Institut de Ciències del Mar | Universitat Politècnica de Catalunya | Universitat de Barcelona

Programa de Doctorado en Ciencias del Mar

Tesis Doctoral

Trophic interactions between micro and mesozooplankton

Memoria presentada por Elisabetta Broglio para optar al título de Doctora en Ciencias del Mar en el Departament d'Enginyeria Hidràulica, Marítima i Ambiental, Universitat Politècnica de Catalunya, bajo la tutoría del Dr. Miquel Alcaraz Medrano y la dirección del Dr. Enric Saiz Sendrós del Departamento de Biología Marina y Oceanografía del Institut de Ciències del Mar (CMIMA, CSIC) de Barcelona.

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GENERAL INTRODUCTION

The oceans, planktonic food webs and carbon cycle

Over the last two decades, one of the central objectives of biological oceanographic research has been to characterize the patterns and mechanisms that control carbon flow in the pelagial. The oceans, which occupy the 3/4 part of the planet's surface, play a key role in regulating the exchange of CO_2 with the lower atmosphere, with implications for climate regulation (i.e. Longhurst, 1991; Siegenthaler & Sarmiento, 1993). In it, both biological processes (the biological pump) and physical processes (e.g. hydrodynamical forces) work in exporting organic carbon to the deep ocean and in importing atmospheric CO_2 into the ocean to compensate for the loss of carbon taken up by organisms. The velocity at which CO_2 is returned to the atmosphere will depend on the time it remains circulating in the photic zone and on its sequestration to deep waters (i.e. Rivkin & Legendre 2002). It is in the photic zone in fact, that CO_2 and inorganic nutrients are utilized by phytoplankton to produce organic matter, which then circulates through complex food webs before coming back to the atmosphere as respired CO_2.

The concept of planktonic marine food webs has completely changed in the last two decades. The old assumption of a unique and simple food chain which transported matter and energy from phytoplankton to fishes through zooplankton has been substituted by a more complex idea (Pomeroy 1974), which takes into account several trophic interactions, including those involving very small organisms, which had been largely ignored due to inefficient methods of sampling and enumeration. What is currently accepted is that two main food webs, the classical and the microbial food web are responsible for the transport of matter and energy in the oceans. These food webs are mainly associated with certain trophic and physical characteristics of the system. However, one does not prevail exclusively and in many occasions both classical and microbial food web can occur at the same time (Cushing 1989; Legendre & Rassoulzadegan 1995). It is generally assumed that in nutrient-rich mixed waters, where phytoplankton concentration is very high and mainly characterized by large cells (> 20 μm), like diatoms (as i.e. in upwelling areas or during seasonal phytoplankton blooms), the bulk of matter and energy is mainly transferred from phytoplankton to herbivorous mesozooplankton and then to fishes (classical food webs, Steele 1974). In these systems the mesozooplankton community is not able to catch up with the increase in phytoplankton biomass (phyto-zooplankton mismatch) and most of the phytoplankton which is not consumed is exported as aggregates, together with

zooplankton fecal pellets (Alldredge & Silver 1988; Kiørboe, 1996). On the contrary, in nutrient-scarce stratified waters or during periods between phytoplankton blooms, the pool of phytoplankton is mainly constituted by organisms in the pico and nano size (0.2 - 20 μm) (Takahashi & Hori 1984; Cushing 1989) and carbon and energy preferentially circulate through microbial food webs (Azam et al. 1983). In these systems the small size of phytoplankton prevents its efficient consumption by most metazooplankters (Nival & Nival 1973; Berggreen et al. 1988) and primary production circulates through several trophic levels (heterotrophic nano flagellates, larger heterotrophic flagellates, ciliates) before it can reach the metazooplankton (Azam et al. 1983; Sherr et al. 1986). Within the microbial food webs, the export of carbon in the form of fecal pellets is minimal and depends on the composition of the micro-grazer community, however the high number of trophic levels involved increases the loss of CO_2 by respiration (Azam et al. 1983). On the other hand, heterotrophic bacteria efficiently recycle most of the dissolved organic matter released by the other planktonic organisms of the system, counteracting the loss of biogenic matter from the surface layers to the deep ocean (Sherr et al. 1987; Karl et al. 1998).

The dominant transfer pathway from the marine pelagic primary producers to the successive consumer levels (either by microbial or classical food webs) is probably the determinant of the final fate of biogenic carbon, and thus of the role of an oceanic area as a source or a sink for CO_2. The CO_2 derived from organic carbon that is remineralized above the permanent pycnocline stays in surface waters and will re-equilibrate with the atmosphere in term of decadal time scales by vertical diffusion and biological processes. However, the organic carbon sequestrated below the permanent pycnocline, which remains isolated from the upper ocean by the density barrier and thus is not available for epipelagic grazers, will stay out of the cycle for centuries or, if buried in the sediments, for millennia (reviewed in Legendre & Rivkin 2002). Furthermore, the dominance of one or the other transfer pathway is also decisive for the efficiency at which energy and matter are transferred through zooplankton (principally copepods) towards higher trophic levels, with implications for the prosperity of fisheries directly related to human consumption (Ryther 1969; Iverson, 1991).

A key node in planktonic food webs: the link micro-mesozooplankton

Within the complex marine planktonic food webs, zooplankton occupies a central position. By eating and being eaten zooplankton, and specially copepods, is important for the flow of matter and energy (Kiørboe, 1997; Legendre & Rivkin 2002). During the last two decades, the diet of zooplankton has been shown to be much more diverse than previously known (Stoecker & Capuzzo 1990; Kleppel 1993). Particularly, both laboratory and field studies have demonstrated that copepods eat food other than phytoplankton and that they are the main predators of ciliates (Stoecker & Sanders 1985; Stoecker & Egloff 1987; Gifford & Dagg, 1988) which, in turn, are the top-predators of the microbial loop. By feeding on ciliates, copepods represent the connection between the smaller components of the microbial food web and the higher trophic levels (Fig. 1), favoring the transfer of part of the "recycled" production from the microbial loop to larger crustaceans and finally to fish.

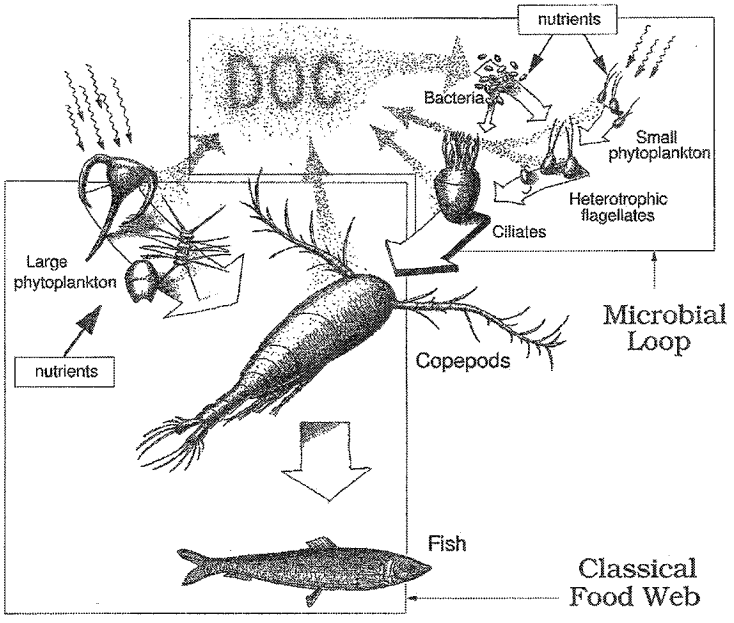

Figure 1. Copepod-ciliate interactions: a key node in planktonic food webs. (Drawing by R.V. Sole)

This trophic link has two main implications for the transfer of energy and matter between the food webs. It can increases the efficiency of the microbial loop by exporting energy and matter to higher trophic levels (Azam et al. 1983; Wassmann 1998) and, at the same time indirectly (through trophic cascades) exert a control on microbial populations, with implications for the structure of the whole marine food web (Smetacek & Pollehne 1986; Calbet & Landry 1999).

Copepods and ciliates are the major actors in the trophic link between micro and mesozooplankton. It is well known that, globally, copepods represent about 80% of zooplankton abundance in marine systems (i.e. Verity & Smetacek, 1996) and, for this reason, are one of the most studied group. On the other hand, within the heterotrophs that comprise the microbial loop, ciliates possess the adequate size to be preyed upon by copepods, becoming a suitable prey alternative to phytoplankton. Despite the importance that this trophic link can have for the functioning of planktonic marine food webs however, it is poorly known whether or not zooplankton can actually exert a control on the ciliate populations. On the other hand, the role that ciliates play in zooplankton, and more specifically copepods, diet and reproduction, is an open question as well.

Copepod-ciliate feeding interactions: what to pay attention to?

The study of the trophic interactions between micro and mesozooplakton requires not only the evaluation of the strength of this link (from both, the ciliate and the copepod side) but also the analysis of the factors that regulate it. First of all, it should be taken into account that despite different potential food sources are available for copepods in the oceans, these resources are diluted and not homogeneously distributed (Conover 1968), which implies that copepods, like others zooplankters, must search for prey. According to optimal foraging theory, a predator would try to optimize the ingestion of food and to minimize the energetic costs of prey searching and capture, by feeding preferentially on prey that increases its energetic gain (Begon et al. 1990). When a predator chooses a prey, it presumably takes into account different characteristics of such a prey, as e.g. its size, availability, vulnerability and nutritional quality. Selection for prey however, is also related with the total amount of the resources available. Thus one would expect prey selection occurring mainly when prey availability is high, since in the opposite case

(scarce resources available) predators will mainly try to guarantee their survival by feeding on whatever is accessible.

Regarding the ciliate-copepod interaction, the major alternative food source to be considered is phytoplankton. Ciliates as a prey differ very much from algae. Their cells are on average in the right size to be preyed by copepods, since most copepod species predate efficiently on particles > 5 μm (Nival & Nival 1976; Berggreen et al. 1988). However, ciliate abundance in marine planktonic systems is lower (in the order of 10^3-10^4 cells l^{-1}) that that of micro algae (10^4-10^5 cells l^{-1}) (i.e. Pierce & Turner 1992), which suggests that they could be a good prey alternative to phytoplankton when the algal stock is mainly constituted by small cells (as e.g. in oligotrophic waters or during periods between blooms).

For a prey to be ingested, first it has to be encountered and captured. Vulnerability implies the ability of the predator to efficiently capture such prey and, at the same time, the ability of the prey to efficiently avoid the attack. In this aspect also, there exist relevant differences between ciliates and most of the algae. Ciliates can move relatively fast and some species also jump (Buskey et al 1993; Jakobsen 2002). Motility has important implications for detection processes and encounter rates. The detection of ciliates and algae implies the use of different receptors and capture strategies in copepods. Cinematographic observations have shown that copepods can detect their prey at distances in the order of one or more body lengths, using chemo and mecanoreceptors which are located mainly in the first antennae (Landry 1980; Bundy & Paffenöfer 1993). It is generally assumed that copepods detect algae by chemoreception and ciliates by mecanoreception. Encounter models, based on relative velocities between predators and prey, predict that the motility of the prey influences the rate at which the prey is encountered (Gerritsen & Strickler 1977). But different degrees of prey motility (as e.g. low for algae or high for ciliates) also determine a change in the searching and capture strategy of the predator. In the presence of ciliates some copepods behave as ambush predators (Jonsson & Tiselius 1990; Tiselius & Jonsson 1990; Kiørboe et al. 1996). Numerous setae in the first antennae, sometimes oriented in three-dimensions, are observed in copepod species which feed preferentially on motile prey (as ciliates or nauplii for instance). This insinuates that those species have enhanced perception capabilities to detect mechanical signals (Paffenhöfer et al. 1996; Uchima & Hirano 1988). Ambush copepods actively sink through the water waiting for a signal from the prey and without creating any additional

disturbance, which could alert the prey of their presence. From the prey side, ciliates have also shown some capability to detect the predator presence and avoid the predator attack by escaping (Jonsson & Tiselius 1990; Jakobsen 2002). Once the prey is detected, copepods jump on it and capture it. This strategy is substantially different from that exhibited by copepods that feed preferentially on algae. Those in fact, capture the algae mainly by creating feeding currents, which are generated by the mouth appendages. Within feeding currents, small particles are passively collected (Price & Paffenhöfer 1984) whereas larger particles seem to be reoriented and individually handled by specialized mouth appendages (Alcaraz et al. 1980; Vanderploeg & Paffenhöfer 1985; Strickler 1985). Kiørboe and al. (1996) have observed that the calanoid copepod *Acartia tonsa* exhibits a change of feeding strategy (ambush versus suspension feeding) when offered either ciliate or algae respectively, showing the effectiveness of one strategy with respect to the other to depend on prey characteristics. Other copepods however, were shown to prey efficiently on motile prey by creating feeding currents (Jakobsen 2002), which emphasizes that the above mentioned predation strategies are not the only existent and that many intermediate situations may occur explaining the coexistence of several species competing for the same prey assemblage.

Feeding on ciliates may also be a question of their relative abundance with respect to alternative prey and of their nutritional quality. Copepods are considered opportunistic predators, which means that they will probably feed on the most abundant prey (Cowles 1979). Considering that ciliates could be energetically expensive to prey, due to their motility and escape reaction to predator attacks (Jonsson & Tiselius 1990), one would expect an omnivorous predator to be less dependent on animal prey ingestion when feeding on autotrophic prey pays off because of its availability. In laboratory experiments Kiørboe et al. (1996) showed that the same copepod could switch from carnivory to herbivory when the relative abundance of the prey type changed (ciliates versus algae), confirming previous evidences of switching between herbivory and carnivory as a response to changes in food composition (Landry 1981; Stoecker & Sanders 1985; Gifford & Dagg, 1988; Kleppel et al. 1988). It is not well known yet how changes in the availability of ciliates and phytoplankton can actually regulate this switching of diet, but nevertheless is an important question to solve as it has many implications for the food web functioning.

Regarding food quality, in the last two decades several studies have emphasized the importance of the qualitative aspect of food for copepods (Cahoon 1981; Kleppel 1993; Koski et al. 1998; Jones et al. 2002). Actually, copepods appear to be able to discriminate between food items of different quality (Cowles et al. 1988) and optimize the ingestion of certain elements or biochemical components (Houde & Roman 1987). Food quality differs between algal groups (e.g. Volkman 1989; Razouls et al. 1991) and also between their growth conditions (Jonasdottir 1994). Ingestion of "better" algae, in terms of quality, lead to higher egg production rates (Schmidt & Jonasdottir 1997; Koski et al. 1998) and higher hatching success (Jonasdottir & Kiørboe 1996). However, the knowledge of the biochemical composition of protozoans is scarce. Some authors (Stoecker & Capuzzo 1990; Gifford 1991; Sanders & Wickahm 1993) have suggested that protozoans may have a C:N ratio lower than algae, thus resulting more nutritious than phytoplankton with respect to their protein and amino acids concentration. Unexpectedly, the only study which directly compares the egg production rates of copepods fed either with algae or ciliates (Ederington et al. 1995), reported the opposite result, showing an inferior nutritional quality for the bacterivorous ciliate than for the alga studied. Surely, more studies, including different ciliate and copepod species are needed to define the role of nutritional quality in copepod feeding preferences.

As evidenced by this general introduction, which depicts the importance of the trophic link between micro and mesozooplankton, the study of the strength of this link and of the factors that control it are key topics in marine biology. Some of the aspects mentioned above about this trophic link have been dealt with in this thesis, which main objectives are presented in the following section.

AIM AND OUTLINE OF THE THESIS

AIM OF THE THESIS

The main aim of this thesis was to study the trophic interaction between micro and mesozooplankton. The study is mainly centered on the most representative groups of both categories: copepods and ciliates. The main hypotheses behind this work were that ciliates are significant contributors to the diet of zooplankton and that copepods may play an important role in controlling ciliate populations.

The major objectives can be depicted as follows:

1. To quantify the contribution of ciliates to the diet of crustacean zooplankton, at both, the community and species levels.

2. To investigate the factors that regulate such ciliate-zooplankton interaction and specifically:

   2.1 the effect of the presence of alternative prey on the importance of ciliates in zooplankton diet
   2.2 the role of cilate nutritional quality on copepod reproduction
   2.3 the influence exerted by the ciliate swimming behavior on predation risk

3. To determine the trophic impact exerted by zooplankton predators on ciliate communities.

OUTLINE OF THIS THESIS

The structure of the thesis follows, to large extent, the order of the objectives presented above. Chapter organization goes from a broader perspective of general patterns to a more detailed analysis of the factors involved in the trophic relationship copepods-ciliates.

In particular, the importance of heterotrophic prey in crustacean zooplankton diets, in comparison with that of autotrophic prey, is examined in **Chapters I** and **II**. Those chapters analyze the results of feeding experiments carried out with the whole zooplankton community (**Chapters I**) and with

several copepod and cladoceran species (**Chapters II**), both fed on natural microplanktonic populations (ciliates + phytoplankton). Thus, **Chapter I** reports grazing experiments carried out during an oceanographic cruise in the Alboran Sea (SW Mediterranean) where stations were located along a transect which crossed highly dynamic areas. On the contrary, the experiments presented in **Chapter II** were performed monthly during a year at a fixed station in an oligotrophic coastal area (waters off Masnou) in the NW Mediterranean Sea. Natural variability in the relative abundance of heterotrophic and autotrophic prey were used to investigate the possible effect of the presence of alternative prey on the relative importance of heterotrophic prey in the diet of zooplankton. In these studies, the selection patterns for ciliates were also examined, as well as the trophic impact exerted by crustacean zooplankton on ciliate communities.

Laboratory experiments carried out in order to investigate the role of nutritional quality and prey behavior in determining prey selectivity patterns are presented in **Chapter III** and **IV**. In **Chapter III**, the effect of the ingestion of ciliates on the egg production rates and hatching success of the calanoid copepod *Acartia tonsa* is analyzed in comparison with other heterotrophic and autotrophic diets. In this study, production and hatching success of copepod eggs was related to the nutritional quality of prey, estimated by the content and composition of fatty acids. In **Chapter IV** the effect of prey swimming behavior on predation risk was analyzed. A two-dimension video set-up was utilized to study in detail the differences in prey swimming behavior of two species of ciliates, a naked ciliate and a tintinnid, similar in size. Behavioral observations of ciliate capture by the calanoid copepod *Acartia clausi* were also conducted. Finally, behavioral information obtained in feeding experiments carried out with the same species was utilized to explain the difference in prey vulnerability to the copepod predator.

CHAPTER I

Released as a published article. Calbet A., Broglio E., Saiz E. and Alcaraz M. (2002) Aquatic Microbial Ecology 26: 235-246.

Chapter I

Field studies on the grazing of mesozooplankton on microplankton populations. I. Community impact in the Alboran Sea

Introduction

In most of the research on the functional role of mesozooplankton (> 200 μm) in marine food webs, herbivory has been considered as the main path of transfer of organic matter from primary producers to higher trophic levels. In this sense, the majority of studies on zooplankton feeding have largely used the gut fluorescence technique to estimate zooplankton grazing impact (see review by Calbet 2001). Because this technique uses photosynthetic pigments to estimate food intake, the contribution of microzooplankton to the diet is systematically ignored. An increasing number of studies, however, have shown non-autotrophic food sources as important contributors of the diet as well (see review by Sanders & Wickham 1993). This is particularly the case for planktonic ciliates and heterotrophic dinoflagellates (Fessenden & Cowles 1994, Nakamura & Turner 1997, Lonsdale et al. 2000), both key agents in the functioning of microbial food webs (Sherr et al. 1986, Sherr & Sherr 1988). By grazing upon protozoans, zooplankton may benefit from supplementary food sources and complement the diet with additional essential nutrients needed for metabolism (Kleppel & Burkart 1995, Klein Breteler et al. 1999).

However, and in spite of the relevance of microzooplanktonic organisms as food for mesozooplankton, relatively few studies consider both, algal and animal food sources, when assessing the impact of mesozooplankton grazing in natural communities. Among those there are even less the ones taking into consideration the whole mesozooplanktonic community instead of single copepod species (e.g., Carrick et al. 1991, Thouvenot et al. 1999, Calbet & Landry 1999). Here, we present a community grazing approach to study the dynamics and relationships between the different compartments of microplankton and mesozooplankton in a marine pelagic community. The study took place in the Alboran Sea (SW Mediterranean), a highly dynamic

area (Jiménez et al. 1987, Minas et al. 1991, Rodríguez et al. 1998) characterized by the presence of a persistent anticyclonic gyre caused by the inflow of Atlantic waters into the Mediterranean basin through the Gibraltar Strait (Minas et al. 1991, Tintoré et al. 1991). The gyre is associated with a frontal zone in which intermittent upwelling of nutrient rich waters (Coste et al. 1988, Perkins et al. 1990, Minas et al. 1991) produces sporadic phytoplankton blooms (Videau et al. 1994). The Alboran Sea provides, thus, an excellent scenario for our purposes, because in a relatively small area mesozooplankton can potentially experience a wide spectrum of different trophic conditions.

