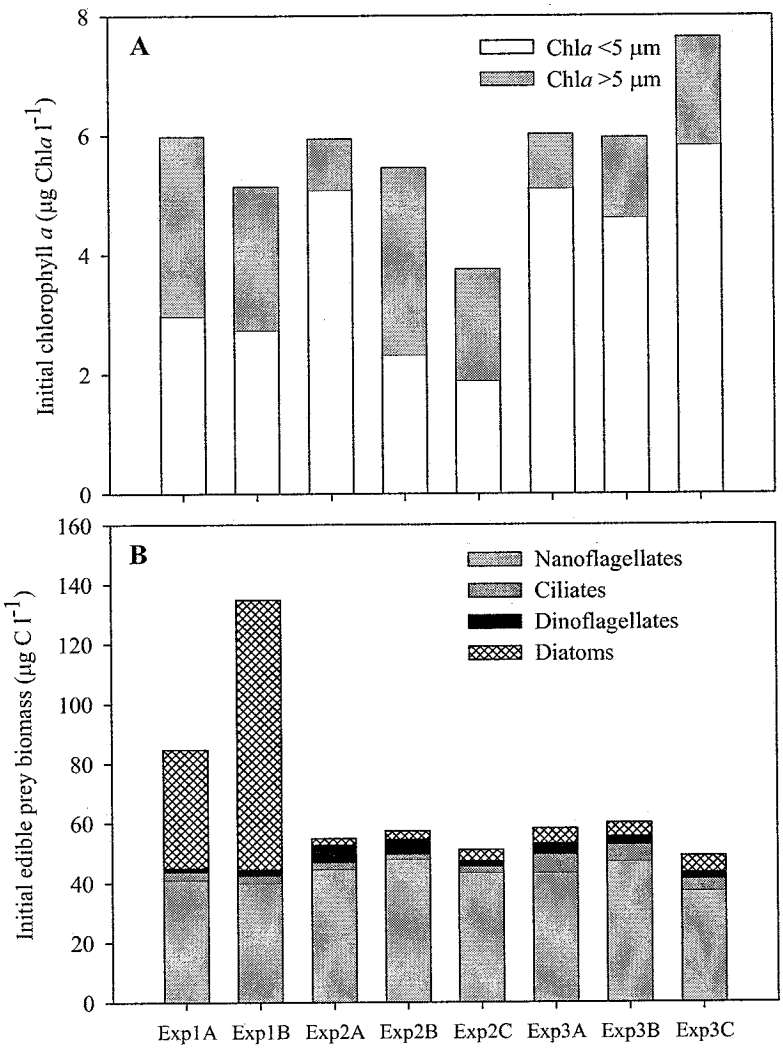


Figure 5.5. A) Initial concentration (µg Chl*a* $l^{-1}$) of chlorophyll *a*. B) Relative contribution to the initial edible prey biomass (as %) of the different microbial groups considered during the experiments. Note that bacteria are excluded since they were not significantly grazed by *Penilia avirostris*.

Chl *a* concentrations ranged from 3.75 μg $l^{-1}$ in Exp2C to 7.64 μg $l^{-1}$ in Exp3C (Figure 5.5A). In Expts 1A, 2B and 2C the >5 μm fraction represented the bulk of Chl *a*, whereas Expts 2A, 3A, 1B, 3B and 3C were clearly dominated by <5 μm cells.

Regarding the other components of the microbial community, the initial edible prey biomass (defined as prey items significantly grazed by *Penilia avirostris*, i.e. excluding bacterioplankton) was higher during Expts 1 series (between 80-140 μgC $l^{-1}$) than during the other two series of experiments (around 50 μgC $l^{-1}$). Nanoflagellates were important contributors to total carbon biomass in all these experiments, followed by diatoms, the group that contributed most to community biomass in Expts 1 series.