Materials and methods

Sampling strategy

The study was conducted on board the *R/V Hespérides* from the 14th to the 24th of September 1999 in the Alboran Sea (SW Mediterranean). Sampling was conducted around noon at 3 fixed stations (A, B and C, respectively at 36° 23' N, 4° 15' W; 36° 14' N, 4° 15' W; and 36° 0' N, 4° 15' W; Fig. 1) located along a transect crossing the northern part of the Western Alboran Gyre. Station A was visited 2 times (A1 and A2); stations B and C, were visited 3 (B1, B2 and B3) and 2 times (C1 and C2), respectively.

Previous studies in the same area (Morán & Estrada 2001) reported Station A as representative of upwelling conditions, while Stations B and C were expected to be more oligotrophic. The hydrographic characteristics and fluorescence profiles of the surveyed stations were determined by CTD casts made with a Neil-Brown MARK-V CTD equipped with a Sea Tech fluorometer. Water samples for chlorophyll *a* (Chl*a*) analyses and for microplankton profiles (0-100 m) were collected at 10-20 meter depth intervals using a rosette provided with 12-l Niskin bottles. For total Chl*a*, 100 ml of water were filtered under low vacuum pressure (< 100 mm Hg) through Whatmann glass fiber filters (GF/F, 25 mm diameter). For the > 5 µm fraction, 200 ml samples were filtered through 5 µm pore-size polycarbonate Nucleopore™ filters (25 mm diameter). After filtration, the filters were stored frozen at -80°C until analysis (see below). Water samples for the determination of microplankton abundance were fixed with 1% Lugol's solution (Leaky et al. 1994).

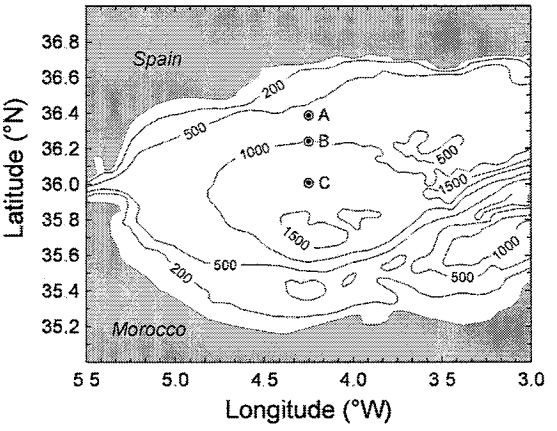

Figure 1. Map of the area surveyed showing the location of the three main stations (A, B and C).

Samples for mesozooplankton biomass and abundance quantification were obtained from 200-0 m vertical plankton tows made with a 57cm diameter double WP-2 net (200 μm mesh) without flowmeter, towed at 30 m min^{-1}. One of the cod ends was entirely preserved with buffered Formalin (5% final solution) for later assessment of community composition. The contents of the other cod end was poured into a graduated cylinder from which 3 aliquots of 10 ml were taken after thorough mixing, filtered onto GF/F glass fiber filters, quickly washed with ammonium formiate, and dried at 60°C for later measurement of zooplankton community dry weight (DW). In order to minimize organismal damage due to the net tow process the mesozooplankton samples for grazing experiments were obtained with a similar protocol, but with shallower (up to 75 m depth) and slower (10 m min^{-1}) net tows. A 4-l plastic bag was used instead of the closed cod end to reduce sampling stress and damage. These shallower tows integrated the depth of highest organismal abundance (Sabatés et al. unpublished).

Experimental design

Experimental water for grazing assessment was collected at the depth of the Chl*a* maximum using 50-l Van Dorn-type transparent bottles. The water was gently poured into a 50-l bucket and reverse-flow filtered gently submerging a 30 cm diameter PVC cylinder with a bottom of 100 μm mesh. By this procedure we eliminated > 100 μm metazoan predators from the water inside

the sieve, while only few rarely abundant large phytoplankton cells were excluded (Rodríguez et al. 1998). Once the suspension was ready it was amended with a nutrient mixture (15 μM NH_4Cl and 1 μM Na_2HPO_4) to compensate for nutrient enrichment due to zooplankton excretion, and the experimental water was left from 15 to 20 minutes to allow the microplanktonic community to stabilize. After this period of time, 6 transparent wide-mouth Polyethylene bottles of 5-l capacity were gently filled with the water from inside the sieve. Three of them were used as controls; in the other 3 an aliquot of 10 ml of a total volume of 500 ml of concentrated suspension containing mesozooplankton from the net tow was added. Duplicate initial samples were taken for microplankton and Chl*a* quantification as described previously, and four extra mesozooplankton initial aliquots were filtered onto GF/F glass filters and dried for assessment of biomass (dry weight). The bottles were incubated for 24 h in an incubator cooled with circulating surface seawater (temperature = 16.0-16.5°C) and light-screened with a dark mesh that filtered 99% of surface light intensity. The incubation bottles were gently turned upside down several times during the incubations to reduce settling of algae.

Sample analysis

Mesozooplankton abundance and species composition was estimated by counting and identifying under stereomicroscope at least 300 individuals per sample. Chl*a* was fluorometrically analyzed according to Yentsch and Menzel (1963). Frozen filters were placed in 6 ml of 90 % acetone, and kept for ca. 24 h in the dark at 4°C. Fluorescence was subsequently measured without acidification with a Turner-Design fluorometer. For microplankton abundance assessment preserved 100 ml samples were settled in Utermöhl settling chambers for 48 hours and counted under a Zeiss Axiovert 35 microscope. Ciliate abundance was corrected considering a factor of losses due to 1%-Lugol's fixation of 25% (personal observations). Video images of 50 dinoflagellates and 50 ciliates per sample were digitized with a Power Macintosh computer provided with a frame grabber and NIH Image analysis software. The contour of the organisms was outlined, the area measured, and the length and width of the cell automatically estimated assuming an ellipsoidal shape.

Microplankton biovolumes were converted to cell carbon using a factor of 0.19 pg C μm^{-3} for ciliates (Putt & Stoecker 1989) and the equation log pgC

$cell^{-1}$ = -0.119 +(0.819 log volume) for dinoflagellates (Menden-Deuer & Lessard 2000). Zooplankton carbon contents was considered to be 45% DW (Omori & Ikeda 1984). Chlorophyll concentration was transformed into phytoplankton carbon using a carbon-to-chlorophyll ratio of 25 for stations A and B, and 60 for station C. These ratios corresponded to a previous cruise (May 1998) and were calculated combining fluorimetrical measurements of chlorophyll with microscopic biovolumetric estimations, and later conversion to carbon using literature values (Arin et al. 2002.). Ingestion rates were calculated according to the equations of Frost (1972).

Results

Environmental conditions

1) Horizontal distribution

The different stations could not be characterized by persistent patterns regarding the integrated abundance or biomass of the different plankton groups (Tab. 1 and 2). The stations showed important differences among the consecutive visits, so the expected positive abundance gradient from upwelling stations (A) to the more oceanic ones (B and C) was masked by temporal variability and by a possible displacement of upwelled waters towards open-ocean. Only during the last visit to station C (C2) the Chl*a* values were similar to those expected for oceanic oligotrophic waters (Moran & Estrada 2001). Surprisingly, Chl*a* concentrations reached the highest values at stations B3 and C1. Phytoplankton dominated the biomass of the planktonic community, followed in relevance by mesozooplankton. Among the two size-fractions of Chl*a*, the biomass of > 5 μm autotrophs was, in general, similar or greater than the smaller size-classes (except for station C2). The contribution of > 5 μm to total Chl*a* showed a consistent, albeit not significant, tendency to increase with total Chl*a* concentration ($r = 0.6$, $p = 0.12$), even if the range of the contribution percentage was narrow (40-68%).

Ciliates were more abundant than dinoflagellates (Tab. 1), except for station C2, in which the dinoflagellate *Gymnodinium catenatum* contributed in great measure to increase the total dinoflagellate abundance (data not shown), and represented from 6 to 35% of the total planktonic community biomass.

Table 1. Integrated values (0-100 m) of Chl*a* concentration (mg Chla m^{-2}), abundance of ciliates (cells m^{-2}), dinoflagellates (cells m^{-2}), and mesozooplankton (ind m^{-2}) (0-200 m) for the consecutive visits at the 3 fixed stations (A, B and C).

Station	A1	A2	B1	B2	B3	C1	C2
Date	15/10	21/10	16/10	20/10	23/10	17/10	22/10
Chla > 5 µm	46.4	31.6	67.3	60.4	66.3	68.1	13.3
Chla < 5 µm	48.9	32.6	31.2	28.5	52.3	46.4	20.4
Ciliates (x 10^8)	4.3	4.7	2.1	3.8	3.9	3.3	1.8
Dinoflagellates (x 10^8)	3.3	2.2	2.1	2.0	1.8	2.3	2.8
Mesozooplankton	348039	223039	409804	552941	185784	210294	177451

Table 2. Integrated values (0-100 m) of the concentration (as carbon, mg C m^{-2}) of Chl*a*, ciliates, dinoflagellates, and (0-200 m) mesozooplankton, for the consecutive visits at the 3 fixed stations (A, B and C). For conversion factors see text (Methods section).

Station	A1	A2	B1	B2	B3	C1	C2
Date	15/10	21/10	16/10	20/10	23/10	17/10	22/10
Chla > 5 µm	1160	790	1683	1510	1658	4086	798
Chla < 5 µm	1223	815	780	713	1308	2784	1224
Ciliates	688	915	402	499	892	330	129
Dinoflagellates	465	324	333	159	142	137	330
Mesozooplankton	1380	672	1328	1732	658	902	1224

The horizontal distribution of mesozooplankton across the transect did not correspond either with the expected higher abundance at coastal stations (Tab. 1 and 2). Instead, the maximum values were found at station B2. The community was numerically dominated by copepods and cladocerans (Tab. 3).

Table 3. Zooplankton community composition (as major groups, ind m^{-2}) for the consecutive visits at the 3 fixed stations (A, B and C).

Taxa	A1	A2	B1	B2	B3	C1	C2
Copepods	187255	86765	172549	287255	130392	123529	98529
Cladocerans	119608	105392	217647	220588	21078	58333	44608
Ostracods	9804	6863	3922	12745	15686	3431	3922
Crustacean larvae	3922	3922	1961	6863	1471	9314	4412
Cnidarians	5882	1961	980	4902	4412	1471	1961
Chaetognaths	7843	1961	1961	2941	1961	3922	6373
Appendicularians	4902	8333	7843	5882	4902	6373	5392
Other Tunicates	5882	1961	980	0	3431	1471	490
Equinoderm larvae	0	0	0	3922	0	0	490
Molluscs	2941	5882	1961	7843	2451	2451	11275

The bulk of the copepod community corresponded to an assemblage of copepodite stages of calanoids (*Clausocalanus* spp., *Calocalanus* spp., *Paracalanus* spp. and *Ctenocalanus* spp.), and, with minor importance, *Oithona* spp. and *Centropages typicus*; for Cladocerans, *Penilia avirostris* was the dominant species (Tab. 4). The copepod/cladoceran quotients ranged between 0.8 and 2.2, except for station B3 in which was 6.2. Other remarkable features of the zooplanktonic community during the study were the ubiquitous presence of appendicularians and molluscs (mostly pteropods), and a sporadic bloom of echinoderm larvae at station B2. Larger predators, such as chaetognaths and cnidarians were also present at all stations, although their abundances never reached values above 800 ind m^{-2}. No significant correlation was observed between integrated mesozooplankton biomass or abundance and their possible prey (Chl*a*, ciliates or dinoflagellates).

Table 4. Species composition (as ind m^{-2}) of the copepod and cladoceran communities at the stations surveyed. The group "calanoid copepodites" includes juveniles of the genera *Clausocalanus*, *Paracalanus*, *Ctenocalanus* and *Calocalanus*, which were considered together due to morphological resemblance. For the rest of copepods the data shown correspond to adult stages plus copepodites.

Groups	A1	A2	B1	B2	B3	C1	C2
Copepods							
***Clausocalanus* spp.**	13725	5882	7843	15686	5392	5882	11275
Paracalanus parvus	7843	2941	12745	3922	7353	5392	1471
Ctenocalanus vanus	6863	490	980	0	490	490	1471
***Calocalanus* spp.**	12745	4902	6863	5882	4902	3431	6373
Calanoid copepodites	48039	19608	51961	48039	50000	24510	24020
Centropages typicus	10784	4902	25490	10784	12255	17647	3922
Temora stylifera	6863	4412	18627	5882	3922	8824	5392
***Eucalanus* sp.**	3922	7353	8824	22549	1471	16667	980
***Acartia* spp.**	7843	7843	6863	23529	4412	4412	8333
Euterpina acutifrons	4902	980	0	8824	0	2941	490
***Oithona* spp.**	23529	19118	19608	27451	21569	7353	11765
***Oncaea* spp.**	21569	2451	5882	11765	5882	6373	7843
***Corycaeus* spp.**	3922	980	980	2941	490	490	7843
Other copepods	14706	3922	5882	20588	11275	9804	6373
Cladocerans							
Penilia avirostris	95098	94608	191176	208824	19118	53431	30392
***Evadne* spp.**	24510	10784	26471	11765	1961	4902	14216

2) Vertical distribution

The mixed layer extended down to the first 20-30 m depth at all the surveyed stations, being less conspicuous at the more oceanic ones (Fig. 2).

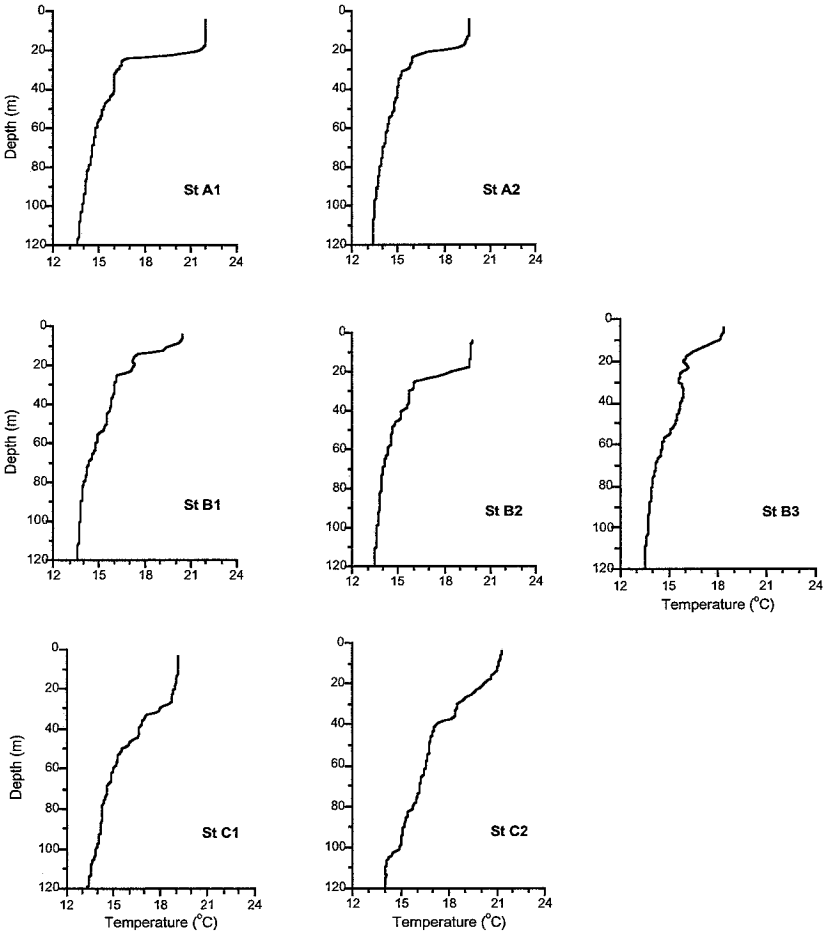

Figure 2. Vertical profiles of temperature at the stations surveyed.

In general, Chl*a* maximum was situated below the thermocline, between 20 and 40 m depth, except for stations B3 and C2, where the higher values were found at surface layers (Fig. 3).

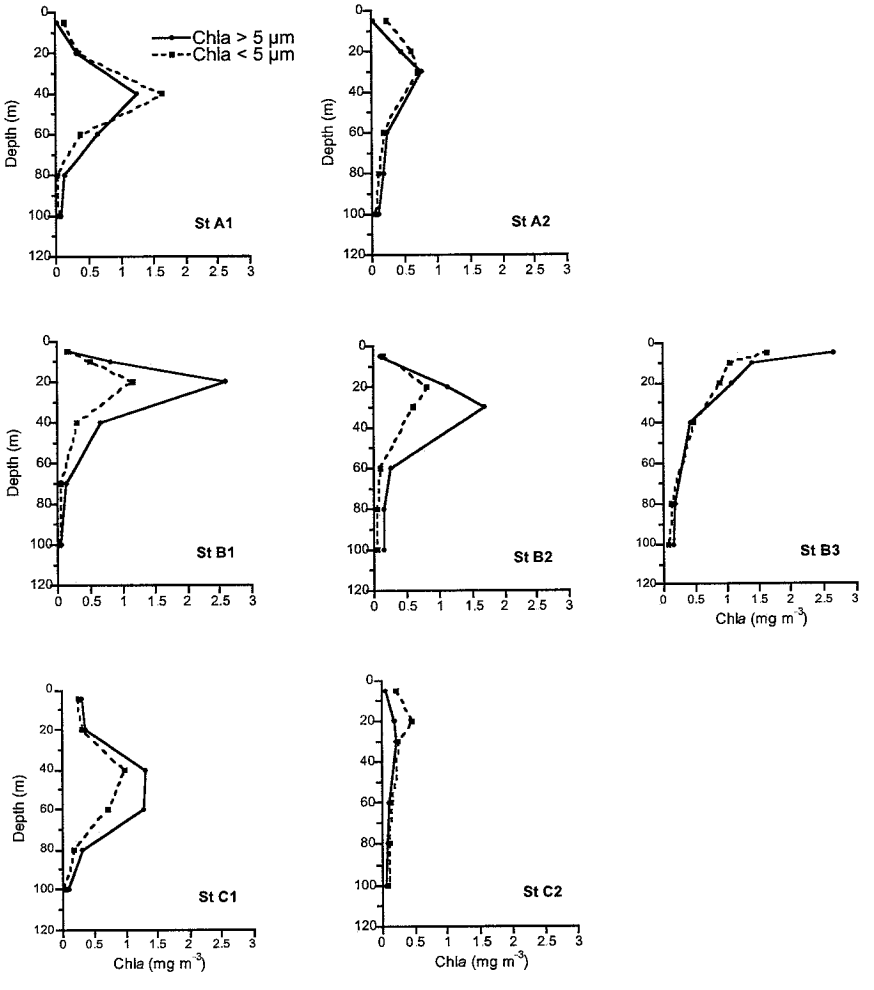

Figure 3. Vertical profiles of the two fractions of Chl*a* concentration considered in the different stations surveyed.

In general the vertical distribution of Chl*a* approximately paired the profiles of dinoflagellates and ciliates, except for station B1 (Fig. 4). Ciliate and dinoflagellate maxima coincided in depth, being at that layer ciliates more abundant than dinoflagellates. Exception to the later was station C2, mostly due to the high abundance of *Gymnodinium catenatum* between 20 and 40 m (data not shown).

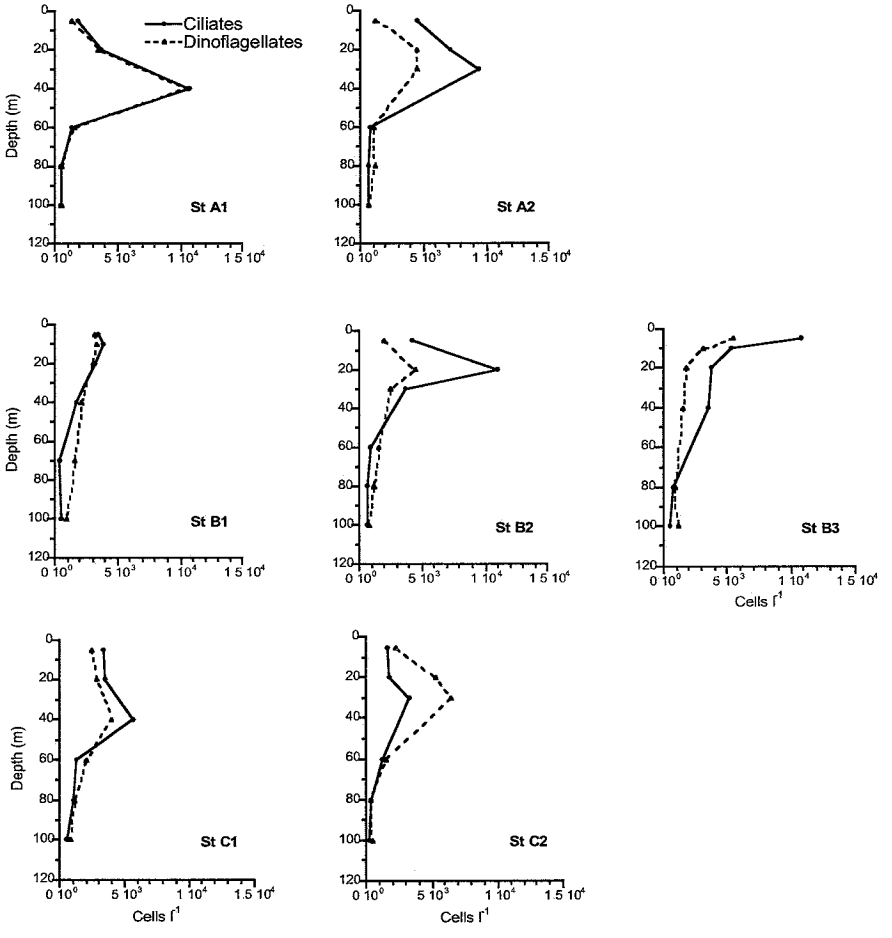

Figure 4. Vertical profiles of ciliate and dinoflagellate concentrations in the different stations surveyed.

Feeding rates and mesozooplankton grazing impact

Weight-specific ingestion rates of the mesozooplanktonic community on the different fractions of Chl*a* are shown in Fig. 5. In general, ingestion rates were low (< 1.5 mg Chl*a* mg DW^{-1} d^{-1}), being the highest values found at stations B3 and C1.

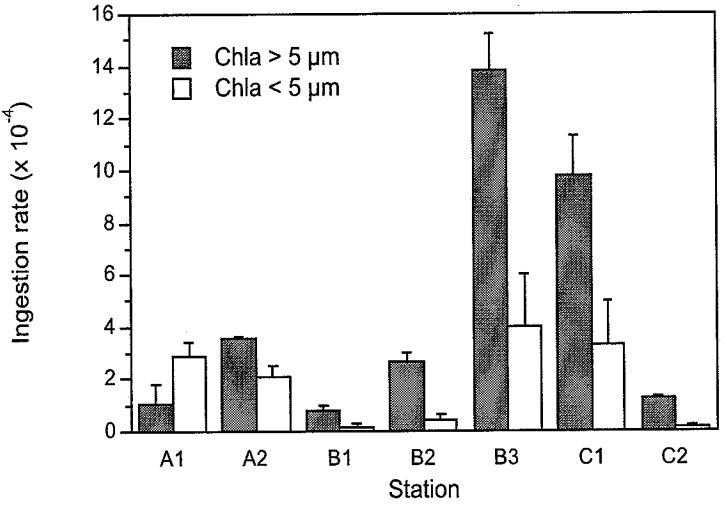

Figure 5. Mesozooplankton specific ingestion rates (mg Chl*a* mg DW^{-1} d^{-1}) on Chl*a* > 5 µm and Chl*a* < 5 µm. Error bars represent SE.

Grazing was higher on > 5µm fractions ($p < 0.05$, ANOVA analyses), except for station A1. Ingestion rates on Chl*a* size-fractions were significantly ($p < 0.05$), although poorly correlated with pigment concentration (Fig. 6). No significant differences were found between the regression lines describing the relationship between Chl*a* ingestion and the concentration of the different size-fractions (Fig. 6, ANCOVA analysis), indicating that the feeding response to algal food concentration was proportional for the different size-fractions considered.

The impact that mesozooplankton feeding was exerting on Chl*a* standing stock was low, averaging 1.3% for all stations and ranging from 0.3 for station B1 to 2.4 for station C1.

The weight-specific ingestion rates on ciliates and dinoflagellates by mesozooplankton are shown in Fig. 7. For ciliates, as for Chl*a*, the highest grazing rates occurred at station B3. On the other hand, station C1, the second station in importance regarding Chl*a* ingestion, presented the minimum grazing rates upon ciliates. Specific ingestion rates on ciliates were directly related to their concentration (Fig. 8).

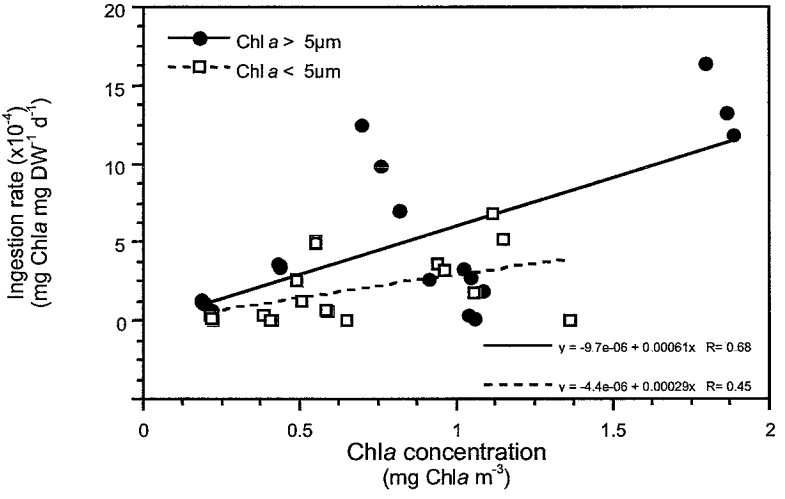

Figure 6. Relationship between specific ingestion rates ($x10^{-4}$ mg Chl*a* mg DW^{-1} d^{-1}) and the concentration of Chl*a* (mg Chl*a* m^{-3}) for the 2 fractions considered, respectively. Regression lines and equations are also provided.

However, specific feeding on dinoflagellates was very constant along the study and independent of cell concentration, ranging from 1000 to 2500 dinoflagellate cells mg DW^{-1} day^{-1} at stations C2 and A1, respectively (Fig. 8). Total grazing impact on microplankton communities was low, averaging 1.4% (range 0.3-3.5%) and 1.5% (range 0.8-2.3%) for ciliates and dinoflagellates, respectively.

Overall, feeding on phytoplankton and microplankton (ciliates and dinoflagellates), accounted for food ratios of less than 5% of mesozooplankton body carbon d^{-1} in most of the stations (Fig. 9). The contribution of Chl*a* to the mesozooplankton diet ranged from 26 to 99% of total ingested carbon; ciliates

represented from 0.4 to 49%, and dinoflagellates accounted for less than 25%. Since the technique used to preserve microplankton did not allow to differentiate between autotrophy and heterotrophy, part of the contribution of dinoflagellates to the daily rations was already considered in terms of Chl*a*. Nevertheless, due to the notoriously higher contribution of autotrophic food in the diet, no attempt was made to correct the data.

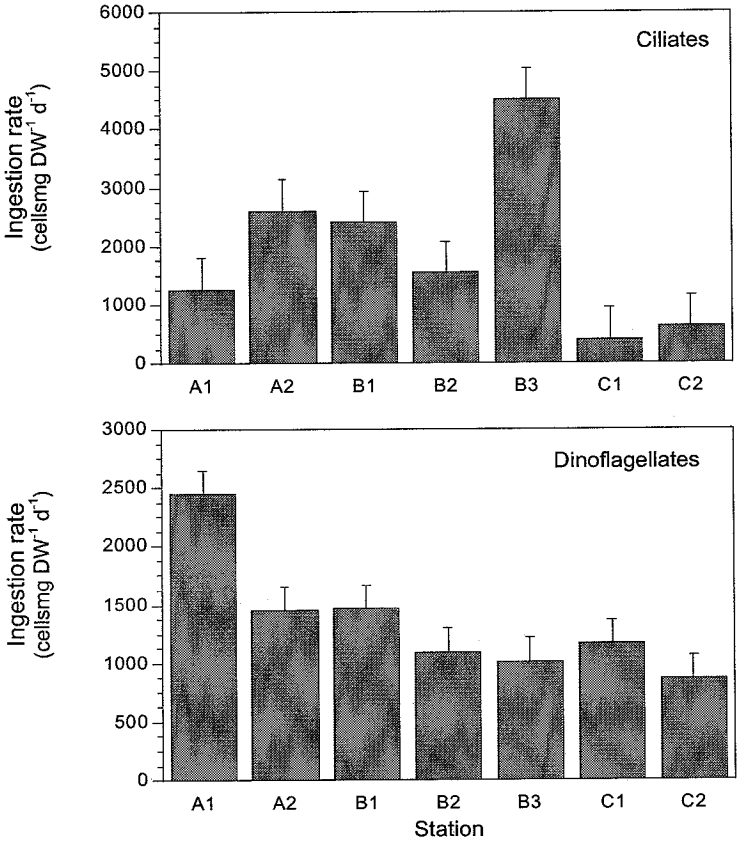

Figure 7. Mesozooplankton specific ingestion rates (cells mg DW^{-1} d^{-1}) on ciliates (above) and dinoflagellates (below) for the different stations surveyed. Error bars represent SE. Note differences in axis scale.

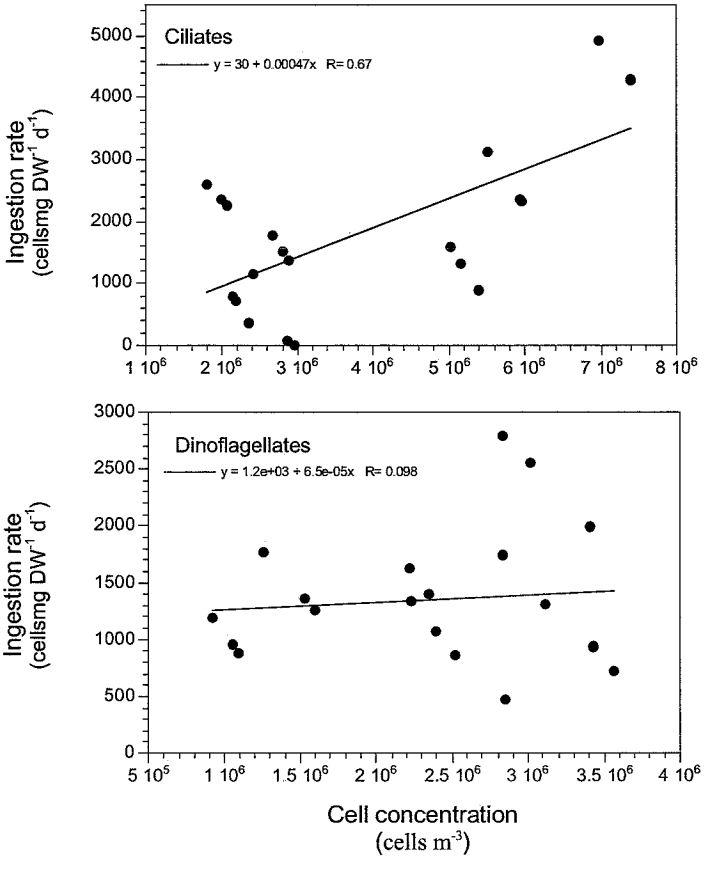

Figure 8. Relationship between specific ingestion rates (cells mg DW^{-1} d^{-1}) and the concentration of ciliates (above) and dinoflagellates (below) (cells m^{-3}).

Mesozooplankton showed a higher proportional ingestion rates on ciliates and Chl*a* larger than 5 μm cells when compared with Chl*a* < 5μm (Fig. 10 a). When ciliates and dinoflagellates were compared (Fig. 10 b) a tendency towards selecting dinoflagellates was observed, especially at stations C1.

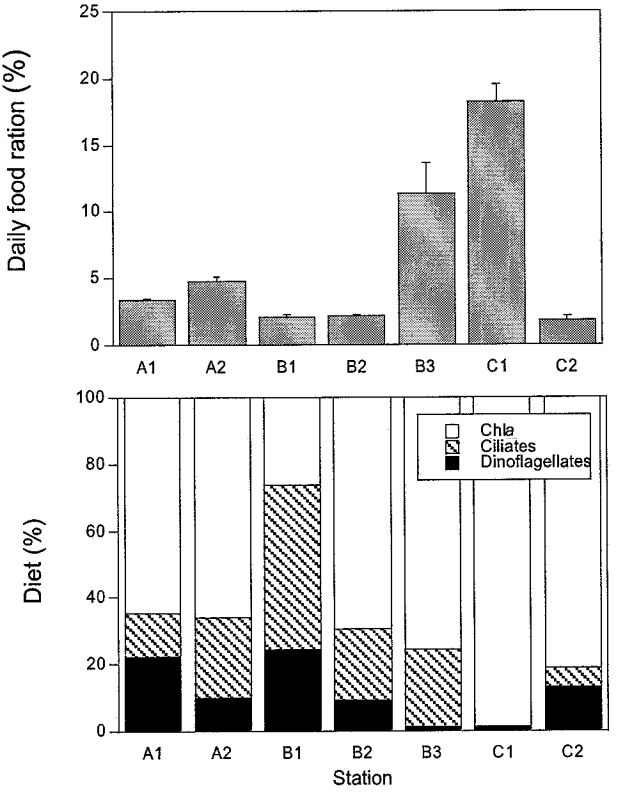

Figure 9. Above: Total daily food rations (% body carbon ingested daily) for the mesozooplankton at each of the stations surveyed (error bars represent SE). Below: Contribution (as %) of the different prey to the mesozooplankton diet.

Discussion

Due to the complex hidrodynamics of the Alboran Sea (Jiménez et al. 1987, Minas et al. 1991, Rodríguez et al. 1998) any spatial trend in the abundance of the planktonic groups here considered was masked by temporal variability. In spite of this very variable environment, the estimations of phyto- and mesozooplankton biomass fall within the range of values reported in previous studies in adjacent areas of the Alboran Sea (García & Camiñas

1985, Jiménez et al. 1987, Thibault et al. 1994, Moran & Estrada 2000). Thus, the lack of clear correlations between integrated mesozooplankton biomass and Chl*a* concentration could be indicative of an uncoupling between consumers and producers, typical of highly dynamic systems.

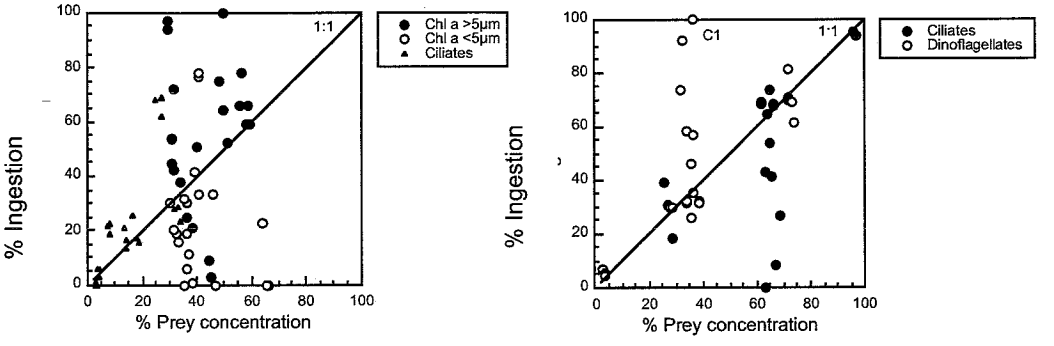

Figure 10. Scatterplot of the percentage of a specific prey biomass in the planktonic community versus the percentage that represents the same prey in the total mesozooplankton carbon ingestion. Values above the line 1:1 would indicate feeding selection for that prey. A) Chl*a* > 5µm, Chl*a* < 5µm and ciliates. B) Ciliates and dinoflagellates

In those systems the response of the zooplanktonic community cannot cope with the frequency of environmental changes (Calbet et al. 1996). This uncoupling could be the possible cause of the peculiar community biomass distribution for the different groups components of the planktonic community. Conceptually, one would expect that under upwelling conditions, or in productive coastal environments, large phytoplankton and zooplankton would dominate the community biomass, which was not the case for the coastal stations (A1 and A2). Moreover, it has been shown that open-ocean communities support more heterotrophic biomass than do coastal communities for a given biomass of autotrophs (Gasol et al. 1997). This was clearly not the case of station C1, located in more oceanic waters. Actually, this station could be indicative of the earliest stages of a phytoplankton bloom, likely produced by the displacement of nutrient rich waters from the upwelling region.

Feeding rates should respond faster to sudden changes in primary producers than biomass (Calbet et al. 1996). This was the case of the observed

relationships between mesozooplankton ingestion rates and Chl*a*. Although both Chl*a* size fractions were significantly correlated with ingestion, the regression coefficient was higher for > 5μm Chl*a*. This is not surprising, considering the numerical relevance of copepods in the mesozooplankton community, unable to feed efficiently on < 5μm prey (Hansen et al. 1994, Calbet et al. 2000a). Better relationships were apparent for ciliates, although the grazing on dinoflagellates seemed to be independent of cell concentration. In spite of the lack of relationship between dinoflagellate concentration and their consumption rates, this group was selected by mesozooplankton as food source in front of ciliates in some stations. It is difficult to relate these results with other studies because selectivity patterns are highly dependent on the specific communities investigated. In this sense, selection for microzooplankton over algae is not a rare feature (Stoecker & Egloff 1987, Gifford & Dagg 1988, Wiadnyana & Rassoulzadegan 1989), although preference for algae over protozoans has also been observed (Williamson et al. 1996, Calbet et al. 2000a). A similar contrasting situation is found when comparing selectivity for ciliates and dinoflagellates. Dinoflagellates appear to be selected by zooplankton in some studies (Suzuki et al. 1999, the present study), whereas other studies show that ciliates are the preferred prey (Stoecker & Sanders 1985, Lonsdale et al. 2000, Vincent & Hartmann 2001). Food-selection mechanisms are the result of complex interactions. The feeding response depends not only of the proportion of prey items (Sanders & Wickham 1993, Fessenden & Cowles 1994), but of the specific composition of predators. The community found in the present study had a high proportion of cladocerans, which are believed to feed unselectively (Paffenhöfer & Orcutt 1986). Thus, any emergent species-specific selective pattern could have been masked by the proportion of the different zooplanktonic groups in our incubations. The particular case of ciliates, which were negatively selected against dinoflagellates at some stations, could be explained by the presence in some ciliate species of escape reactions (Jonsson & Tiselius 1990, **Chapter IV**), avoiding, by this way, cladoceran and copepod feeding. Aside from any electivity pattern, phytoplankton was the main food source in the daily rations of mesozooplankton (except for station B1). Certainly, autotrophic organisms dominated microplankton in terms of biomass. Hence, seems quite reasonable to assume that zooplankton will take advantage of the most common prey, as it has been shown in other studies during phytoplankton bloom conditions (Tiselius 1989, Fessenden & Cowles 1994). It is very surprising, however, that mesozooplankton daily rations and the resulting grazing impact were very low. For similar values of Chl*a*, in the same area Calbet et al. (2000b) reported

copepod daily food rations ranging from 20-50% body carbon as derived from egg production rates (assuming a gross growth efficiency of 20-30%; Straile 1997). The daily food rations found in the present study averaged 6.3 ± 2.4 SE, ranging from 2 to 20%, which seem barely cover their metabolic demands. An average zooplankter of the study area (0.0089 mg DW ind^{-1}, Tab. 1) would require about 4% of its body carbon to compensate for respiratory losses (Omori & Ikeda 1984, Ikeda 1985). Only in stations B3 and C1 this threshold was clearly surpassed, being the mesozooplanktonic community extremely starved in the rest of stations. The low mesozooplankton ingestion rates observed in spite of the fact that food was abundant enough could be explained by the use of other food sources different than the ones considered in this work (metazoan organisms, bacterioplankton or detritus). Metazoans (e.g. nauplii and copepodites) may have occasionally been ingested by some zooplankters. However, the mesozooplankton community that dominated during our study (mostly *P. avirostris* and fine particle feeder copepods) can hardly use metazoans as their main prey item. Aggregated bacterioplankton, on the other hand, is a frequent component of the diet of cladocerans, but this is not the case for free-living bacteria (Turner et al. 1988). Copepods are not able either to exploit this food source (Berggreen et al. 1988, Calbet et al. 2000a). In any case, at least autotrophic bacteria were already considered within Chl*a* < 5μm estimations. Detritus, which can be used as food for many zooplankters (Heinle et al. 1977, Rudstam et al. 1989, Finenko & Romanova 1991), are only important food sources in estuarine and enclosed environments.