Table 5.4 shows clearance rates of *Penilia avirostris* on the different groups of the microbial community. Bacterioplankton (heterotrophic bacteria and *Synechococcus*) were not grazed by *P. avirostris*, and in most of the incubations were actually significantly enhanced by the presence of the cladocerans (one-way ANOVA test, two tailed $p < 0.05$). For the rest of the components of the microbial community, clearance rates ranged between non significant to 25 ml $ind^{-1}$ $d^{-1}$ (mean 10.3 ml $ind^{-1}$ $d^{-1}$ ± 0.48 SE). Almost all prey types (excluding bacterioplankton) were eaten in all experiments, with the exception of chain-forming diatoms in Exp2B. *P. avirostris* consumed Chl *a* at variable rates during the study, overall ranging from 3.6 to 7.8 ml $ind^{-1}$ $d^{-1}$ for total Chl *a* (mean 5.9 ml $ind^{-1}$ $d^{-1}$ ± 0.30 SE), and from 2.0 ml $ind^{-1}$ $d^{-1}$ to 11.1 ml $ind^{-1}$ $d^{-1}$ for >5 μm Chl *a* (mean 6.1 ml $ind^{-1}$ $d^{-1}$ ± 0.56 SE). *P. avirostris* showed maximum clearance rates when feeding on nanoflagellates (11.9 ml $ind^{-1}$ $d^{-1}$ – 24.8 ml $ind^{-1}$ $d^{-1}$), especially when feeding on 2-5 μm nanoflagellates (21.6 – 24.8 ml $ind^{-1}$ $d^{-1}$). Ciliates and dinoflagellates were cleared at rates ranging from 5.6 to 12.9 ml $ind^{-1}$ $d^{-1}$ (mean 10.1 ml $ind^{-1}$ $d^{-1}$ ± 0.47 SE) and from 7.6 to 24.5 ml $ind^{-1}$ $d^{-1}$ (mean 20.2 ml $ind^{-1}$ $d^{-1}$ ± 1.14 SE), respectively. Single-cells diatoms were cleared at the lowest rates, between 2.3 ml $ind^{-1}$ $d^{-1}$ and 6.6 ml $ind^{-1}$ $d^{-1}$, but with a peak (13.5 ml $ind^{-1}$ $d^{-1}$) in Exp1B (mean 5.0 ml $ind^{-1}$ $d^{-1}$ ± 0.73 SE). Finally, clearance rates on chain-forming diatoms presented the highest variability, with rates ranging from 0 to 23.9 ml $ind^{-1}$ $d^{-1}$ (mean 10.8 ml $ind^{-1}$ $d^{-1}$ ± 1.83 SE).

Weight-specific ingestion rates of *Penilia avirostris* ranged between 0.27 and 0.93 $d^{-1}$. Nanoflagellates constituted >70% of the carbon ingested by *Penilia avirostris*, followed by dinoflagellates (Expts 2 series) and ciliates (Expts 3) (data not shown). Only in Expts 1 series diatoms contributed >40% of the daily ration. Weight-specific ingestion rates were positively related to total edible prey biomass ($R^2 = 0.93$; $p < 0.05$) (Figure 5.6A). No evidence of saturation was observed over the range of edible food biomass found during the seasonal period studied.

Growth rates of *Penilia avirostris* were quite constant between experiments, ranging from 0.10 to 0.24 $d^{-1}$, and positively related with total edible prey biomass ($R^2$

Table 5.4. *Penilia avirostris* clearance rates (ml ind$^{-1}$ d$^{-1}$; average ± SE). Chl *a*: Chlorophyll *a*; HetBact: Heterotrophic bacteria; Synec: *Synechococcus*; Flag: Nanoflagellates; Dinoflag: Dinoflagellates; Single-Diat: Single diatoms; Chain-Diat: Chain-forming diatoms. *Growth*: values in the experimental bottles higher than in the controls; 0: no ingestion; Avg: average.

| | Exp1A | Exp1B | Exp2A | Exp2B | Exp2C | Exp3A | Exp3B | Exp3C |
|---|---|---|---|---|---|---|---|---|
| Chl *a* total | 3.6 (0.23) | 4.7 (0.21) | 6.5 (0.61) | 6.9 (0.15) | 7.8 (0.65) | 7.0 (0.09) | 5.8 (0.19) | 4.6 (0.10) |
| Chl *a* >5µm | 2.0 (0.11) | 3.9 (0.38) | 5.3 (0.57) | 8.8 (0.48) | 11.1 (0.73) | 6.0 (0.07) | 5.3 (0.41) | 6.3 (0.61) |
| HetBact | *growth* | *growth* | *growth* | *growth* | *growth* | *growth* | *growth* | *growth* |
| Synec | 0 | *growth* | *growth* | *growth* | *growth* | *growth* | *growth* | *growth* |
| <2 µm Flag | 14.7 (0.43) | 13.3 (0.05) | 13.2 (0.10) | 11.9 (0.08) | 12.1 (0.14) | 13.9 (0.16) | 12.6 (0.23) | 12.6 (0.46) |
| 2-5 µm Flag | 24.8 (0.55) | 24.5 (0.12) | 22.6 (0.05) | 22.3 (0.18) | 23.6 (0.19) | 23.8 (0.22) | 21.6 (0.89) | 21.7 (0.98) |
| >5 µm Flag | 10.4 (0.35) | 11.0 (0.15) | 10.5 (0.12) | 11.0 (0.13) | 10.3 (0.16) | 13.2 (0.03) | 11.4 (0.26) | 11.1 (0.22) |
| Ciliates | 8.5 (0.33) | 5.6 (0.22) | 9.7 (0.50) | 10.5 (0.39) | 9.5 (0.20) | 12.3 (0.26) | 12.9 (0.17) | 11.6 (0.14) |
| Dinoflag | 24.3 (0.63) | 7.6 (0.76) | 16.6 (1.17) | 19.4 (0.34) | 24.5 (0.51) | 23.9 (0.16) | 22.1 (0.35) | 23.0 (0.37) |
| Single-diat | 4.5 (0.28) | 13.5 (1.32) | 3.0 (0.06) | 3.3 (0.02) | 2.3 (0.54) | 3.1 (0.35) | 4.1 (0.45) | 6.6 (0.26) |
| Chain-diat | 23.9 (1.21) | 20.5 (1.60) | 4.7 (1.26) | *growth* | 13.8 (3.03) | 5.8 (0.66) | 4.2 (0.31) | 3.0 (0.40) |

= 0.45; p <0.05; Figure 5.6B). GGE ranged from 0.16 to 0.58, showing a tendency to decrease when prey biomass increased (Figure 5.6C). However, the pattern was not consistent because was highly influenced by 2 experiments, Exp1B and Exp3C, with the lowest and highest efficiencies (respectively).

## DISCUSSION

During the present study, different life history parameters, grazing rates, growth rates, and gross growth efficiencies (GGE) of *Penilia avirostris* were estimated in order to understand the ecological success of this marine cladoceran. The