An alternative explanation for the low feeding rates detected during our study could be related to the presence of toxic phytoplankton. The toxic dinoflagellate *Gymnodinium catenatum* (Fig. 11), previously reported in the area (Delgado 1990), was present in all stations, although not at bloom concentrations (from 2.0 10^6 to 1.3 10^8 cells m^{-2}). The effects *G. catenatum* produces in other planktonic organisms are not fully understood, and range from no effect upon the heterotrophic dinoflagellate *Polykrikos kofoidii* (Matsuyama et al. 1999), to strongly reduce copepod naupliar activity (Bagøien et al. 1996). Although the experiments of Bagøien et al. (1996) were designed to test the effects of high dinoflagellate concentrations (175 cells ml^{-1}), possible effects of lower concentrations cannot be rejected. If this was the case, the presence of *G. catenatum* should contribute to explain the observed ingestion rates. To test this hypothesis we built a multiple regression model that related the total specific ingestion rates in carbon with the biomass of the different components of the planktonic community.

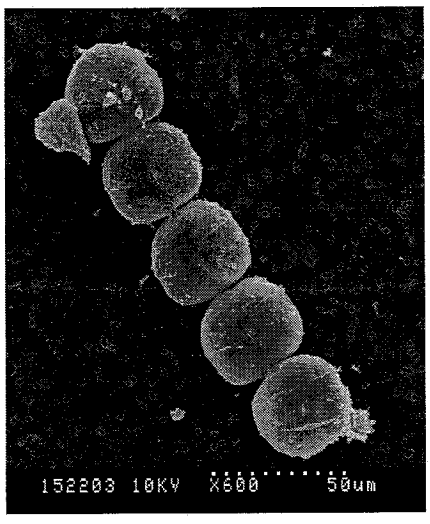

Figure 11. *Gymnodinium catenatum* from the Alboran Sea. Photo by Albert Calbet and JM Fortunio.

When considering only Chl*a*, ciliate and dinoflagellate concentrations, the model was only significant for Chl*a* ($r^2 = 0.68$, $p < 0.00001$). However, when the concentration of *G. catenatum* was included, this added a significant negative term to the model, and improved the coefficient of determination ($r^2 = 0.75$, $p < 0.00001$), explaining a higher percentage of the variability observed. The resulting equation was:

$$\text{Specific ingestion rate} = -0.0029 + 0.0019 * \text{Chl}a - 0.029 * G.\ catenatum$$

Certainly, the addition of significance in the regression model does not prove a causal effect of *G. catenatum* in inhibiting mesozooplankton grazing. However, it reinforces to the idea that toxic phytoplankton could have an effect upon food webs through mesozooplankton, even when they are not at "bloom" concentrations. Regardless, the results stress the need of complementary taxonomic studies when interpreting experimental work with planktonic communities, and highlight the importance of species specific interactions in the pelagic realm.

CHAPTER II

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Chapter II

Field studies on the grazing of mesozooplankton on microplankton populations. II. Interspecific variability, selectivity patterns and seasonal variations.

Introduction

Ciliates are important components of marine pelagic food webs (Azam et al. 1983, Sherr et al. 1986). They are significant consumers of pico and nano-autotrophic production (Finlay & Fenchel 1996) and, at the same time, represent a valuable food for zooplankton (Stoecker & Capuzzo 1990). For these reasons ciliates may constitute an important link between the microbial loop and higher trophic levels of the food chain (Sherr & Sherr 1988).

Since the early 90s, quite an amount of studies have highlighted this potential role of ciliates and have reported high clearance rates on these organisms by marine zooplankton both in the field (e.g. Tiselius 1989, Fessenden & Cowles 1994) and the laboratory (e.g. Stoecker & Egloff 1987, Jonsson & Tiselius 1990). Ciliates appear to be significant prey items of zooplankton in very different trophic scenarios, ranging from rich upwelling zones and spring bloom situations (Fessenden & Cowles 1994, Irigoien et al. 1998) to oligotrophic (Gifford & Dagg 1991, Zeldis et al. 2002) and polar ecosystems (Atkinson 1996, Levinsen et al. 2000). Most studies have focused on copepods, showing that a variety of species, with very different feeding behaviors, are able to predate efficiently on them. At the small-scale, behavioural studies have revealed that ciliates are detected by mechanical perception (Jonsson & Tiselius 1990, Saiz & Kiørboe 1995, Kiørboe et al. 1996), and that ciliate behavior plays a principal role in the ciliate-copepod interaction and selectivity patterns (**Chapter IV**).

However, some interesting questions on ciliate-zooplankton interactions remain still open. For instance, the frequent lack of correlation observed in many field studies between in situ growth rates of zooplankton and the concentration of phytoplankton (e.g. Dam et al. 1994, Ohman and Runge 1994,

Saiz et al.1999, Calbet et al. 2002b) is common argument to attribute a relevant contribution of ciliates to zooplankton diet. Yet, relatively few studies have paid attention to the actual nutritional contribution of ciliates as carbon and nitrogen sources for zooplankton metabolism, and compared them to the one supplied by phytoplankton ingestion (e.g. Froneman et al. 1996, Batten et al. 2001). Furthermore, it is scarcely known how ciliate contribution to crustacean zooplankton diet may vary through seasonal succession, where scenarios pictured by different zooplankton species and microbial communities occur.

A second aspect of interest in the trophic interactions between ciliates and zooplankton deals with feeding selection mechanisms. In the literature, copepods frequently exhibit higher clearance rates on ciliates than on phytoplankton (e.g. Gifford & Dagg 1991, Fessenden & Cowles 1994, Levinsen et al. 2000), fact that has been attributed to positive selection patterns. What exactly determines this positive selection is not clear. Implications of food quality and encounter rates have been suggested (Stoecker & Capuzzo 1990, Jonsson & Tiselius 1990). However, the preference for determinate food items could also be dependent on the different availability of an alternate suitable prey, and switching responses may appear as well (Kiørboe et al. 1996, Gismervik & Andersen 1997). Unfortunately, few field studies have considered these aspects.

Finally, it is also inadequately known the trophic control that zooplankton may have on ciliate communities. Because their nexus position as feasible prey for zooplankton and top predators for other microbes, ciliates represent a crucial step within marine planktonic food webs. Empirical evidence up to now, however, indicate that copepods, the major integrand of mesozooplankton, in general, exert only low predation on ciliate communities (e.g. Atkinson et al. 1996, Lonsdale et al. 2000, Batten et al. 2001). Nevertheless, most of these previous studies have been conducted in ecosystems not necessarily comparable to temperate oligotrophic conditions (e.g. polar and subpolar, Atkinson 1996, Lonsdale et al. 2000) and restricted to short periods of time, making difficult to obtain a comprehensive picture of this control on a yearly basis.

All these concerns can be summarized in the following questions, which represent the main objectives of this study: 1) Are ciliates important contributors to the diet of zooplankton under natural conditions? 2) Is the reported preference for ciliates by zooplankton a mere consequence of their

availability in the field, or depends on the presence of alternate prey? and 3) Can predation by zooplankton control the ciliate community?

To address these questions, integrative works contemplating a broad spectrum of prey abundance and the study of the natural feeding behavior of the different groups (species) of zooplankters are needed. In this sense, field studies are essential tools. Even though they may not provide as much insight into the actual mechanisms driving processes as laboratory experimentation under controlled conditions, they cope with natural diversity and variability and provide, thus, an ideal frame for further research. Here, we report on experiments on zooplankton feeding carried out in a nearshore station in the north-western Mediterranean Sea, comprising a whole year round seasonal cycle, which contemplates a wide spectrum of ciliate and phytoplankton abundance. The studied zooplanktonic species were the copepods *Oithona* spp., *Paracalanus parvus*, *Clausocalanus* spp., *Temora stylifera*, *Euterpina acutifrons* and *Centropages typicus*, and the cladocerans *Evadne spinifera*, *Penilia avirostris*, and *Podon* spp. We quantified the contribution of ciliates, and small (< 5μm) and large (> 5μm) phytoplankton to the natural diet of these zooplankters, and determined their selectivity patterns and grazing impact on microbial communities.

Materials and methods

Field sampling

Monthly experiments were conducted, from May 1999 to July 2000, at a near shore station located off Masnou (20 km north of Barcelona, Spain, NW Mediterranean). Water for experiments and assessment of the naturally occurring microbial community (phytoplankton and ciliates) was collected with a transparent bottle (15-l capacity, two collections) from ca. 1 m depth. A 250-ml subsample of the water collected was preserved immediately with 10% acidic Lugol's iodine solution for ciliate enumeration (Stoecker et al. 1994). Two 500-ml water subsamples were kept in dark for chlorophyll determination after arrival at the laboratory. The remaining water was transported to the laboratory to be used as natural planktonic community for feeding experiments, which started about 2-3 h after collection.

Zooplankton abundance was assessed by vertical tows, from near bottom (ca. 20 m) to surface, using a hand-hauled 36-cm diameter plankton net (53-μm mesh size). Zooplankton samples were preserved with buffered formaline (4% final concentration). Zooplankton for feeding experiments were collected by slow-speed oblique tows using a Judai-Bogorov plankton net (200 μm mesh) with a 5-10 liter plastic bag as non-filtering cod end to minimize organism damage and reduce sampling stress. Animals were kept in a cooler until arrival to the laboratory.

Phytoplankton biomass was estimated as chlorophyll concentration in two size fractions, < 5μm and > 5μm. Aliquots of respectively 75 and 150 ml of water were filtered onto Whatmann GF/F and 5-μm pore-size polycarbonate membrane filters under low vacuum pressure (< 100 mm Hg), and the filters stored frozen at -80°C until analysis. The size fraction < 5μm was estimated by the difference between total (GF/F) and > 5μm chlorophyll concentrations.

Grazing experiments

The experimental water was carefully poured into a 50 l bucket and reverse-flow screened gently by submerging a 30-cm diameter PVC cylinder fitted with a 100-μm mesh size bottom. By this procedure, predators >100μm were eliminated from the experimental suspension. Once the suspension was ready it was amended with a nutrient mixture (15 μM NH_4Cl and 1 μM Na_2HPO_4 final concentration) to compensate for nutrient enrichment due to zooplankton excretion. The experimental water was left ca. 30 minutes to allow the microplanktonic community to stabilize. Duplicate initial samples were taken for ciliate enumeration (acidic Lugol's iodine solution, 10% final concentration) and for chlorophyll analysis (< 5μm and > 5μm). Then, 620-ml screw-cap Pyrex bottles were gently filled with the water from inside the sieve (< 100μm), taking special care to avoid the formation of bubbles. Each experiment consisted of 3 or 4 experimental bottles with predators (4 to 14 individuals, depending on the species size) and four bottles without predators (controls). The bottles were incubated on a rotating plankton wheel (0.2 rpm) in a temperature-controlled room at similar temperature and light cycle than those of the day of collection.

After 24 hours, the contents of the bottles were gently filtered through a submerged 180-μm sieve and samples for the assessment of ciliate abundance and chlorophyll concentration were treated as described above. The

zooplankters remaining in the 180-µm sieve were quickly and carefully transferred to a glass bowl, counted live under a stereo microscope (dead or in bad condition organisms were not included in later calculations) and then preserved in formaline (4% final concentration) for later determination of size.

Sample processing and calculations

Chlorophyll was analyzed fluorometrically. Filters were placed in tubes with 6 ml of 90% acetone, and chlorophyll allowed to be extracted in darkness for ca. 24 h at 4°C. Subsequently, fluorescence was measured (without acidification) with a Turner-Design fluorometer. Chlorophyll concentration was transformed into phytoplankton carbon using a carbon-to-chlorophyll ratio of 30 (Arin et al. 2002).

Ciliate abundance and diversity were estimated by settling 100-ml samples in Utermöhl chambers for 48 hours. The whole sample was scanned under a Zeiss Axiovert 35 microscope (200x) and cells were identified into broad taxonomic groups and logged in size categories.

In order to consider possible losses of ciliates due to both Lugols' preservation and water sample handling in the computation of cell abundance, we carried out in parallel an attempt to determine the degree of ciliate loss in our counts. To determine the factor of cell loss due to preservation with 10% acidic Lugol's iodine solution, we took twenty aliquots of 1 ml of ciliates from a culture of the ciliate *Strombidium inclinatum* (which concentration was prepared of about 20-25 ciliates ml^{-1}), and counted them live under a microscope. To minimize counting error of live ciliates the counts were repeated four times for each aliquot and average value computed. Counting error was low, with an average CV among replicates of 8 % (range: 4 to 15%). At the same time, three aliquots (50 ml) from the same *Strombidium* culture were fixed with 10% acidic Lugol's iodine solution and then counted under the microscope. Live counting resulted significantly different from dead counting (one-factor ANOVA: $F=34.9$, $P<0.004$) and the effect of preservation by Lugol (10 % final concentration) resulted to reduce the ciliate abundance by a factor of 30%. Accordingly, this factor was used to correct the ciliate abundance of this study.

Moreover, we also explored any possible effect of handling processes and nutrient addition on ciliate abundance in our experiments. For each sampling

date, we compared parallel aliquotes for water samples preserved immediately after collection at sea and those coming from the same water after being transported to the laboratory, sieved, suffering a nutrient addition and gently mixed to fill in grazing bottles (they actually corresponded to start concentrations in grazing experiments). In both cases two 250-ml aliquotes for each treatment were preserved with 10% acidic Lugol's iodine solution. Table 1 shows average values (± 1SE) for both treatments (untreated and treated samples). No significant differences were found between treatments (Two-factor ANOVA, Model II, where sampling date was a random factor and untreated/treated sample a fixed factor; $F_{1,9} = 0.34$, $p > 0.5$), indicating that neither time spent in reaching the lab form the sample site and setting-up the experiment (2-3 hours approximately) nor laboratory handling (addition of nutrients, sieving through a 100-μm mesh, mixing of water and filling of the experimental bottles) affected the ciliate abundance.

Table 1. Ciliate abundance (average, cells ml^{-1} ± SE) of untreated samples (immediately fixed after collection) and treated samples (preserved in about 2-3 hours after sampling, following the addition of nutrient, and the processes of sieving, mixing and filling of bottles). All samples were preserved with 10% acid Lugol's iodine solution.

Date	Untreated	Treated
May 1999	3.6 ± 0.53	3.3 ± 0.15
June 1999	1.7 ± 0.20	1.0 ± 0.10
July 1999	13.2 ± 1.04	15.0 ± 1.24
August 1999	14.0 ± 2.40	14.7 ± 1.66
September 1999	1.1 ± 0.07	1.6 ± 0.05
February 2000	1.5 ± 0.12	1.9 ± 0.20
March 2000	3.8 ± 0.10	4.1 ± 0.85
May 2000	4.3 ± 1.05	3.7 ± 0.12
June 2000	17.5 ± 1.72	12.5 ± 1.82
July 2000	3.9 ± 0.67	3.4 ± 0.07

Ciliate biomass was calculated assuming 0.19 pg C μm^{-3} (Putt & Stoecker 1989). Average ciliate volume for each experiment was estimated from pictures (n=50) taken with a digital photo camera attached to the microscope, and analysed in a Power Macintosh computer with the NIH Image analysis software (National Institute of Health, Bethesda, USA). The contour of the ciliates was outlined, the cell area measured, and the length and width of the

cell automatically estimated assuming an ellipsoidal or spherical shape, depending on the species.

Zooplankton clearance and ingestion rates were calculated according to the equations of Frost (1972). In those cases where ingestion negative values were obtained, we leveled them to zero. Zooplankton biomass (as μg C) was estimated from species-specific length-weight relationships from the literature, assuming, when necessary, that carbon content is 40% of dry weight (Parsons et al. 1984a). The equations employed came from Uye & Sano (1998) for *Oithona* sp., from Uye (1991) for *Paracalanus* sp., from Chisholm & Roff (1990) for *Temora* sp and *Clausocalanus* sp., from Davis & Alatalo (1992) for *Centropages* sp., from Satapoomin (1999) for *Euterpina* sp., from Walve & Larsson (1999) for *Evadne* sp. and from Uye (1982) for *Penilia avirostris* and *Podon* sp. The equation used to determine the biomass of the whole copepod community (used to calculate the trophic impact, see results) is also from Uye (1982).

Prey selectivity (i.e. the ingestion of certain prey in a higher or lower proportion than expected from their field relative abundance) was evaluated in different ways. Although an acute difference in the clearance rates of a predator on different prey within an experiment may be indicative of selective feeding patterns, another visual way to approach selection, however, is the one by plotting the prey contribution to the zooplankton diet against to its availability (**Chapter I**). In this kind of plot, values that fall above the line 1:1 are indicative of prey preference, whereas values falling below the line represent deterrence for the specific prey. We further determined prey selectivity patterns by using the Chesson's index of selectivity α (Chesson 1983):

$$\alpha_k = \frac{r_k / p_k}{\sum_i^n \left(r_i / p_i \right)},$$

where r_k and p_k are respectively the proportion of prey class k in the zooplankter diet and in the field, and n is the number of prey classes. Neutral selection would result in a constant $\alpha_i=1/n$. This index was chosen because is prey density independent. Although this index must be considered qualitative, it permits to determine whether prey items were ingested in higher or lower

proportion of what would be expected due to their relative abundance in the field.

Otherwise stated, arithmetic means ± SE are shown. However, in some cases median values and range are reported instead, due to presence of very extreme values in the lower or upper ends of the range, which made arithmetic means less representative.

Result

Seasonal plankton patterns

Total chlorophyll concentration ranged from 0.28 to 4.38 μg Chl*a* l^{-1} through the studied period. Three phytoplankton blooms were observed: in summer (July 1999: 3.5μg Chl*a* l^{-1}), in winter (February 2000: 4.3 μg Chl*a* l^{-1}) and in spring (April and May 2000: 4.4 μg Chl*a* l^{-1}). On these dates, the chlorophyll fraction > 5μm ($Phyto_{>5\mu m}$) dominated the phytoplankton community (Fig. 1), although concurrent peaks of the chlorophyll < 5μm ($Phyto_{<5\mu m}$) were also observed. On the other dates, the chlorophyll concentration averaged 1.1 ± 0.18 μg l^{-1} and was mainly composed of < 5μm cells. Ciliate abundance averaged 5 (± 1.4 SE) cells ml^{-1} over the year. Higher values were found in summer (14 and 17.5 cells ml^{-1} in August 1999 and June 2000, respectively) and minimum ones from September 1999 to February 2000 (1.3 cells ml^{-1}, on average). The highest ciliate abundance occurred approximately one month after the phytoplankton blooms (Fig. 1).

A minor increase of ciliate concentration also followed the winter phytoplankton bloom of February. The ciliate community was numerically dominated by small (<20 μm length) aloricated ciliates (Fig. 2). Overall, *Strombidium* spp. and *Strobilidium* spp. represented, on average, more than 70% of the ciliate community, while loricated ciliates (tintinnids) represented on average only the 4.4 % of the community along the year. Other numerically important genera were *Mesodinium, Laboea*, *Tontonia*, *Strombidinopsis*, *Askenasia*, *Pleuronema*, *Cyclidium* and *Balanion*. Among these genera, *Mesodinium* was the most abundant, representing, on average, 12 % of the ciliate community along the year.