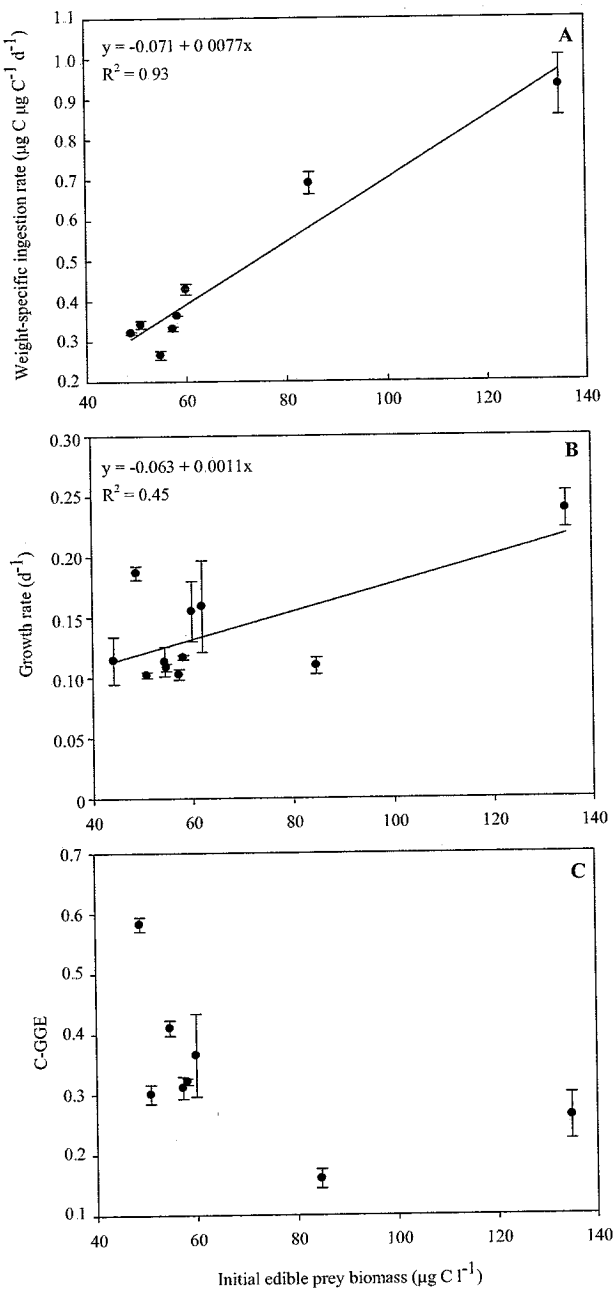


Figure 5.6. A) *Penilia avirostris* weight-specific ingestion rate ($d^{-1}$) relative to initial edible prey biomass (µg C $l^{-1}$). Error bars represent ± 1 SE. B) *P. avirostris* growth rate ($d^{-1}$) vs initial edible prey biomass (µg C $l^{-1}$) during the feeding experiments and cohort experiments. Error bars represent ± 1 SE. C) *P. avirostris* carbon gross growth efficiency (C-GGE) relative to initial edible prey biomass (µg C $l^{-1}$). Error bars represent ± 1 SE.

combination of those variables could help us to explain the explosive growth of *P. avirostris* populations and the high seasonal abundance on different regions.

Regarding growth rates, the only two published values for *Penilia avirostris* are from Paffenhöfer & Orcutt (1986) (0.14 – 0.47 $d^{-1}$), and Rose et al. (2004) (0.29 – 0.60 $d^{-1}$). Our values are in the lower range of the former study and are lower than those reported by Rose et al. (2004). We never achieved growth rates higher than 0.24 $d^{-1}$, and probably the slightly difference between our results and those studies are related to the methodology or the experimental conditions. Rates reported by Paffenhöfer & Orcutt (1986) come from laboratory experiments using >112 µgC $l^{-1}$ of *Isochrysis galbana* as food, therefore slightly higher rates achieved by those authors could be related with a higher food availability. On the other hand, Rose et al. (2004) used size classes to derive developmental or molt stages. Since in our experiments we used a mixture of cladocerans of different sizes (stages), our estimates of growth rates represent an average rate of different stages, and not completely comparable to those reported by Rose et al. (2004). It is expected that cladoceran growth rates decrease with body size and age because adult cladocerans somatic growth is simultaneous with reproductive growth. Rose et al. (2004) found a progressive decrease in somatic growth as the size of the organism's increase, which was compensated by an increase of reproductive growth. Growth rates of *P. avirostris* fall in the same range than those for freshwater cladocerans (0.10 – 0.60 $d^{-1}$; Urabe 1988, DeMott et al. 2001, DeMott 2003). Finally, our values of *P. avirostris* growth rates were slightly lower than those expected for marine copepods at the same temperature (0.3-0.4 $d^{-1}$, Hirst & Bunker 2003) which contradicts our initial hypothesis that higher growth rates of this marine cladoceran in comparison with copepods, could explain the explosive growth of *P. avirostris* populations.

Certainly, in our experiments no feeding saturating conditions were reached (as we can see in the functional response to food concentration, Figure 5.6), suggesting elevated growth activity under more favorable food conditions. Nevertheless, this general pattern is shown by copepods and cladocerans in the same manner, the latter benefiting from a broader spectrum of prey available. *Penilia avirostris* ingested a wide spectrum of natural microbial prey, from <2 µm nanoflagellates to chain-forming diatoms (Turner et al. 1988, Broglio et al. 2004, Katechakis & Stibor 2004, Katechakis et al. 2004, Atienza et al. 2006a, 2006b). Summertime, warm water conditions were usually associated with microbial communities where a high proportion of the carbon is available as small cells, such as bacterioplankton and nanoflagellates. In this situation, *Penilia avirostris* which ingested preferentially nanoflagellates <5 µm, could exploited the available food resources seldom exploit by copepods.

Regarding GGE, the range of values for *Penilia avirostris* (16-58%) observed in our study were not overall different to the typical efficiencies reported for marine copepods, 22-26 % and freshwater cladocerans (27-28%) (Straile 1997, Table 5.5).

Our results, however, are slightly lower than the values reported for *P. avirostris* (Paffenhöfer & Orcutt 1986, Table 5.5), although comparison is difficult since these experiments were conducted under laboratory conditions with a single prey and different food availability. Higher GGE under low food conditions could represent an important evolutionary adaptation for a zooplankter living in a relative food-diluted environment (Castellani et al. 2005). This seems to be the case for *P. avirostris,* higher GGE being reached under lower food conditions. In this sense the greatest differences between marine cladocerans and copepods are more evident at low food availability (<60 μgC l-1, Figure 5.6C), although the pattern is not robust enough due to data variability.