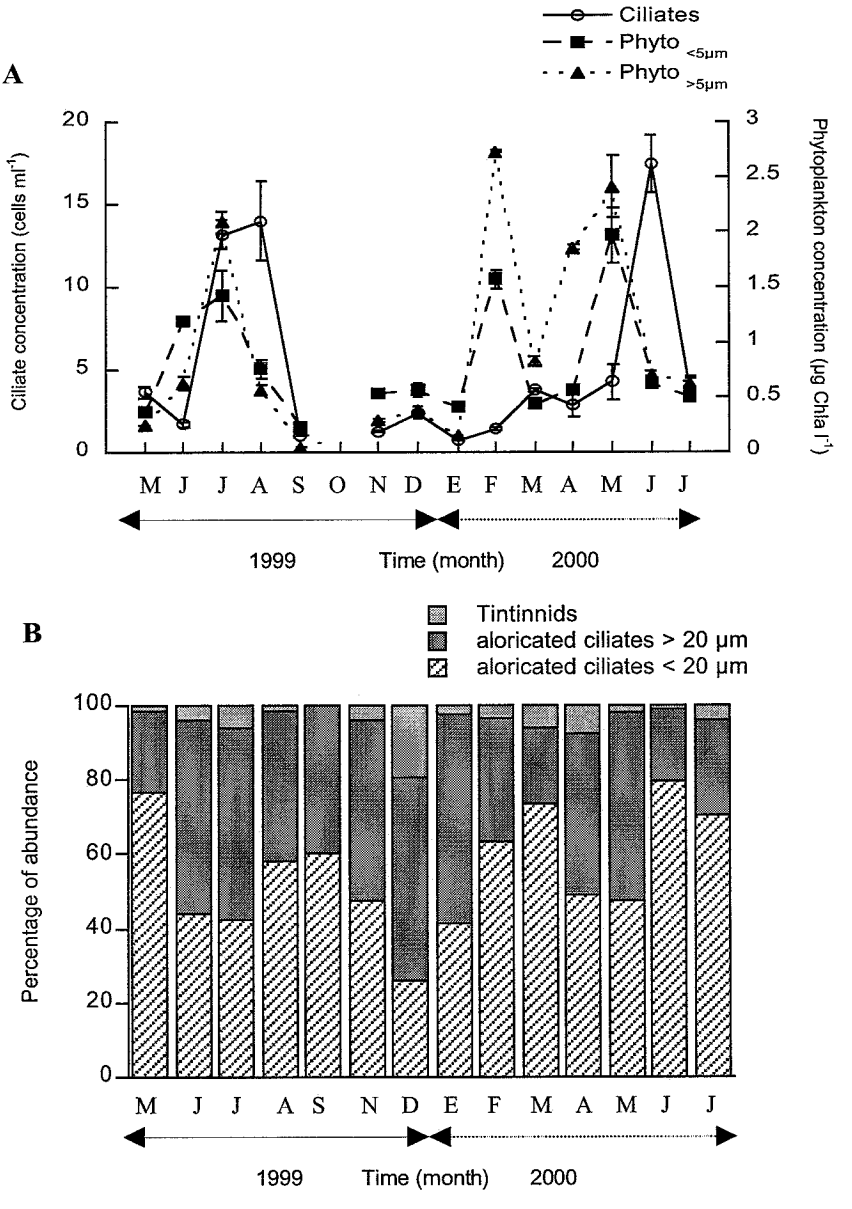

Figure 1: A) Temporal evolution of ciliates and phytoplankton (as chlorophyll < and > 5µm) concentrations at the study site. Means of two replicates (SE) showed. Bars indicate standard errors. October 1999 not sampled. B) Relative abundance (as %) of tintinnid and aloricated ciliates (> 20 and < 20 µm maximum length) trough the studied period.

Feeding experiments

Table 2 provides information on dates, temperature, zooplankton predators and initial phytoplankton and ciliate concentrations in the experiments conducted.

Table 2. List of the experiments conducted. Date, water temperature T (°C), genera of predator studied and initial ciliate (cells ml^{-1}) and phytoplankton abundance (µg Chl*a* l^{-1}) are showed. Arithmetic mean (±1 SE) are given, except for Phyto $_{< 5\mu m}$, where only averages were computed (as Phyto $_{total}$ – Phyto $_{> 5\mu m}$).

Exp #	Date	T	Predator	Ciliates	Phyto$_{<5\,\mu m}$	Phyto$_{>5\,\mu m}$
1	M 99	18.0	*Evadne; Oithona; Paracalanus*	3.0 (0.19)	0.4	0.3 (0.01)
2	J 99	20.7	*Oithona; Paracalanus*	1.0 (0.10)	1.3	0.5 (0.01)
3	J 99	22.2	*Paracalanus; Penilia*	15.0 (1.24)	1.4	2.1 (0.15)
4	A 99	24.0	*Paracalanus*	14.7 (1.66)	0.7	0.6 (0.05)
5	S 99	21.8	*Clausocalanus; Penilia; Temora*	1.61 (0.05)	0.2	0.04 (0.00)
6	F 00	12.5	*Centropages; Euterpina; Podon*	1.9 (0.20)	0.7	1.6 (0.02)
7	M 00	14.0	*Euterpina; Evadne; Podon*	4.1 (0.85)	0.3	0.9 (0.02)
8	M 00	18.0	*Centropages; Oithona*	3.7 (0.12)	1.9	2.8 (0.15)
9	J 00	23.1	*Centropages; Oithona; Euterpina; Penilia*	12.5 (1.82)	0.7	0.7 (0.01)
10	J 00	23.1	*Clausocalanus; Penilia; Oithona; Temora*	3.4 (0.07)	0.6	0.6 (0.07)

In terms of biomass (as µg C l^{-1}), ciliates represented a low percentage of the available potential food resources (i.e. ciliates + Phyto$_{> 5\mu m}$ + Phyto$_{< 5\mu m}$), comprising only 14 (± 4 SE) % of available carbon. Phyto$_{> 5\mu m}$ and Phyto$_{< 5\mu m}$ contributed similarly to the carbon pool, being on average the 43 (± 5.9 SE) % and 43 (± 5.7 SE) % of the available carbon, respectively. However, during the summertime (Exp # 3, 4, 9), due to their high concentration, ciliate importance increased, accounting between 16 and 46% of the biomass of the total

microbial community considered. The biomass of ciliates was not correlated with neither $Phyto_{>5\mu m}$ nor $Phyto_{<5\mu m}$ ($P > 0.8$ in both cases), although both phytoplankton fractions were significantly correlated between them ($r=0.79$, $p < 0.007$, $n=10$). Zooplankton clearance rates showed to be very variable among and within species (Tab. 3).

Table 3. Clearance rates on ciliates, phytoplankton < 5µm and phytoplankton > 5µm by the different copepod and cladoceran species studied. Average values (ml ind^{-1} d^{-1} ± SE) are shown.

Copepods

Exp. #	Species	Ciliates	$Phyto_{<5\mu m}$	$Phyto_{>5\mu m}$
1	*Oithona spp.*	26 (6)	0	1 (1)
1	*P. parvus*	73 (12)	7 (3)	20 (2)
2	*Oithona spp.*	21 (3)	0	1 (0.4)
2	*P. parvus*	58 (6)	1 (1)	24 (5)
3	*P. parvus*	23 (7)	39 (38)	6 (2)
4	*P. parvus*	28 (7)	7 (3)	33 (4)
5	*Clausocalanus*	110 (5)	6 (3)	10 (2)
5	*T. stylifera*	103 (21)	7 (4)	42 (9)
6	*E. acutifrons*	15 (5)	13 (9)	5 (2)
6	*C. typicus*	79 (23)	18 (11)	44 (14)
7	*E. acutifrons*	21 (5)	11 (2)	11 (1)
8	*C. typicus*	76 (6)	0	14 (5)
8	*Oithona spp.*	5 (5)	1 (1)	6 (3)
9	*C. typicus*	90 (6)	0	16 (1)
9	*E. acutifrons*	4 (4)	1 (1)	2 (1)
9	*Oithona spp.*	8 (6)	0	1 (0.2)
10	*Clausocalanus*	37 (7)	6 (3)	6 (1)
10	*Oithona spp.*	17 (14)	7 (7)	1 (1)
10	*T. stylifera*	24 (5)	16 (2)	30 (2)

Cladocerans

Exp	Species	Ciliates	$Phyto_{<5\mu m}$	$Phyto_{>5\mu m}$
1	*E. spinifera*	33 (3)	0	1 (1)
3	*P. avirostris*	23 (2)	0	6 (4)
5	*P. avirostris*	17 (10)	15 (2)	0
6	*Podon spp.*	23	64 (26)	2 (2)
7	*E. spinifera*	4 (2)	16 (6)	0.4 (0.4)
7	*Podon spp.*	0	26 (5)	4 (2)
9	*P. avirostris*	18 (11)	6 (3)	10 (1)
10	*P. avirostris*	8 (1)	2 (2)	4 (2)

Clearance rates on ciliates ranged from 4 to 110 ml ind^{-1} d^{-1} for copepods (median: 26.4 ml ind^{-1} d^{-1}) and 4 to 33 ml ind^{-1} d^{-1} for cladocerans (median: 17.6 ml ind^{-1} d^{-1}). Copepod clearance rates on phytoplankton were usually lower than on ciliates, with median values of 6.3 ml ind^{-1} d^{-1} for $Phyto_{<5\mu m}$ and 10 ml ind^{-1} d^{-1} for $Phyto_{>5\mu m}$. Cladoceran clearance rates on phytoplankton were, in general, lower than on ciliates as well. For *Evadne spinifera* spp. and *Penilia avirostris* feeding rates were up to 16 ml ind^{-1} d^{-1} on $Phyto_{<5\mu m}$ and up to 10 ml ind^{-1} d^{-1} on $Phyto_{>5\mu m}$, while clearance rates up to 33 ml ind^{-1} d^{-1} were observed on ciliates (Tab. 3). Among cladocerans, *Podon* sp. presented a contrasting feeding pattern, with apparent higher clearance rates on $Phyto_{<5\mu m}$ than on ciliates. Table 4 shows the ingestion rates for the different experiments.

Table 4. Ingestion rates on ciliates, phytoplankton < 5µm and phytoplankton > 5µm by the different copepod and cladoceran species. Average values (ng C ind^{-1} d^{-1} ± SE) are shown. Average predator carbon estimates are also indicated.

Copepods

Exp. #	Species	Body C content	Ciliates	$Phyto_{<5\mu m}$	$Phyto_{>5\mu m}$
1	*Oithona spp.*	574	164 (34)	0	13 (13)
1	*P. parvus*	1975	387 (45)	94 (37)	230 (20)
2	*Oithona spp.*	573	26 (3)	0	12 (7)
2	*P. parvus*	1932	62 (4)	32 (19)	372 (62)
3	*P. parvus*	2298	684	654 (614)	821 (310)
4	*P. parvus*	2343	539	118 (60)	629 (53)
5	*Clausocalanus spp.*	2257	61 (1.4)	40 (21)	16 (3)
5	*T. stylifera*	6699	61 (9)	47 (24)	59 (10)
6	*E. acutifrons*	682	58 (18)	238 (157)	230 (116)
6	*C. typicus*	2940	258 (57)	351 (211)	1987 (533)
7	*E. acutifrons*	682	54 (11)	123 (21)	342 (15)
8	*C. typicus*	1633	169 (4)	0	1327 (446)
8	*Oithona spp.*	661	17 (17)	79 (79)	573 (297)
9	*C. typicus*	5228	1507 (50)	0	405 (13)
9	*E. acutifrons*	689	118	25 (25)	63 (21)
9	*Oithona spp.*	509	200	0	30 (6)
10	*Clausocalanus spp.*	1399	180 (20)	100 (57)	205 (34)
10	*Oithona spp.*	987	84 (58)	112 (112)	29 (29)
10	*T. stylifera*	3144	132 (26)	273 (25)	853 (47)

Table 4 continue

Cladocerans

Exp. #	Species	Body C content	Ciliates	Phyto $_{<5\,\mu m}$	Phyto $_{>5\,\mu m}$
1	*E. spinifera*	595	208 (14)	0	16 (16)
3	*P. avirostris*	1001	696 (66)	0	812
5	*P. avirostris*	1053	13 (6.8)	96 (11)	0
6	*Podon spp.*	2838	82 (58)	1078 (433)	85 (85)
7	*E. spinifera*	670	10 (7)	173 (66)	12 (12)
7	*Podon spp.*	2838	0	263 (45)	139 (75)
9	*P. avirostris*	1408	428	103 (46)	260 (32)
10	*P. avirostris*	1298	50 (9)	35 (35)	142 (74)

Values were very variable, from not significant feeding up to ingestion rates of 2000 ng C ind^{-1} d^{-1}, representing daily food rations between 2 and 101% and between 10 and 151 % of the body carbon for copepods and cladocerans, respectively (Fig. 3). For the case of copepods, the total carbon intake was correlated with the total carbon availability (i.e. ciliates + $Phyto_{>5\mu m}$ + $Phyto_{<5\mu m}$) in the experiments ($r = 0.81$, $p < 0.001$, $n=19$). Ciliate carbon contributed between 2.5 and 92.4% of the total C intake by copepods (median 37%, Fig. 3).

When considered separately, copepod ingestion rates (as % body C ingested per day) of ciliates and $Phyto_{>5\mu m}$ were significantly correlated with their corresponding prey abundance (respectively, $r = 0.686$, $p < 0.002$, $n=19$; and $r = 0.884$, $p < 0.001$, $n=19$); however, no correlation ($p > 0.8$) was found between the ingestion and concentration of $Phyto_{<5\mu m}$. The copepod ingestion rate of ciliate carbon was not correlated with neither the carbon ingestion rates of $Phyto_{>5\mu m}$ nor that of $Phyto_{<5\mu m}$ ($p > 0.3$). For cladocerans, the few number of observations precluded robust statistics. Then, the total carbon intake was also correlated with the total carbon availability (i.e. ciliates + $Phyto_{>5\mu m}$ + $Phyto_{<5\mu m}$; $r = 0.928$, $p < 0.001$, $n=8$), but the significance of this relationship disappeared when one influential observation (Expt. #3, *Penilia avirostris*) was removed from the analysis ($r = 0.702$, $p > 0.07$, $n=7$). The cladoceran ingestion rates on ciliates and $Phyto_{>5\mu m}$ were also correlated with the respective concentrations of ciliates and $Phyto_{>5\mu m}$ (respectively $p < 0.002$ and $p < 0.05$), but again these statistical relationships disappeared after removing the influential observation mentioned above ($p > 0.05$ in both cases).

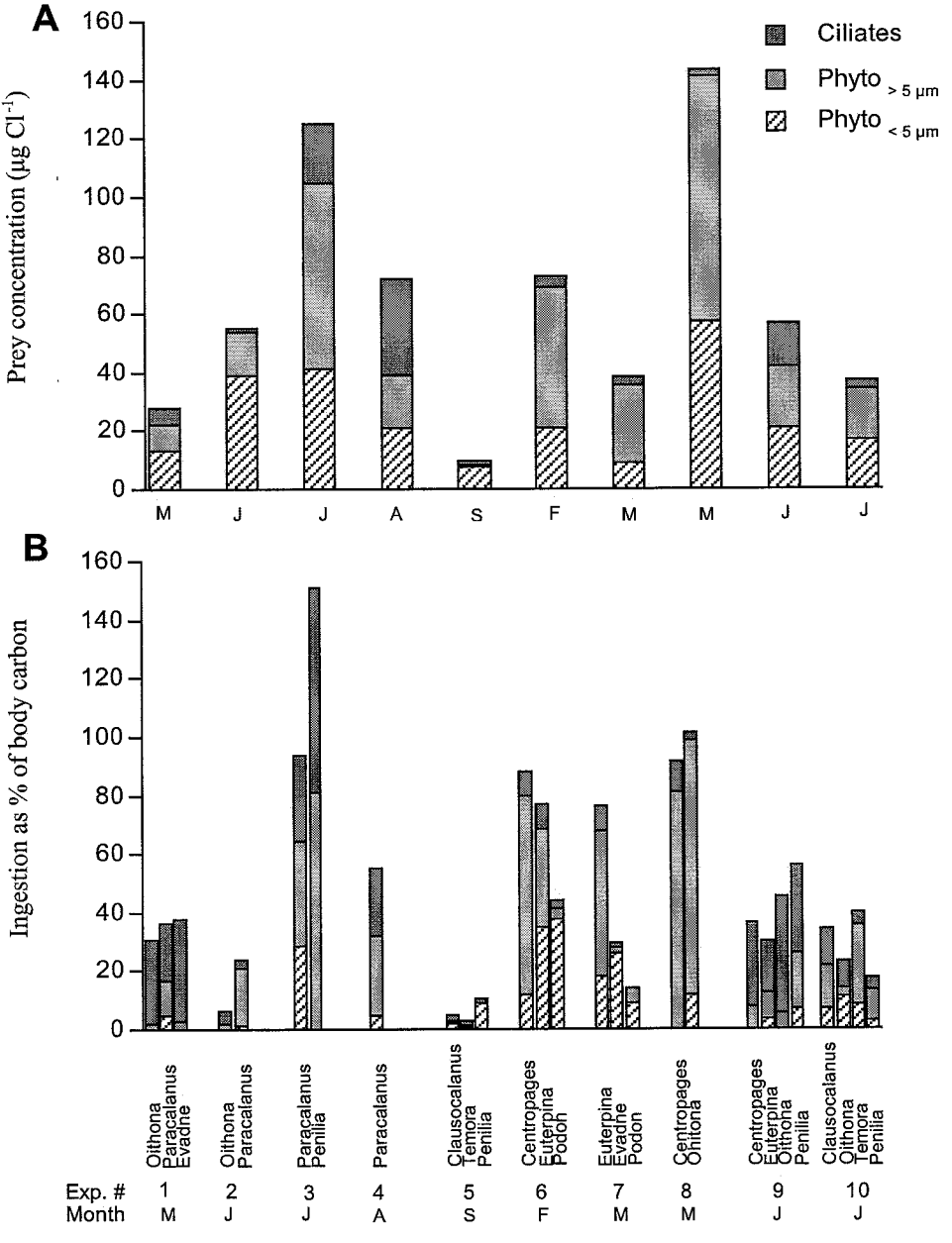

Figure 3. A) Relative prey concentration (µg C l^{-1}) available to the studied species at each experiment. B) Relative prey ingestion by zooplankton species expressed as % of body carbon ingested daily.

Selection patterns

Figure 4 shows the prey contribution to the zooplankton diet as function of its relative availability. In spite of the dispersion of the data, it appears obvious that overall the majority of zooplankton species studied showed feeding selection for ciliates (i.e. most values are above the 1:1 line).

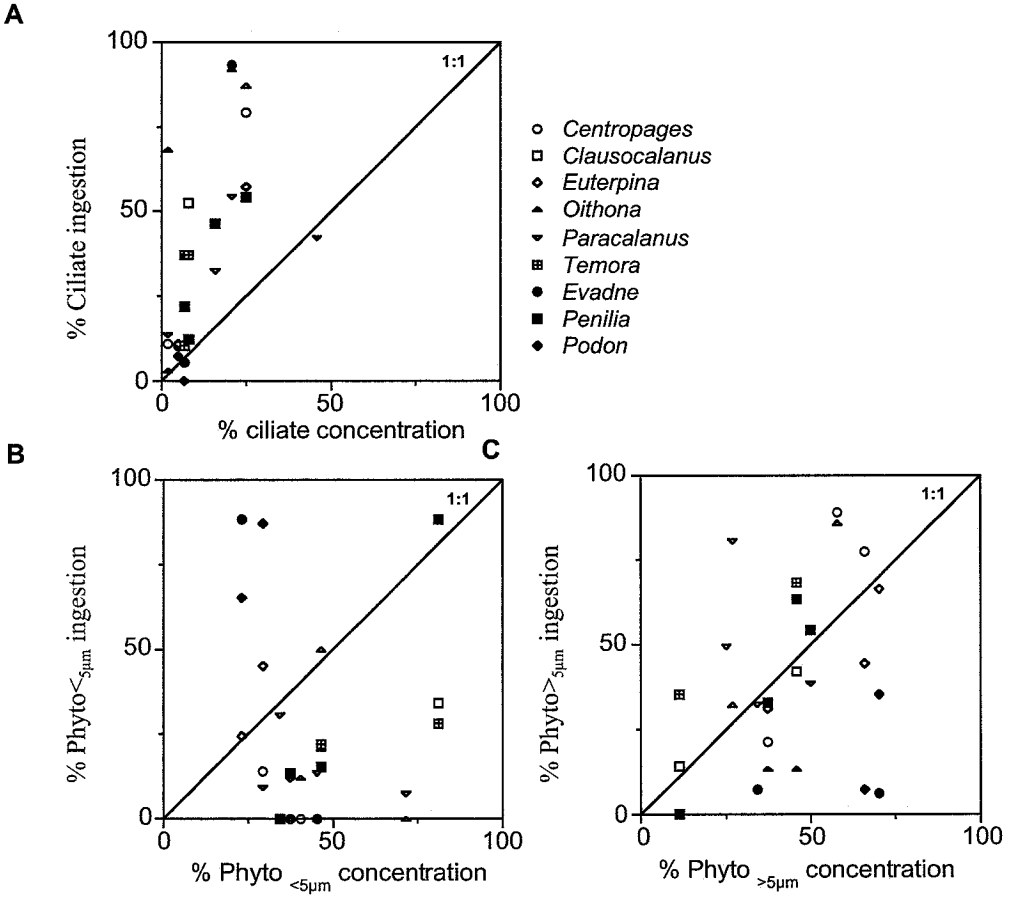

Figure 4. Scatterplots of the percentage of specific prey biomass abundance, as a function of concentration (as %) of the same prey in the zooplankton diet. Values above the 1:1 line indicate feeding selection for that prey. A) Ciliates, B) $Phyto_{<5\mu m,}$ C) $Phyto_{>5\mu m}$.