Table 5.5. Comparison of gross growth efficiencies (C-GGE, %) under different prey biomass availability (C, μgC $l^{-1}$) of *Penilia avirostris*, freshwater cladocerans, and other marine zooplankters.

| Species | C | GGE | References |
|---|---|---|---|
| *Penilia avirostris* | 20 | 62 | Paffenhöfer & Orcutt 1986 |
| *Penilia avirostris* | 50 | 58 | This study |
| *Penilia avirostris* | 51 | 30 | This study |
| *Penilia avirostris* | 55 | 41 | This study |
| *Penilia avirostris* | 57 | 31 | This study |
| *Penilia avirostris* | 58 | 32 | This study |
| *Penilia avirostris* | 60 | 37 | This study |
| *Penilia avirostris* | 60 | 49 | Paffenhöfer & Orcutt 1986 |
| *Penilia avirostris* | 85 | 16 | This study |
| *Penilia avirostris* | 135 | 26 | This study |
| *Daphnia* spp. | 50 | 35 | Urabe & Watanabe 1992 |
| *Daphnia* spp. | 250 | 40 | Urabe & Watanabe 1992 |
| *Acartia tonsa*/grani | 8-96 | 37 | Saiz et al. 1992 |
| *Acartia tonsa* | 92 | 49 | Kiørboe et al. 1985 |
| *Acartia tonsa* | 367 | 41 | Kiørboe et al. 1985 |
| *Calanus helgolandicus* | 49 | 24 | Paffenhöfer 1976 |
| *Calanus helgolandicus* | 78 | 15 | Rey-Rassat et al. 2002 |
| *Calanus helgolandicus* | 278 | 28 | Rey-Rassat et al. 2002 |
| *Calanus helgolandicus* | 101 | 16 | Paffenhöfer 1976 |

On the other hand, at higher food concentrations (120-150 μgC l-1) *Penilia avirostris* presents unusually low GGE. It has been suggested that zooplankton feeding on high concentrations of nutrient-deficient resources ingest more carbon and less nutrients than required for maintenance and growth (DeMott et al. 2001). In these situations, the excess of carbon could be handled, for example, by the reduction of C assimilation to maintain essential nutrient assimilation. This fact could explain the higher carbon GGE at the highest food availability. Using conversion factors (C:N = 4.84, Uye 1982; C:P = 48 Katechakis et al. 2002) we estimated the GGE for nitrogen (27 – 96%) and phosphorus (52 – 192%). The extremely high GGE on P, and in less measure, on N obtained in our experiments, suggest that these elements (especially P) are key limiting nutrients for *P. avirostris* metabolism (DeMott et al. 1998; Jones et al. 2002). This result perfectly agree with the growth rate hypothesis, which proposes that elevated demands for increased allocation of phosphorus to P-rich RNA under rapid growth, drives variation in the P content of many organisms (Elser et al. 2003). Cladocerans have successive parthenogenetic reproduction cycles before sexual maturity, short generation times, and continuous somatic growth that demands for a higher GGE on P.

Another parameter that could help us to understand the explosive growth of *Penilia avirostris* populations is brood size. Our measures of brood size (mean 4.51 ± 0.20 SE embryos female$^{-1}$ d$^{-1}$) are similar to values reported by Mullin & Onbé (1992), Marazzo & Valentin (2003b), Valentin & Marazzo (2003), and Rose et al. (2004). *P. avirostris* does not produce larger broods if we compared it to either sac spawner (mean 5.13 eggs female$^{-1}$ d$^{-1}$) or broadcaster copepods (mean 46.99 eggs female$^{-1}$ d$^{-1}$) (Bunker & Hirst 2004). Nevertheless, the variation of brood size during the seasonal appearance of this marine cladoceran might be more interesting to understand the explosive growth of *P. avirostris* populations. Consequently more research has to be done to describe the variation of this reproductive parameter in natural populations of *P. avirostris* and to establish the relationship with *P. avirostris* population dynamics.

Other life history traits are important to understand the blooms of marine cladocerans in the zooplankton communities for certain periods of time. Studies on the duration of stages during the life cycle of *Penilia avirostris* were scarce. Pavlova (1959) (in Rose et al. 2004) established that *P. avirostris* has three different juvenile stages of 36h, and five adult stages of 45 h (Sevastopol Bay, 11-24 °C). Mullin & Onbé (1992) assume that juveniles remain as embryos during 2 days, as pre-reproductive adults for 1 days, and produced a brood every 2 days for a total of four broods (Gulf of Mexico, 24-28 °C). Finally, Rose et al. (2004) found that *P. avirostris* has 3 juvenile stages of 20.5 h and 4-5 adults stages of 41.4 h (Jamaican waters, 27-30 °C). These latter studies (Mullin & Onbé 1992 and Rose et al. 2004) estimated that the developmental time of a complete cohort of *P. avirostris* was 11-12 days. From our cultures, in particular from cohort-1, we can estimate that juveniles of our *P.*

*avirostris* remained as embryos for 2 days, as pre-reproductive animals for 1 day, and produced broods during 4 days, which is very similar to previous observations. Also, our developmental time was between 6 and 8 days, which are much faster than previous data. If we compare duration of this life cycle with those from marine copepods, we can point out clear differences. Copepods have a slow stage development with a series of stepwise increments, each including a major metamorphosis process. Huntley & Lopez (1992) compiled data for (mainly) calanoid copepod from the literature, and their regression predicts a generation time (egg to adult) of 15-20 days (at similar temperatures), which is almost double of *P. avirostris*. Hence, an important difference between *P. avirostris* and copepods is that, at similar conditions of food, temperature and other environmental variables, this marine cladoceran could reach dense populations much faster.

In addition to the differences in development time, other life history parameters can represent a relevant ecological advantage for marine cladocerans in front of copepods. Thus, copepods have sexual reproduction and their somatic growth includes a major metamorphosis process, whereas cladocerans are characterized by parthenogenetic reproduction of adults, which in some species (i.e. podonids) the reproduction could be further accelerated by the presence of embryos in the juveniles (Egloff et al. 1997).

In summary, we know that *Penilia avirostris* has a broad range of prey types in comparison to copepods. This marine cladoceran is able to feed upon nanoflagellates with preference of <5 μm cells, but is also able to prey upon ciliates, dinoflagellates and diatoms. In contrast, copepods fed upon a narrow size range, mainly constrain to ciliates and larger cells of phytoplankton. No differences were evident in relation with growth rates between both groups. Opposite to our original hypothesis, GGE of *P. avirostris* was not different enough to GGE of marine copepods to explain an advantage in front of copepods. For this reason, the ecological success and better performance of this species seem to be related to parthenogenetical reproduction and shorter developmental time. In this study some aspects of its reproduction were examined (cohort developmental time, brood size, life cycle), however, more specific research needs to be done in order to completely understand the development of this marine cladoceran, and more important, to describe its population dynamics.

# Main Conclusions

# MAIN CONCLUSIONS

As stated in the introductory chapter, the first question addressed in this Thesis was, *how environmental factors affect the distribution and dynamics of Penilia avirostris?*. In **Chapters I** and **II** the distribution patterns (seasonal and spatial) of *P. avirostris* in the Catalan Sea (NW Mediterranean) were analyzed in relation to environmental factors. We found that ***Penilia avirostris*** **has a seasonal maximum during the summertime, when stratification of the water column is well developed (CONCLUSION 1).** The mechanisms behind the sudden development of *Penilia avirostris* populations in summer from resting eggs are still unveiled.

Regarding the spatial distribution, the results from **chapter I** indicate a complex interaction with physical variables, temperature, salinity, and the presence of a stable thermocline interplaying to shape the presence of *Penilia avirostris* along the Catalan coast. According to the data it seems that ***P. avirostris*** **finds its optimum habitat in the Catalan Sea (NW Mediterranean) in warm-waters (between 22 and 25 °C), with relatively low salinities (from 37.5 up to 37.9), and a stable thermocline (CONCLUSION 2).** The presence of *P. avirostris* follows the mesoscale exchanges between shelf and oceanic waters, its distribution pattern being associated to warm-low salinity water masses along the Catalan coast.