On the contrary, $Phyto_{<5\mu m}$ seemed to be consumed at lower rates than expected from its relative abundance (i.e. negative selection), whereas for $Phyto_{>5\mu m}$ there was a scattered response and several species appeared to change selection patterns along the year (see below). One must be aware, however, that selection may depend in great manner not only on the proportion of prey but also on the absolute value of prey availability, which could partially explain the variability in response within and between species.

Figure 5 presents a visual summary of the results previously considered in combination with prey absolute concentration and a selectivity index (Chesson's index). Despite the majority of the species were studied in situation of different relative prey abundance (only ciliate versus $Phyto_{>5\mu m}$ concentration showed in the graphs), it appears that, except for one experiment with *Podon* sp (#7) and one experiment with *Paracalanus parvus* (# 4), all studied species always selected for ciliates. In several occasions, copepods selected also for $Phyto_{>5\mu m}$ at the same time than for ciliates. Furthermore, neither copepods nor cladocerans selected for $Phyto_{<5\mu m}$, alone, whereas the three species of cladocerans showed occasionally selection preference for the small phytoplankton fraction and ciliates together.

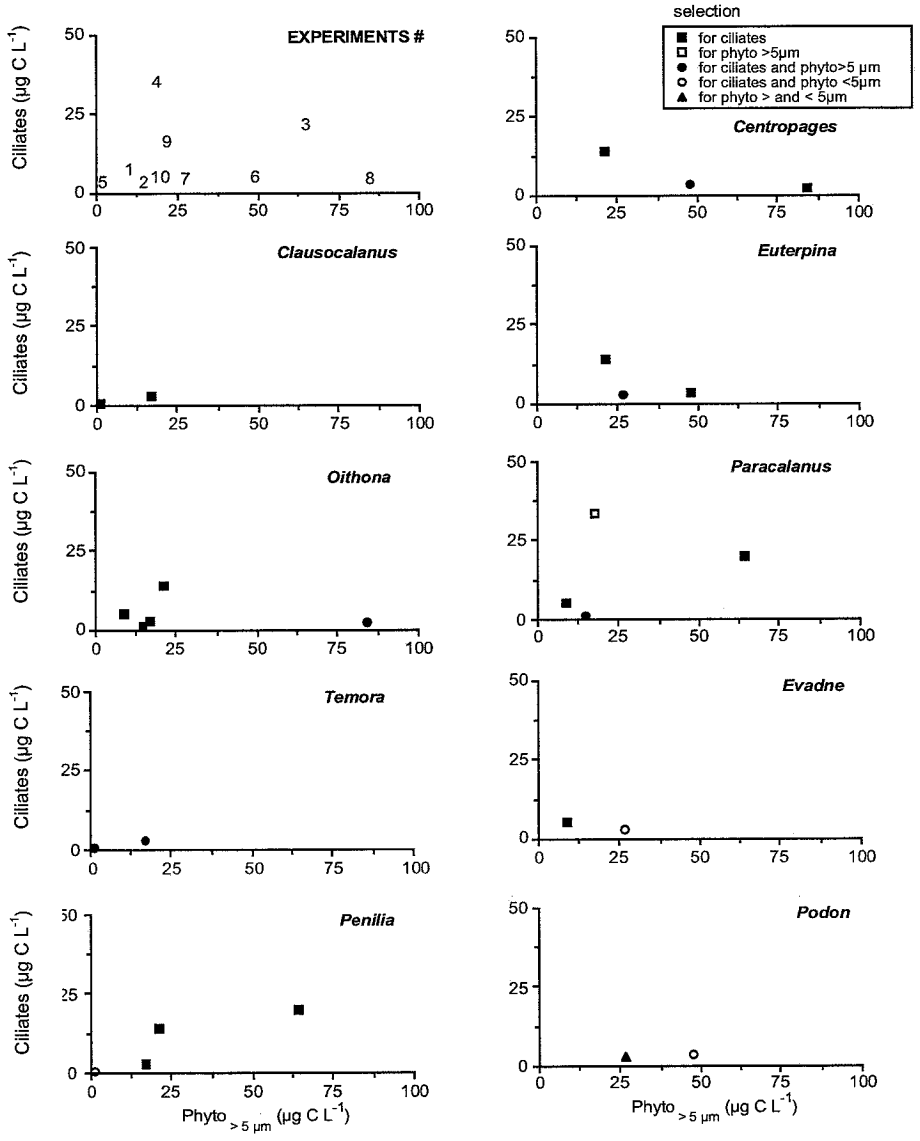

Figure 5. Chesson's selectivity index for each crustacean species as a function of prey availability. For the sake of the interpretation, the graph on the upper left corner shows the relative abundance of $Phyto_{>5\mu m}$ and ciliates in the experiments. The number of each experiment is positioned as a function of the relative prey availability. The other graphs show the selection patterns for each species. In these graphs symbols are placed in the corresponding positions for each experiment (as in the upper left corner graph). Only positive selection according to the Chesson's index are shown.

Crustacean zooplankton grazing impact

Data on clearance rates (Tab. 3) and abundance of copepods and cladocerans (Tab. 5) were used to assess the grazing impact of crustacean zooplankton community on the ciliate and phytoplankton standing stock. Zooplankton predation was computed as the copepod or cladoceran abundance multiplied by their clearances rates. An average copepod and cladoceran biomass was estimated from the measure of the prosome length of about 50 individuals of the community. Then, we computed both cladoceran and copepod ensemble clearance rates for each sampling date from the empirical values obtained for each dominant species (as ml $\mu g\ C^{-1}\ d^{-1}$), taking into account their relative abundance; for those species (not dominant) not employed in the experiments, we assigned them the average clearance obtained from the corresponding dominant ones. The total crustacean zooplankton (copepods and cladocerans) impact on the ciliate and phytoplankton standing stock was determined assuming a homogenous ciliate distribution in the water column. Crustacean zooplankton daily cleared from 0.5 to 7% (median: 2.4 %) of the ciliate standing stock through the period of study (Tab. 5), whereas the impact on phytoplankton standing stock was lower ($Phyto_{<5\mu m}$: median at 0.17%, range: 0.01-0.86%; $Phyto_{>5\mu m}$: median at 0.34%, range: 0.02-0.84%).

Table 5. Crustacean and ciliate abundance utilized to calculate the grazing impact (%). Ciliate standing stock was determined assuming a homogeneous distribution in the water column. The zooplankton predation was computed as the copepod or cladoceran abundance (ind m^3) multiplied by their clearance rates (Tab 2, see Methods section). The grazing impact is calculated as the percentage of ciliate standing stock ingested by the crustacean community in a day (values x 10^4 cells m^{-3}).

Exp. #	Cop. abundance (ind m^3)	Clad. abundance (ind m^3)	Ciliate st. stock (x 10^4 cells m^{-3})	Grazing Impact (%)
1	2310	424	300	5
2	3961	217	100	7
3	1262	196	1500	0
4	7697	590	1470	3
5	5140	421	160	6
6	1324	25	190	2
7	521	165	410	1
8	3026	12	370	4
9	2109	335	1020	1
10	2068	105	340	1
median	*2210*	*206*	*355*	*2.4*

Discussion

Most of crustacean zooplankton species, traditionally considered herbivores, incorporate protozoan prey to their diets (Ohman & Runge, 1994, Turner and Graneli 1992, Atkinson 1996, Calbet et al. 2000). It would be otherwise surprising that zooplankters would not take advantage of such available food source, especially in highly diluted environments, e.g. the open ocean. Then, omnivory favours ecological success by complementing the diet with supplementary nourishment and by representing extra food supplies under scarcity of plant food.

Our experiments along a year cycle permitted us to explore the trophic interactions between ciliates and a series of zooplankton species under quite different trophic situations, varying from communities dominated by $Phyto_{>5\text{-}\mu m}$ (experiments #3, #6 and #8), to communities with similar presence of both $Phyto_{>5\text{-}\mu m}$ and ciliates (experiments #4 and #9), to periods of very scarce availability of both prey (experiment #5) and to transitional periods with low phytoplankton and ciliate concentrations (experiments #1, #2, #7 and #10). In this study, mean and maximum values of ciliate and phytoplankton standing stocks found, as well as ciliate composition, were typical of Mediterranean coastal waters (Vaqué et al. 1997, Modigh 2001, Perez et al. 1997, Pitta & Giannakourou 2000).

Contribution of ciliates to zooplankton food ration

The total carbon ingestion rates for copepods observed in this study were in general not very low, with 50% of data comprising daily food rations between 24 and 77% of the body carbon. However, these values were still quite below maximum satiation values determined in the laboratory for similarly sized copepods (*Acartia tonsa*, 180% body C d^{-1}, Kiørboe et al. 1985; *Temora longicornis*, 170% body C d^{-1}, Klein Breteler et al. 1990), indicating that copepods appeared to be at least moderately food limited. Maximum carbon intakes (77-101% body C d^{-1}) occurred in periods where $Phyto_{>5\mu m}$ dominated the carbon standing stock (experiments #3, #6 and #8), whereas minimum intakes occurred in situations of very scarce availability of both phytoplankton and ciliates (experiment #5). The significant correlation between prey availability and copepod ingestion rates (except for $Phyto_{<5\mu m}$) also supports the view that copepods were food limited in most occasions. Regarding cladocerans, their daily C rations also indicated food limitation throughout

most periods of the study (50% of values between food rations of 15 and 53% of body C d^{-1}). We cannot exclude, however, that other possible food sources not considered in the present study (e.g. other heterotrophic prey and/or detritus) may have contributed to increase our daily food rations estimates.

In spite of representing only 9% (range: 2-46%) of total prey carbon available, ciliates highly contribute to the total carbon intake of copepods (median: 37 %, range: 3-93%) and of cladocerans (median: 17 %, range: 0-93%). These values are within the range of estimates found in the literature, which varies from studies where ciliates did not contribute much to diet (percentage of total carbon ingestion: <10%, Tiselius 1989; <6%, Irigoien et al. 1998) to moderate contributions (5-29.2% of diet, Vincent & Hartmann 2001; 20-29% Verity & Paffenhöfer 1996; 0.4-49% Calbet et al. 2002a; 22-31%, Zeldis et al. 2002) and to high contributions (16-100%, Fessenden & Cowles 1994). Although prey contribution to the daily food rations is usually considered in terms of carbon, it is obvious that the ingestion of a prey is not only a question of one element. For instance, it is known that copepods may tend to maximize nitrogen ingestion (Houde & Roman 1987, Cowles et al. 1988). When contributions to food rations are computed in terms of nitrogen, due to ciliate higher nitrogen contents in comparison with phytoplankton ($C:N_{ciliates}$=3.5, Stoecker & Capuzzo 1990; $C:N_{algae}$=6.6, Parsons et al. 1984b), a higher role of ciliates appears, supplying on median 51 % and 34 % of the nitrogen intake of copepods and cladocerans, respectively. It appears that, nutritionally, ciliates are major contributors to the diet of zooplankton and consequently, more attention should be paid to their effects on zooplankton metabolism. It has been speculated (Stoecker & Capuzzo 1990; Wickham 1995) that ciliates could be a qualitatively rich source of proteins and amino acids, and that they could contain some polyunsaturated fatty acids (PUFA), considered important in the reproduction of crustaceans. However, the literature in ciliate biochemical composition is very scarce. A recent study has shown that some ciliates (i.e., *Mesodinium pulex*) can contain high levels of PUFA, and that the presence of these substances seems to affect hatching processes more than to increase egg production rates in copepods (**Chapter 3**). However, further research is still needed to better assess the biochemical composition of ciliates and its relationship to zooplankton nutrition and reproduction.

Prey selection patterns and zooplankton feeding behavior plasticity

Most predators exhibit prey selectivity as an optimizing strategy to warrant their survival and reproduction. The economics of prey choice depend on factors such as the energetic (nutritional) value of prey, handling time and encounter rate (searching time) (Krebs & Davies 1993), although the actual prey selectivity pattern will depend also on other factors such as the ability of prey to avoid predation. In our study, there is an overall positive selection for ciliates by crustacean mesozooplankton, corroborating previous studies, which rendered similar results (e.g. Fessenden & Cowles 1994, Levinsen et al. 2000). The reasons for this selection upon ciliates are not completely understood. One may expect organisms to tend to optimize the ingestion of food and to minimize the energetic costs of capture. As mentioned above, consuming ciliates might signify a higher intake of nutritious organic matter than that obtained by consuming algae (excluding, perhaps, autotrophic dinoflagellates). On the other hand, motility and escape reactions that some ciliates present may increase the energetic cost of capture (Jonsson & Tiselius 1990; Burns & Gilbert 1993) and affect capture efficiency (**Chapter IV**).

Optimal foraging theory predicts that a predator may switch from feeding on diverse prey (no preference) to specialize on single "good" prey when the realized availability (encounter) of it increases. Then, the relative proportion of ciliates *vs.* phytoplankton must generate a trade-off between energetic gains and costs of selective feeding on ciliates. We expected to observe switching responses between diets based on phytoplankton and those based on ciliates as a function of their relative abundance. However, this switch, if present, was only suggested in a case (*Oithona* spp.) and appear to be highly species specific (i.e. *Centropages typicus*, *Euterpina acutifrons, Paracalanus parvus* and *Penilia avirostris* fed preferentially on ciliates in spite of high abundance of phytoplankton). Switching responses have been previously suggested in the literature (e.g. Fessenden & Cowles 1994, Levinsen et al. 2000). Such feeding plasticity could be interpreted as an adaptive response to situations of low phytoplankton availability, omnivory being unnecessary under phytoplankton blooms conditions (Fessenden & Cowles 1994). However, such responses are not on-off responses, and they may imply a change in the prey relative contribution to diet and not necessarily the exclusion of a determined prey item.

Our data on marine cladocerans are attractive in the sense of the scarcity of knowledge on their feeding behavior. We observed selection for ciliates by *Penilia avirostris* while the few evidences up to date, based on laboratory experiments, indicated they are adapted to feed unselectively on small particles (Paffenhöfer & Orcutt 1986, Turner 1988). More surprising are the results for *Evadne spinifera* and *Podon* spp, feeding preferentially on $Phyto_{<5\text{-}\mu m}$ in most of the experiments, when these genera are typically described as raptorial feeders capable of capturing prey in a size range of 20-170 μm (Egloff et al. 1997 and references therein). In spite of the appealing of presenting them as a microphagous consumer, we cannot disregard that artifacts due to interactions within the microbial community (trophic cascades) could be partially responsible for the results. Certainly, more studies are needed to better understand marine cladocerans dietary preferences, particularly because they must have a significant impact on the microzooplankton communities of stratified oligotrophic waters (Calbet et al. 2001).

Zooplankton grazing impact

Despite crustacean zooplankton largely predated on ciliates, their grazing impact on the ciliate community appeared to be low in most cases, only 2 % of the ciliate standing stock being ingested in median (over a yearly cycle of experiments) (Tab. 5). The studies of Dolan (1991) and Fessenden & Cowles (1994) reported episodes of daily predation pressures up to 45 and 200% of ciliate standing stock. However, other previous studies, conducted in very different environments and different zooplankton communities, yielded conclusions similar to ours, with daily grazing impacts on ciliate standing stocks in the order of 5% (Lonsdale et al. 2000, Atkinson et al. 1996, Atkinson 1996, Batten et al. 2001, Calbet et al. 2002a, Zeldis et al. 2002). In those studies, and in ours as well, feeding rates on ciliates were high, the low grazing impact appearing to be related to the low ciliate abundance in the field. Our results show higher grazing impact when ciliate concentration was not especially high, when the alternate food source was scarce, as well as when crustacean predators were particularly abundant. All this evidence suggests that the top-down control exerted by zooplankton might manifest mainly in those periods when ciliates are the almost exclusive prey. In periods of low ciliate abundance, but presence of alternate prey mesozooplankton would not exert any relevant pressure on them.

As a final remark, we should draw the attention to the fact that other factors,

not fully considered neither in our study nor in other previous studies, could potentially enhance the role of zooplankton in exerting a predation control on ciliate communities. First, most studies have contemplated only copepods as potential predators, while other zooplankters, with major or minor dominance, might be also able to predate on them (e.g. cladocerans, this study). Within copepods, although small copepodites and nauplii are able to feed on ciliates, they are usually undersampled (Calbet et al. 2000, Gallienne & Robins 2001) and negligently contemplated in budgets (but see Dolan 1991, where *Acartia tonsa* nauplii can account for up to 200% of the ciliate standing stock in Chesapeake Bay; and Merrell & Stoecker 1998, where *Eurytemora affinis* nauplii can account up to 56% of total copepod grazing impact on ciliates respectively). A second factor to be considered is the attribute of plankters to aggregate in patches (Mackas 1985, Davis et al. 1991, Montagnes et al. 1999) and the ability of copepods to find them and remain in them (Saiz et al. 1993; Tiselius 1992). Most evaluations of grazing impact on ciliates are based under the assumption of homogenous distribution of predators and prey, whereas heterogeneous distributions can render much higher impacts (Mullin & Brooks 1976). Finally, standard incubations to determine grazing rates of zooplankton are conducted under conditions of absence of small-scale turbulence, while one ubiquitous characteristic of the oceans is the presence of turbulence. Saiz & Kiørboe (1995) and Caparroy et al. (1998) found that moderated turbulence levels enhanced up to three times the feeding rates of *Acartia tonsa* and *Centropages typicus* on ciliates. Actually, Saiz & Kiørboe (1995) and Kiørboe et al. (1996) proposed that turbulence might induce a change in selective patterns, shifting copepod feeding behavior into a ciliate diet. The consideration of this turbulence enhancement of feeding on ciliates, if extended to other zooplankters, would drastically increase the predation pressure on them.

CHAPTER III

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Chapter III

Nutritional quality: relationship between copepod reproduction and prey fatty acid composition under heterotrophic and autotrophic diets

Introduction

Marine pelagic systems are characterized by the presence of a wide variety of autotrophic and heterotrophic species, which represent potential food items for omnivorous copepods. Copepod diets have been shown in recent years to be much more diverse than traditional concepts based on the classical food chain implied. Special interest has been devoted to the role of large heterotrophic protists (ciliates and dinoflagellates) in the diet of copepods, because they have been proposed as an intermediate link between the microbial loop and higher trophic levels (Sherr & Sherr 1988, Verity & Paffenhöfer 1996). In fact, several studies have shown that copepods have high clearance rates on microzooplankton (i.e. ciliates and heterotrophic dinoflagellates) compared to those on phytoplankton, indicating positive selection for microzooplankton (e.g. Dolan 1991, Fessenden & Cowles 1994, Zeldis et al. 2002).

Although in most environments microzooplankton have been shown to be an important contributer to the carbon and nitrogen food ration of copepods (Turner & Anderson 1983, Stoecker & Sanders 1985, Merrell & Stoecker 1998), very few investigations have examined their actual contribution to copepod reproduction, and evidence is often indirect. For instance, the frequent lack of correlation observed in many field studies between in situ growth rates of zooplankton and the concentration of phytoplankton (e.g. Dam et al. 1994, Ohman & Runge 1994, Saiz et al.1999, Calbet et al. 2002) has been used to argue that ciliates may contribute to zooplankton diet. Some field studies have also found correlations between ciliate concentrations and copepod egg production (Kleppel et al. 1991, White & Roman 1992). Stoecker and Egloff (1987) provided more direct evidence from the laboratory, by observing that egg production rates (EPR) of *Acartia tonsa* were enhanced in the presence of ciliates.