In addition, patterns of seasonal developmental and settling of *Penilia avirostris* population in the Catalan Sea can be depict from the results from **chapters I and II.** ***Penilia avirostris*** **appears in the Catalan Sea by the south (when temperature reaches values >22 °C) at the end of June, and extends its distribution further north by mid June, due to the increase of temperature. By the end of July *P. avirostris* is an important contributor of the zooplankton community along all the Catalan Sea. In September its abundance starts to decline, apparently result as a combination of decreasing temperatures, changes in seston composition, and intensification in the hydrodinamism (CONCLUSION 3).**

The third important result from **chapter I** is that ***Penilia avirostris*** **was mostly distributed above or related to the seasonal thermocline, with maximal abundance in shallow depths (20-30 m depth), and not related to the deep chlorophyll maximum (CONCLUSION 4).** This layer above the thermocline presents the optimum temperature and salinity for the species.

The second question raised in this Thesis was, *how life cycle characteristics influence the population dynamics of Penilia avirostris? P. avirostris* shows explosive growth and sudden disappearance from the water column, both processes not easily explained by a mere interplay with physical and biological variables, but with profound roots in their characteristic reproductive biology. Life history of *P. avirostris* is characterized by two types of reproduction, parthenogenesis and gametogenesis, each one being

present at certain times of its population development (Figure 3, **chapter II**). Parthenogenesis is always present in the initial phases of explosive population growth, which together with brood size and developmental time are significant parameters to understand the rapid increase of *P. avirostris* populations. In **chapter V** it is reported that embryonic developmental time was around 2 days, which means that in a maximum of 3 days a new complete offspring is produced. Besides, at the initial phases of population burst higher brood sizes were achieved, and in combination with short developmental time render high birth rates. In summary, **parthenogenetic reproduction and short embryonic developmental time are the main reasons for the explosive growth of *Penilia avirostris* populations (CONCLUSION 5).**

In addition, the distribution and abundance of resting eggs are supposed to be a critical factor influencing the overall distribution, seasonal population dynamics, and long-term variations in the abundance of this marine cladoceran. Therefore, **the abrupt appearance of the *Penilia avirostris* populations is also related to the hatching of resting eggs banks (CONCLUSION 6).** In **chapter II** was showed that *Penilia avirostris* produces one, and sometimes two, resting eggs which are larger (279 μm), fewer in number, and fully of yolky cells if they are compared to parthenogenetic eggs. However, the variation in abundance and distribution, and its importance on the population dynamics of *P. avirostris* in the Catalan Sea remain unknown.

The possible causes for the sudden disappearance of *Penilia avirostris* in the water column are not well understood, although some hypotheses can be raised (**chapter II**). As shown in this Thesis this decline is linked to the shift between parthenogenesis to gametogenesis reproduction. Gamogenic females and males always appear when the population density is maxima (Figure 3, **chapter II**). Different environmental signals are identified to trigger the appearance of males, such as temperature, day length, food concentration, and population density. Moreover, predation pressure from invertebrates or vertebrates is known to have an important impact on the populations of this marine cladoceran. Specifically, fish larvae and chaetognaths are known to affect marine cladoceran populations. In summary, **the declining periods of *Penilia avirostris* populations are characterized by the shift from parthenogenic to gametogenic reproduction (CONCLUSION 7).**

The third question addressed in this Thesis was, *what is the feeding performance and trophic impact of Penilia avirostris on natural communities?* Different experiments were conducted to study the feeding capabilities of this marine cladoceran on natural communities (Catalan Sea and coastal SW Atlantic Ocean; **chapters III, IV,** and **V**). Four different issues have been discussed regarding the former question: feeding behaviour, prey selectivity, daily rations, and trophic impact. In relation to feeding behaviour, the results shown in this Thesis evidenced that *P. avirostris* ingested a wide

spectrum of prey, ranging from picoflagellates (< 2 μm) to dinoflagellates, diatoms, and ciliates (> 30 μm), being clearance rates on pico-, and 2 to 5 μm nanoflagellates always higher than those on other microbial groups or phytoplankton. In fact, **due to the ingestion of pico- and nanoflagellates, the feeding behaviour of this marine cladoceran result in a more efficient transfer of energy within the food web towards upper consumers compared to other marine zooplankters like copepods, (CONCLUSION 8).**

Contrary to our original hypothesis, ***Penilia avirostris* does not efficiently capture bacteria, the lower prey size threshold falling between *Synechococcus* (1 μm) and the <2 μm flagellates (CONCLUSION 9),** and therefore differs from Daphnids.