In our opinion, further insight into the role of heterotrophic prey on copepod diet requires to considerations of its nutritional value and, consequently, its biochemical composition. *A priori* one may expect that differences in metabolism between autotrophism and heterotrophism may result in different biochemical composition of protists. Unfortunately, knowledge of the biochemical composition of planktonic heterotrophic protists is very scarce. Conventional stoichiometric theory based on carbon and nitrogen limitation would suggest a higher nutritional quality of ciliates compared with autotrophs (Stoecker & Capuzzo 1990).

However, recent studies including other indicators of nutritional food quality (e.g. fatty acid composition) appear to be contradictory. For example, Ederington et al. (1995) observed that the bacterivorous ciliate *Pleuronema* sp. supplied as food resulted in lower copepod EPR than the diatom *Thalassiosira weissflogii*, and related this effect to the ciliate's lack of 20:5ω3 (eicosapentaenoic acid, EPA) and 22:6ω3 (docosahexaenoic acid, DHA) fatty acids. It is unclear, however, how representative the bacterivorous scuticociliate *Pleuronema* might be of truly marine planktonic ciliates (Vaqué et al. 1997, Pitta & Giannakourou 2000).

The main goal of the present study has been to determine the nutritional value of heterotrophic prey (ciliates and dinoflagellates) for copepod reproduction. We have compared the egg production efficiency (EPE; egg production/ingestion) and egg viability of the copepod *Acartia tonsa* under heterotrophic and autotrophic diets, and analyzed the role of fatty acid composition to explain any differences observed. Furthermore, feeding the heterotrophic cells with different prey, we have also explored whether or not the fatty acid composition of heterotrophic organisms depends on the fatty acid composition of their own food and how their ability (or inability) to synthesize copepod essential fatty acids affects their nutritional value as food for copepods.

Materials and methods

Prey culture

Batch cultures of the autotrophic flagellate *Marsupiomonas pelliculata* and dinoflagellates *Heterocapsa rotundata*, *Gymnodinium sanguineum*, the cryptophyte *Rhodomonas salina* and the diatom *Thalassiosira pseudonana*

were grown on either L/1 or F/2 medium (Guillard & Hargraves 1993) under 12h:12h light:dark cycle.

Three heterotrophic prey were tested: the ciliates *Strombidium inclinatum* and *Mesodinium pulex* (clone CPH-0006), and the dinoflagellate *Gymnodinium dominans*. Batch cultures of these heterotrophs were grown under different diets in 750 ml flat culture bottles. *Strombidium inclinatum* was grown either on *Marsupiomonas pelliculata* or on natural bacteria developing on wheat-grain; *Mesodinium pulex* was grown on *Heterocapsa rotundata*, while *Gymnodinium dominans* was fed either *Thalassiosira pseudonana* or *Rhodomonas salina*. Cultures of herbivorous heterotrophs were kept under similar light conditions as algae, and were diluted and fed daily. *Strombidium inclinatum* grown on bacteria were kept in darkness and diluted when necessary.

Determination of volume and carbon and nitrogen content of prey

For each of the experiments conducted (see below), samples were taken for size determination of prey. Prey volume was converted to cell carbon and nitrogen contents using factors available in the literature or estimated by us.

1. Heterotrophs. Volume of heterotrophic prey was calculated by direct microscopic measurement (200-400X, n=50) of acid Lugol's iodine preserved samples (2% final concentration) and assuming simple geometric formulae (ellipsoid for *Strombidium inclinatum* and *Gymnodinium dominans*; sphere for *Mesodinium pulex*). For the estimation of the elemental composition of *Gymnodinium dominans*, we used the equations pg C $cell^{-1}$ = 0.760 × $volume^{0.819}$ and pg N $cell^{-1}$ = 0.118 × $volume^{0.849}$ provided by Menden-Deuer & Lessard (2000), where the volume is expressed in μm^3. Carbon and nitrogen conversion factors used for ciliates were respectively 0.19 pg C μm^3 and 0.04 pg N μm^3 (corrected for cells preserved in 2% acid Lugol's iodine) according to Putt & Stoecker (1989). Cell losses of both ciliate species due to fixation in 2% acid Lugol's iodine were also estimated to correct ciliate abundance during the experiments. To do that we prepared a suspension of 14-20 ciliates ml^{-1} and compared live counts (1 ml aliquots, n=20) under the stereomicroscope with 2% acid Lugol's iodine preserved samples processed using our standard counting protocol (see below). In our test, the effect of the fixative reduced the abundance of *Strombidium inclinatum* by a 25% (t-Test, $p < 0.05$) while no

effect was observed for *Mesodinium pulex*. Accordingly, only *S. inclinatum* abundance in the experiments was corrected for fixation losses.

2. Autotrophs - Volume of *Rhodomonas salina* was estimated on live cells using an electronic particle counter (Multisizer Coulter Counter). For *Gymnodinium sanguineum* direct microscopic measurement (200-400X, n=50) of relevant lengths in acid Lugol's iodine preserved cells (2% Lugol's final concentration) were taken and volume estimated assuming an ellipsoid shape.

Elemental composition (C, N) of *R. salina* and *G. sanguineum* was estimated by filtering a certain volume of the algal cultures on glass fiber filters (Whatman GF/A), which were kept dried until they were processed with a Carlo-Erba CHN analyzer.

The carbon content of *Acartia tonsa* eggs was estimated by filtering about 5000 eggs on glass fiber filters (Whatman GF/A, n=3), which were kept dried until analysis with a Carlo-Erba CHN analyzer. In parallel, the same volume of eggs (n=3) was fixed in acid Lugol's iodine and counted. A content of 47.6 ng C egg^{-1} and 11.3 ng N egg^{-1} was obtained, comparable with previous estimates for the same species (Kiørboe et al.1985).

Lipid analysis

Samples for lipid analyses were collected when cultures were in exponential growth. In the case of *Heterocapsa rotundata*, *Rhodomonas salina*, *Marsupiomonas pelliculata*, *Gymnodinium sanguineum* and *Gymnodinium dominans*, cells were concentrated by filtering known volumes onto pre-combusted GF/F filters, which were processed as described below.

Ciliates were cultured for weeks with the same diet and abundant supply of food. The day prior to sampling for lipid analysis, the ciliate cultures were fed with a very small amount of food to reduce any interference in the analysis due to the presence of the prey in the water. Ciliates were deliberately not starved before lipid analysis since it could have represented an artifact. As ciliates are very fragile and might not resist a low-pressure filtration, they were concentrated (up to 4000-6000 cell ml^{-1}) either by their phototactic (*Strombidium inclinatum*) or rheotactic (*Mesodinium pulex*) response and washed twice with filtered seawater. Aliquots of all the concentrated samples

were preserved in 2% acid Lugol's iodine and counted under a microscope to estimate cell abundance.

The sample for lipid analyses (3 ml) was placed in a test tube and lipids extracted at -20°C for at least 24 hours in CH_2Cl_2/methanol under argon atmosphere, where the final ratio of the extraction mixture was 8:4:3 (v:v:v) CH_2Cl_2/methanol/water according to Christie (1989). The extraction procedure of Folch et al. (1957) was followed. A known amount of C_{23} fatty acid was added to the sample. Fatty acids were transmethylated with BF_3 in methanol (14%) to form fatty acid methyl esters (FAME). The FAME sample was injected into a gas chromatograph (Hewlett-Packard 5809A, with a Omegawax 320 column and equipped with split/splitless injection system) using helium as a carrier gas at 1.8 ml min^{-1}. The injection temperature was 200°C and initial column temperature of 80°C. The temperature program was an increase of 40°C min^{-1} to 160°C where it was held isothermal for 1 min. Subsequently, the temperature was increased at a rate of 3°C min^{-1} to 220°C where it was kept isothermal for 17 min. Peaks from chromatographs were compared to Sigma FAME standards containing few extra known fatty acids, for specific fatty acid identification, and the integrated peaks were compared to the peak area of the C_{23} standard.

Feeding and fecundity experiments

The calanoid copepod *Acartia tonsa* was obtained from cultures kept at the Danish Institute for Fisheries Research (Charlottenlund, Denmark). Batches of eggs were placed in filtered seawater and after hatching the copepods were grown on the cryptophyte *Rhodomonas salina* until adults. Only young adult females from the same egg batch were used for the experiments. Incubations were conducted with a single prey type per bottle. Copepods (females and some males to ensure fertilization) were conditioned for 48 h to prey type and concentration, as well as to other experimental conditions of light and temperature. Prey suspensions were prepared in 0.2 µm filtered seawater (amended with 5 ml l^{-1} of f/2 medium to compensate for copepod excretion). After the conditioning period, three or four 612-ml Pyrex control bottles (without copepods) and four 612-ml Pyrex experimental bottles (with 6-10 adult females of *Acartia tonsa* added to each) were filled with the desired prey suspension, sealed with plastic film and capped with special care to avoid presence of bubbles. In general, food concentrations were chosen near satiation levels and scaling the relative abundance of each group to natural occurrences.

In the case of *R. salina*, however, we set a larger gradient of concentration to show a wider functional response.

Two samples were taken to determine the initial prey concentration (50 ml aliquots preserved in 2 % acid Lugol's iodine solution for ciliates and dinoflagellates or three Multisizer Coulter-Counter measurements for *Rhodomonas salina*). Experimental and control bottles were incubated on a rotating plankton wheel (speed: 0.2 rpm) at room temperature (17 or 20 ± 1°C) and 12h:12h light:dark cycle. After ca. 24 hours the contents of the bottles were gently filtered through a submerged sieve (180 µm) to collect the copepods. Once aliquots for assessment of prey abundance were taken, the remaining screened water was filtered through a 20 µm mesh and copepod eggs collected. Eggs were then transferred to 320-ml Pyrex bottles filled with filtered seawater, and incubated on a rotating wheel at similar conditions of light and temperature as described above. After 24 h (20°C experiments) or 48 h (17°C experiments), the contents of the bottles was filtered through a 20 µm sieve, and the unhatched eggs, empty shells and nauplii collected and counted.

Lugol's iodine solution samples for estimation of ciliate and large dinoflagellate concentrations were settled on 10-ml Utermöhl chambers or alternatively filtered onto 0.45 µm cellulose filters, and the number of cells in the whole chamber or filter enumerated under a light microscope (200X). A total of 300-600 cells were counted per sample. Eggs that incidentally appeared in these samples (as they were collected previous to the 20-µm screening) were also counted and included into calculation of egg production.

Average prey concentration and copepod clearance and ingestion rates were computed according to Frost (1972). Egg production rate was estimated as the number of eggs laid per female and day. Egg production efficiency (EPE) of *Acartia tonsa* was calculated as the quotient between egg production rate and ingestion rate or as the slope of the ingestion rate versus egg production rate linear relationship, after conversion to carbon units using the conversion factors specified above.

Results

Nutritional quality of the prey

Size, carbon and nitrogen contents, and C:N ratios of the prey species are given in Table 1. C:N ratios varied from 3.6 and 4.6 among the studied species, *Rhodomonas salina* and *Gymnodinium dominans* showing the highest and the lowest values, respectively. C:N ratios of heterotrophic prey were not much lower than those of autotrophs, except for *G. dominans*.

Table 1. Size, carbon and nitrogen cell contents of the prey used in the experiments. Equivalent spherical diameters (EDS) corresponded to 2% Lugol fixed cells, except for *R. salina*, where size was estimated live with Multisizer Coulter Counter. Carbon and nitrogen contents for heterotrophic prey were estimated from the conversion factors provided by: this study (*R. salina, G. sanguineum*), Mendel-Deuer & Lessard (2000) (*G. dominans*) and Putt & Stoecker (1989) (*S. inclinatum, M. pulex*).

Diet	Specimen	ESD	C	N	C:N
Autotrophs	*R. salina*	6.5	0.055	0.012	4.6
	G. sanguineum	28.0	3.56	0.85	4.2
Heterotroph	*S. inclinatum* (bacteria diet)	20.9	0.91	0.22	4.1
	S. inclinatum (*M. pelliculata* diet)	23.0	1.22	0.29	4.2
	M. pulex (*H. rotundata* diet)	14.9	0.33	0.079	4.2
	G. dominans	14.6	0.23	0.063	3.7

The major fatty acid groups SAFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids) and HUFA (highly unsaturated fatty acids carbon chain length >20) were diversely represented in the studied species (Tab. 2). Except for the ciliate *Strombidium inclinatum*, all other species, either autotrophic or heterotrophic, were rich in PUFAs (38 to 123 fg μm^{-3}). Highest HUFAs concentrations were found in the ciliate *Mesodinium pulex* and the dinoflagellates *Gymnodinium dominans* and *Gymnodinium sanguineum*.

Although the specific fatty acid profiles differed among prey, clear differences between autotrophic and heterotrophic prey were not evident (Tab. 2). High concentrations of the fatty acid 22:6ω3 were observed in the ciliate *M. pulex* and the dinoflagellates *G. dominans* and *G. sanguineum*, the latter two in

agreement with a characteristic trend in this phylum (Volkman 1989). The 20:5ω3 fatty acid was well represented in *R. salina, G. dominans* and *G. sanguineum* while the 18:3ω3 was found in high concentrations only in *R. salina*. The scarcity of fatty acids in the ciliate *Strombidium inclinatum* (57 to 82 total fg fatty acid μm^{-3}) was mostly due to low PUFA content (less than 25% of total fatty acids). Moreover, the high contribution of the fatty acids 16:1ω7 and 18:1ω7 could be reflections of a bacterial diet (Zurkova & Kharlamenko 1999, Véra et al 2001).

A part of the content per se, the ratios between certain essential fatty acids (i.e. ω3:ω6 ratio and the ratio between 20:5ω3 and 22:6ω3 [hereafter named 20:22]) have been suggested as an important indicator of metabolic growth and reproduction in crustaceans (Castell 1982; Harrison 1990). The ω3:ω6 ratio was much lower for all ciliates studied (0.4 to 3.1) than for autotrophic prey and the other heterotrophic protozoans (range: 14.8 to 22.5). The highest 20:22 ratio was found in *Rhodomonas salina*, while *Mesodinium pulex* and *Gymnodinium dominans* presented the lowest 20:22 ratio.

The fatty acid profiles of heterotrophic prey fed on different diets are shown in Table 3. In the case of *Strombidium inclinatum*, both diets offered (bacteria or *Marsupiomonas pelliculata*) were poor in 20:5ω3 and 22:6ω3 fatty acids, although *M. pelliculata* presented high values of the fatty acid 18:3ω3 and PUFAs. *S. inclinatum* fed with *M. pelliculata* showed a lipid profile which varied with diet. However, its content in PUFA and HUFA was higher but not significantly different when fed algae compared to than when fed on bacteria, suggesting a lack of capability to synthesize such fatty acids or extract them from the diet. The other ciliate studied, *Mesodinium pulex*, showed a fatty acid composition very different from that of *Strombidium inclinatum* and seems to reflect that of its prey with much higher content of the 22:6ω3 fatty acid, PUFAs and HUFAs than *S. inclinatum*. The fatty acid profile of the heterotrophic dinoflagellate *Gymnodinium dominans* also follows that of its prey. *G. dominans* presented a significantly higher ($p < 0.05$) contents of 18:3ω3 fatty acid when fed on *R. salina* than when fed *Thalassiosira pseudonana*.

Table 2. Fatty acid composition of copepod prey expressed as fg μm^{-3} (averages of two measurements). SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids; 20:22 is the ratio between 20:5ω3 and 22:6ω3. Sum of all fatty acids found, included that which are not presented separately, is shown. The diet of the heterotrophs is specified in brackets: Hr=*H. rotundata*, Mp= *M. pelliculata*, B= bacteria, R= *R. salina* ,T= *T. pseudonana*.

	Autotrophs		**Heterotrophs**				
	R. salina	***G. sanguineum***	***M. pulex (Hr)***	***S. inclinatum (B)***	***S. inclinatum (Mp)***	***G. dominans (Rs)***	***G. dominans(Tp)***
14:1(ω5)	0.1	3.4	1.5	0.7	1.7	0.0	0.0
15:0	1.0	3.5	2.1	0.7	0.9	2.8	2.3
16:0	22.5	86.1	31.5	9.3	11.3	43.9	30.5
16:1(ω9)	1.7	1.5	8.5	1.2	1.4	0.5	1.9
16:1(ω7)	1.7	3.3	1.3	14.2	28.9	0.8	1.9
17:0	0.7	2.3	1.3	0.4	0.6	1.7	3.2
18:0	15.5	30.3	21.7	4.9	8.3	16.5	14.3
18:1(ω9)	6.4	35.1	9.0	2.0	2.1	8.8	6.6
18:1(ω7)	9.0	11.6	3.8	5.7	8.2	4.3	2.1
18:2(ω6)	2.5	4.8	3.2	0.7	1.6	4.3	1.6
18:3(ω6)	2.0	0.0	0.5	0.5	0.7	0.0	0.0
18:3(ω4)	2.6	0.0	28.0	2.3	4.3	0.0	0.0
18:3(ω3)	27.0	0.0	0.8	2.7	0.2	4.4	0.3
18:4(ω3)	49.5	0.0	4.5	0.0	0.3	0.0	0.0
20:4(ω3)	0.7	0.0	0.0	0.1	0.0	0.0	0.0
20:5(ω3)	15.5	35.3	6.8	0.8	0.2	15.5	9.2
22:5(ω3)	0.3	0.4	0.0	0.1	0.0	0.7	0.0
22:6(ω3)	7.0	36.5	27.8	0.7	0.0	42.9	26.8
24:1(ω9)	0.9	0.0	3.2	1.5	0.5	0.0	0.0
F. a. content	183.8	280.9	240.6	56.6	81.8	161.3	111.6
SAFA	48.8	142.5	73.1	17.5	24.1	78.6	58.2
MUFA	19.9	61.3	27.3	25.7	43.1	14.4	15.4
PUFA	112.3	77.1	123.0	11.9	11.5	68.2	38.0
HUFA	23.7	72.2	58.8	2.5	0.9	59.6	36.0
ω3	99.9	72.2	44.8	5.3	1.0	63.9	36.3
ω6	4.8	4.8	26.0	1.7	2.7	4.3	1.6
ω3/ω6	21.0	14.9	1.7	3.1	0.4	14.8	22.5
20:22	2.2	1.0	0.2	1.1	-	0.4	0.3

Table 3. Fatty acid (FA) composition (as % of total fatty acids) of the heterotrophic prey offered to copepods (Mes= *M. pulex*, Si= *S.inclinatum*, Gd=*G. dominans*) and of the diet offered to those prey (Hr= *H. rotundata*, B= bacteria, Mp= *M. pelliculata*, Rs= *R. salina*, Tp= *T. pseudonana*).

FA	Mes (Hr)	Hr	Si (B)	B	Si (Mp)	Mp	Gd (Rs)	Rs	Gd (Tp)	Tp
14:0	5.5	11.7	2.7	2.5	2.9	1.0	8.5	5.0	7.1	11.1
14:1ω5	0.6	0.2	2.0	0.8	1.3	0.1	0.0	0.1	0.0	0.0
15:0	0.9	0.5	1.1	1.9	1.3	0.2	1.8	0.5	2.0	2.1
16:0	13.1	16.9	13.8	19.7	16.4	13.0	27.2	12.2	27.4	29.0
16:1ω9	3.5	3.1	1.7	2.8	2.1	0.9	0.3	0.9	1.7	2.7
16:1ω7	0.5	0.4	35.4	21.8	25.1	0.8	0.5	0.9	1.7	13.4
17:0	0.5	0.3	0.7	1.0	0.7	0.1	1.0	0.4	2.9	1.2
18:0	9.0	2.7	10.2	9.6	8.6	2.0	10.2	8.4	12.8	11.8
18:1ω9	3.8	2.1	2.6	2.9	3.5	23.1	5.5	3.5	5.9	4.5
18:1ω7	1.6	1.1	10.0	16.0	10.1	2.2	2.7	4.9	1.9	1.0
18:2ω6	1.3	2.1	2.0	1.1	1.3	3.3	2.7	1.4	1.4	1.1
18:3ω6	0.2	0.3	0.9	0.9	0.8	0.2	0.0	1.1	0.0	0.0
18:3ω4	11.6	1.9	5.2	5.1	4.0	0.8	0.0	1.4	0.0	0.0
18:3ω3	0.3	1.5	0.3	0.0	4.7	40.5	2.7	14.7	0.3	0.0
18:4ω3	1.9	17.1	0.3	0.0	0.0	0.1	0.0	26.9	0.0	0.0
20:4ω3	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.4	0.0	0.0
20:5ω3	2.8	0.3	0.2	0.2	1.3	0.1	9.6	8.4	8.3	19.0
22:5ω3	0.0	0.2	0.0	0.0	0.2	0.3	0.4	0.1	0.0	0.0
22:6ω3	11.5	13.8	0.0	0.0	1.2	0.4	26.6	3.8	24.0	3.1
24:1ω9	1.3	0.6	0.7	0.0	2.7	0.3	0.0	0.5	0.0	0.0
SAFA	30.4	32.4	29.5	35.4	30.9	16.6	48.8	26.5	52.2	55.2
MUFA	11.4	9.6	52.8	44.3	45.4	27.5	8.9	10.8	13.8	21.7
PUFA	51.1	56.1	14.1	14.8	21.0	54.4	42.3	61.1	34.0	23.1
HUFA	24.4	26.6	1.1	1.8	4.5	0.9	36.9	12.9	32.3	22.1

Feeding, fecundity rates and hatching success

Prey concentration, clearance, ingestion and egg production rates, and hatching success of *Acartia tonsa* on the different prey are shown in Table 4. Clearance rates were dependent on prey concentration, with similar values for autotrophs and heterotrophs of similar size. Egg production rates (EPR) varied between 2 and 51 eggs laid per female and day, this maximum being similar to maximum egg production rates previously reported for this copepod species in the laboratory (Kiørboe et al. 1985; Støttrup & Jensen 1990).