*Penilia avirostris* feeding selectivity patterns are addressed in **chapter III**. Considered a typical filter feeder, wherein phyllopods form a mesh that retains particles, *P. avirostris* is expected to exhibit passive and mechanical selection. However, our data do not fully confirm such behaviour, as evidenced by the changes in preferred prey displayed from experiment to experiment. This variability in *P. avirostris* preferences is puzzling and does not seem to be related neither to prey size nor to prey abundance. In summary, **in contrast to a typical, passive filter feeder, *Penilia avirostris* shows behaviourally driven plasticity in their prey selection by mechanisms not fully understood (CONCLUSION 10).**

Regarding diet composition, it seems evident that flagellates and dinoflagellates were the main contributors to the carbon intake by this marine cladoceran (Figure 1, **chapter IV**; results, **chapter V**). An interesting, but not clear, observation, which requires further confirmation is the relatively high daily food rations, in comparison to copepods, exhibited under low food concentrations. This suggests that, ***Penilia avirostris* seems to be adapted to oligotrophic environments, as evidenced by the relatively high daily rations (CONCLUSION 11).**

*Penilia avirostris* trophic impact was assessed in **chapter IV**. What comes out from this study is that *P. avirostris* exerted a moderate impact on the microbial populations of the Catalan Sea, focused upon flagellates (pico- and nano-), dinoflagellates, and diatoms (<10% removal of the standing stock). However, considering the vertical distribution of this marine cladoceran (restricted to the upper 30 m, **chapter I**), its impacts on the different standing stocks could rise up to >70% in most cases, being, therefore, highly relevant. In addition, the trophic impact by a predator usually extends further down through the food web by cascade interactions. We observed that in the feeding incubations *P. avirostris* produced an enhancing effect on the bacterial community, most likely result of the release of organic matter due to sloppy feeding and excretion, and of the decrease of nanoflagellates due to direct ingestion. In summary, **the trophic impact estimated in this study (either direct or trophic cascade mediated) seems to be moderate when integrated for**

**the whole water column, although *Penilia avirostris* could be strongly controlling the microbial community of their surrounding water masses (CONCLUSION 12).**

The fourth question placed in the introduction of this Thesis was, *does Penilia avirostris contribute significantly to nutrient recycling?* **Chapter IV** analyzes the nutrient metabolic activity, and the contribution to nutrient recycling of this marine cladoceran. As the release and recycling of elements due to any organism is highly determined by its own stoichiometry and by the difference between ingested nutrients and those incorporated into new biomass, we hypothesized that *P. avirostris* should be a high contributor to the recycling of nitrogen, but not for the recycling of phosphorus. The results shown in this Thesis fully confirm this hypothesis, and *P. avirostris* does not excrete phosphorus products, or at least not in a detectable amount, but it does release nitrogen compounds. Saving the major amount of phosphorus in comparison to copepods seems to be related to the high content of nucleic acids in cladocerans, especially ARN, necessary to support the high growth rates and accelerated reproductive behaviour. In summary, ***Penilia avirostris* tends to retain phosphorus while releasing products of nitrogen, which may result in a nutrient unbalance for primary producers (CONCLUSION 13).**

The last question addressed in this Thesis was *what are the main reasons for the ecological success of Penilia avirostris on summertime natural communities?* This issue integrates all the information emerged in this Thesis. A new variable that we believe could explain the ecological success of *P. avirostris* was gross growth efficiency (GGE). In **chapter V** it is reported the first estimate of *P. avirostris* GGE under natural conditions. We hypothesize that this marine cladoceran should have elevated GGE (in comparison to copepods) to explain the explosive growth of its population in oligotrophic waters. However, the general result disagrees with this hypothesis, and GGE of this marine cladoceran were not different enough from those of copepods to explain the better performance of *P. avirostris* on summertime. For this reason, we can conclude that, in agreement to what has been previously shown, the main factors that allow *P. avirostris* to be successful in summertime conditions are the following: a) the dominance of small-sized microbial communities during summer time (suitable prey for *P. avirostris*, but not for copepods); and b) parthenogenetical reproduction, short developmental time, and high growth rates (**chapters II** and **V**) compared to copepods, which allow this marine cladoceran reaching dense populations much faster than other zooplankters. In summary, **the ecological success and better performance of this species in oligotrophic (summer) conditions seem to be related to feeding behaviour and life history traits (CONCLUSION 14).**

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