Figures 1a and 1b show the relationship between ingestion and egg production rates of *Acartia tonsa* expressed in carbon units. For the *Rhodomonas salina* diet, feeding and fecundity showed a significant linear relationship ($r^2 = 0.72$, $p < 0.001$), with a egg production efficiency (EPE) estimated from the slope as 21%. The observations from the *Gymnodinium sanguineum* diet were conspicuously different from the *R. salina* data set; however, the scarcity of data and the narrowness of its range precluded regression analysis, so that EPE was estimated simply as the quotients egg production rate/ ingestion rate. The estimated EPE for *G. sanguineum* ranged 43-48%. A comparison of both procedures to calculate EPE for the *R. salina* data set resulted in similar estimates (slope: 21%; quotients: 22-25%).

For *Acartia tonsa* on heterotrophic diets, the narrow range of values and the variability among diets and among experiments for a same diet precluded linear regression for each diet independently. Since the quotient egg production/ingestion did not vary significantly among heterotrophic diets (one-way ANOVA test, $p = 0.9$), all data were pooled and one single linear regression analysis was conducted for heterotrophic diets to give a more robust estimate. EPE for heterotrophic prey estimated as the slope of this linear relationship was 23%, significantly different from zero ($r^2 = 0.41$, $p < 0.001$). Calculations done in terms of nitrogen resulted in a EPE of 21% for heterotrophs and 23% for *Rhodomonas salina*.

Table 4. Average prey concentration in the experimental bottles, clearance rates, ingestion rates, egg production rates (EPR), egg production efficiency (EPE) in carbon and nitrogen units and hatching success of the copepod *Acartia tonsa* fed on autotrophic and heterotrophic diets. Average values 1± SE are shown. nd= not determined

Prey	Prey diet	Prey conc cell ml^{-1}	Clearance ml ind^{-1} d^{-1}	Ingestion 10^5cell ind^{-1} d^{-1}	EPR eggs ind^{-1} d^{-1}	EPE carbon	EPE nitrogen	Hatching %
AUTOTROPHIC DIETS								
R. salina	-	1723 ± 1	19 ± 10	32 ± 18	7 ± 2	0.27 ± 0.2	0.25 ± 0.2	nd
R. salina	-	2380 ± 14	22 ± 2	52 ± 5	11 ± 1	0.17 ± 0.03	0.16 ± 0.03	nd
R. salina	-	2917 ± 4	18 ± 0.2	51 ± 0.4	8 ± 2	0.12 ± 0.02	0.11 ± 0.02	nd
R. salina	-	4644 ± 69	14 ± 2	63 ± 8	23 ± 0.1	0.28 ± 0.03	0.26 ± 0.03	nd
R. salina	-	5913 ± 36	11 ± 0.2	67 ± 1	22 ± 1	0.25 ± 0.01	0.23 ± 0.01	nd
R. salina	-	7997 ± 72	10 ± 0.5	80 ± 4	25 ± 1	0.24 ± 0.02	0.22 ± 0.02	nd
R. salina	-	8896 ± 44	10 ± 0.6	91 ± 5	22 ± 2	0.24 ± 0.02	0.17 ± 0.01	nd
R. salina	-	14703 ± 248	9 ± 2	128 ± 31	31 ± 1	0.20 ± 0.04	0.18 ± 0.04	nd
R. salina	-	15456 ± 85	6 ± 0.3	99 ± 5	33 ± 0.5	0.22 ± 0.01	0.14 ± 0.02	95
R. salina	-	16324 ± 58	9 ± 0.4	153 ± 7	51 ± 2	0.25 ± 0.01	0.21 ± 0.01	91
R. salina	-	18070 ± 101	7 ± 0.9	127 ± 16	25 ± 0.1	0.15 ± 0.02	0.24 ± 0.01	67
R. salina	-	18666 ± 61	5 ± 0.4	92 ± 6	23 ± 2	0.19 ± 0.003	0.18 ± 0.003	nd
R. salina	-	29912 ± 36	4 ± 0.02	113 ± 0.9	27 ± 4	0.18 ± 0.03	0.17 ± 0.03	nd
R. salina	-	36574 ± 125	3 ± 0.5	125 ± 19	25 ± 2	0.15 ± 0.01	0.14 ± 0.01	nd
G.sanguineum	-	7 ± 0.3	119 ± 9	0.9 ± 0.03	29 ± 1	0.48 ± 0.04	0.50 ± 0.04	70
G.sanguineum	-	10 ± 0.3	96 ± 5	0.9 ± 0.02	31 ± 2	0.43 ± 0.04	0.44 ± 0.04	69
HETEROTROPHIC DIETS								
S.inclinatum	bacteria	49 ± 1.5	68 ± 6.1	3.2 ± 0.2	19 ± 4	0.30 ± 0.07	0.39 ± 0.1	36
S.inclinatum	bacteria	14 ± 0.6	59 ±11.8	0.8 ± 0.1	3 ± 1	0.19 ± 0.10	0.25 ± 0.1	20
S.inclinatum	*M. pelliculata*	38 ± 1.0	35 ± 6.9	1.3 ± 0.2	7 ± 2	0.23 ± 0.09	0.29 ± 0.1	62
S.inclinatum	*M. pelliculata*	11 ± 0.8	134 ± 29	1.4 ± 0.2	6 ± 1	0.20 ± 0.07	0.25 ± 0.1	27
S.inclinatum	*M. pelliculata*	20 ± 1.3	49 ± 9	0.9 ± 0.1	8 ± 2	0.32 ± 0.03	0.41 ± 0.04	52
M. pulex	*H. rotundata*	56 ± 2.3	63 ± 8.8	3.5 ± 0.4	9 ± 3	0.41 ± 0.10	0.53 ± 0.2	83
M. pulex	*H. rotundata*	31 ± 1.3	38 ± 11.6	1.1 ± 0.3	2 ± 0.3	0.25 ± 0.06	0.31 ± 0.1	85
G. dominans	*R. salina*	43.2	49	0.2	3	0.19	0.13	71
G. dominans	*T. pseudonana*	74 ± 3.7	60 ± 9.1	4.3 ± 0.5	8 ± 3	0.29 ± 0.06	0.21 ± 0.05	96

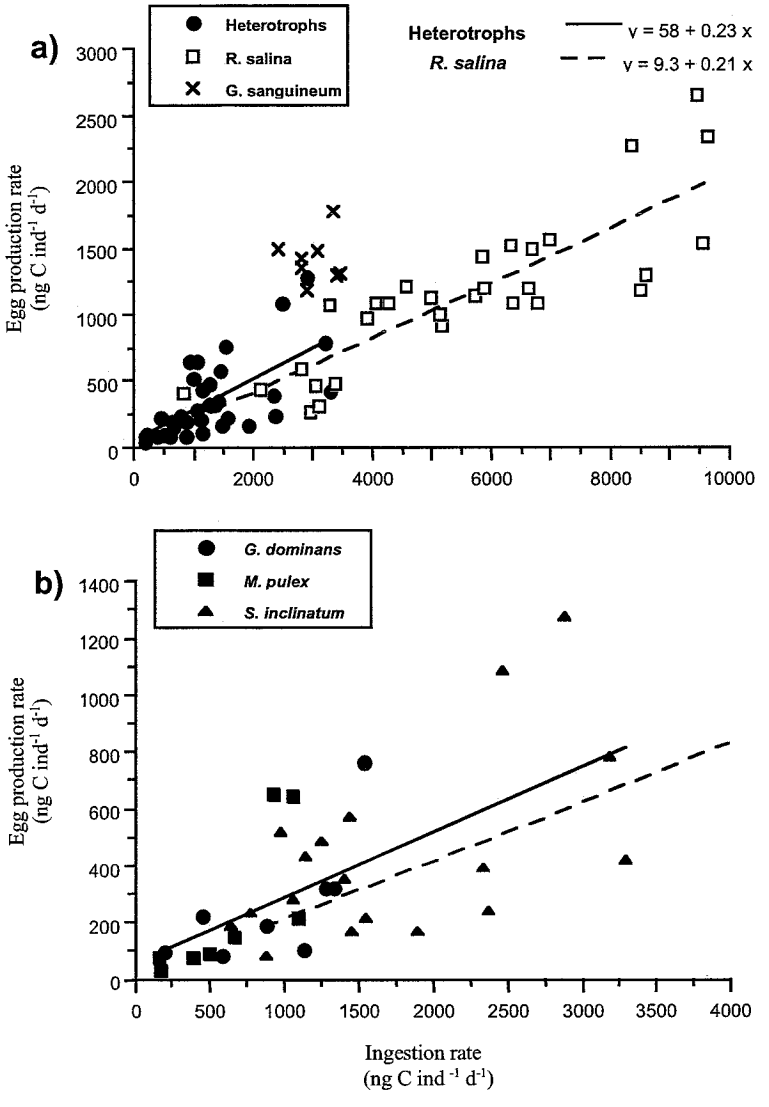

Figure 1. Egg production and ingestion rates of *A. tonsa* expressed in ng C ind^{-1} d^{-1}. a) Diets on the autotrophic prey *R. salina* and *G. sanguineum* and on heterotrophic prey (all pooled in a single category, Heterotrophs). Linear fits for Heterotrophs and *R. salina* are shown. b) Detail showing the heterotrophic prey of the category "Heterotrophs". Note the different scale.

When all prey are considered together, there appears to be a positive relationship between the ingestion of certain fatty acids and the egg production rate of *Acartia tonsa* (Fig. 2). However, when considered individually, the different prey were associated with different patterns. Thus, there is absence of correlation considering only heterotrophic prey, while *Rhodomonas salina* always showed the highest significant correlation. *Gymnodinium sanguineum* tends to appear as a different case.

However, because ingestion of fatty acid and ingestion of carbon are not independent variables ($r^2 = 0.9$, $p < 0.001$), part of the significant correlation obtained between the ingestion of specific fatty acids and egg production rate when all prey were considered together, may reflect variations in carbon ingestion and, in last instance, in food availability. In order to attempt a better discrimination of the effects of fatty acid contents on egg production rates, independent of food availability, we searched for a relationship between the specific fatty acid content of the prey (as pg μm^{-3}) and the residuals from the regression analysis between egg production rate (eggs ind^{-1} d^{-1}) and carbon ingestion rate (ng C ind^{-1} d^{-1}). No significant correlation was observed between the specific prey content of the PUFA and ω3-type fatty acids and the residuals from the egg production carbon ingestion regression analysis (Fig. 3 a, b). However, in the case of the fatty acids 20:5ω3 and 22:6ω3 a weak but significant correlation was observed (Fig. 3c, d). Again, *Gymnodinium sanguineum* proved to be singular in that and its exclusion from the regression analysis resulted in no significant correlation for any type of fatty acid considered.

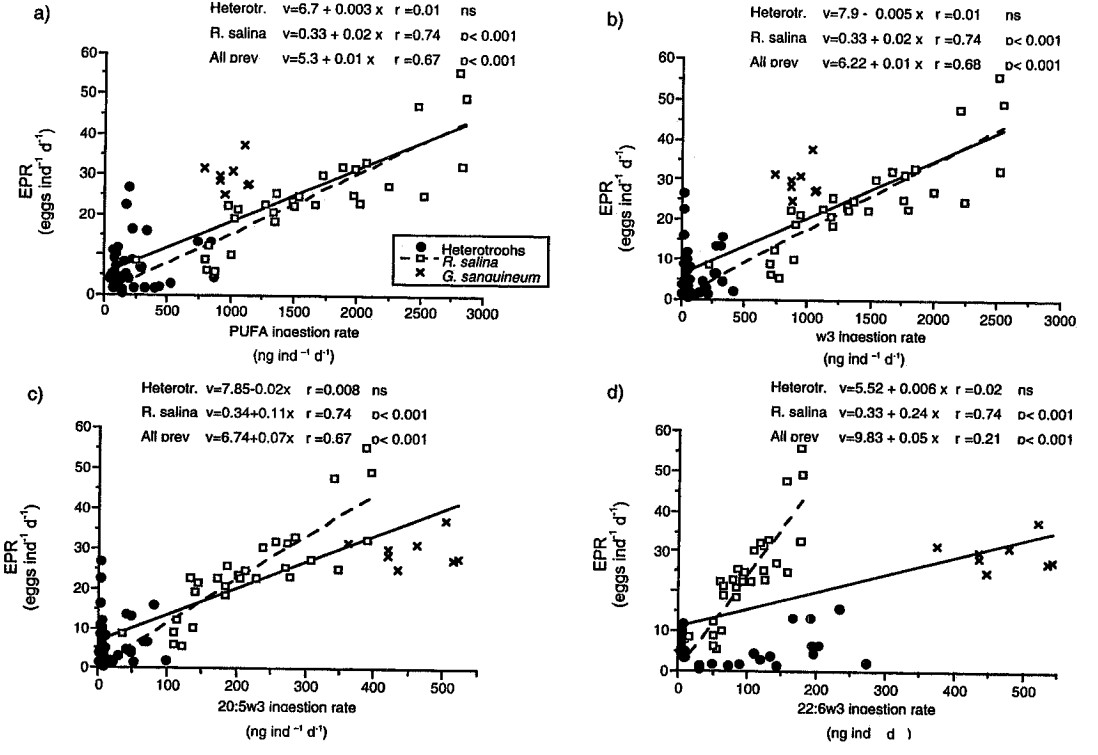

Figure 2. Egg production rates (egg ind^{-1} d^{-1}) of *A. tonsa* as function of fatty acid ingestion (ng ind^{-1} d^{-1}). Regression analysis for diets on heterotrophic prey, *R. salina* or all diets together are shown. Regression lines only shown for *R. salina* (dash) and all diets (continuous). a) PUFA, b) ω3, c) 20:5ω3 and d) 22:6ω3.

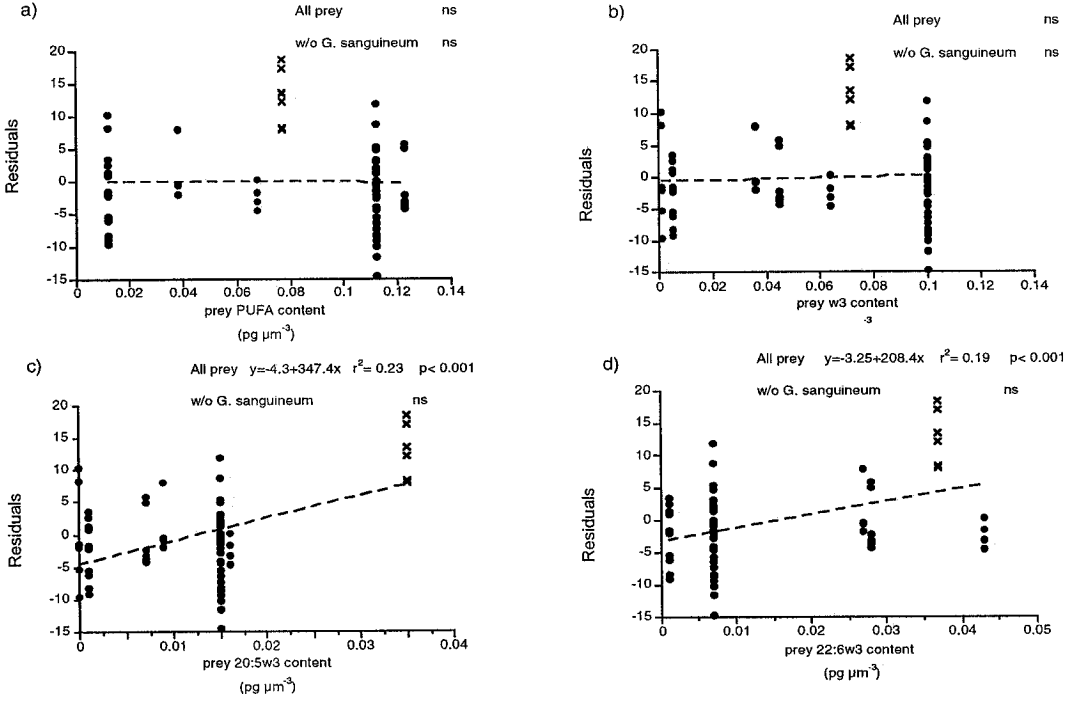

Figure 3. Relationship between the residuals from the egg production-carbon ingestion regression analysis (Fig 1) and the fatty acid contents of the prey (as pg μm^{-3}). a) PUFA, b) ω3, c) 20:5ω3, d) 22:6ω3. Crosses are for the dinoflagellate *G. sanguineum*. Dash line represents all prey fit. Results of regression analyses for all prey or excluding *G. sanguineum* are shown.

There was an even scatter in the hatching success of *Acartia tonsa* eggs from being poor (20-40% on *Strombidium inclinatum* when fed on bacteria) to high (> 80% for several prey diets) (Tab. 4). Actually, *S.inclinatum* fed on bacteria showed the lowest rates of hatching (28% on average), while hatching was much higher (47% on average) when the same species was fed on *Marsupiomonas pelliculata.* There were asymptotic relationships between both the ingestion rate of PUFA (Fig. 4 a) and essential fatty acids (EFA, sum of 18:3ω3, 20:5ω3 and 22:6ω3) (Fig. 4 b) and egg hatching success. It appears that for *A. tonsa* the ingestion of less than 0.1 μg of EFA in its daily food ration is associated with a decline on hatching success. In our experiments this very low hatching success was observed only when *A. tonsa* fed exclusively on *S. inclinatum*; it is noticeable, however, that the other heterotrophic prey provided sufficient essential fatty acid ingestion for successful egg hatching. Hatching success was also correlated with ω3:ω6 fatty acid ratio ($r^2 = 0.42$, $p < 0.01$) but not with 20:22 fatty acid ratio.

Discussion

We examined the nutritional value of heterotrophic prey for copepod reproduction, studying both the egg production efficiency (EPE) and the egg viability of the copepod *Acartia tonsa* under mono-specific diets, and explored their relationship with prey fatty acid composition. We compared those with autotrophic prey known to be suitable for copepod growth, such as the cryptophyte *Rhodomonas salina* and the naked dinoflagellate *Gymnodinium sanguineum*. Clearly, laboratory studies under such conditions are not representative of the situation that copepods encounter in the field. As copepods are typically omnivorous, any deficiency that a nutritionally-poor diet may provide could be compensated in the field by feeding on a wider spectrum of prey. However, monospecific experiments are always a first-step approach to explore the contribution of a potential prey to the diet and nutrition of a predator, and provide valuable information.

The nutritional value of a prey item has been frequently described with stoichiometric ratios (e.g. C:N). Actually, protozoans have been suggested to be qualitatively important as nourishment for zooplankton because of their low C:N ratios compared to phytoplankton (Stoecker & Capuzzo 1990). In our study C:N ratios of *Rhodomonas salina* and *Gymnodinium sanguineum* were lower than the ratio of 6:1 commonly accepted for phytoplankton (Parsons et

al. 1984), and similar to the ratios for heterotrophic prey, (except for *Gymnodinium dominans*, which showed the lowest C:N ratio) indicating that prey items, from a C:N stoichiometric point of view, differed little.

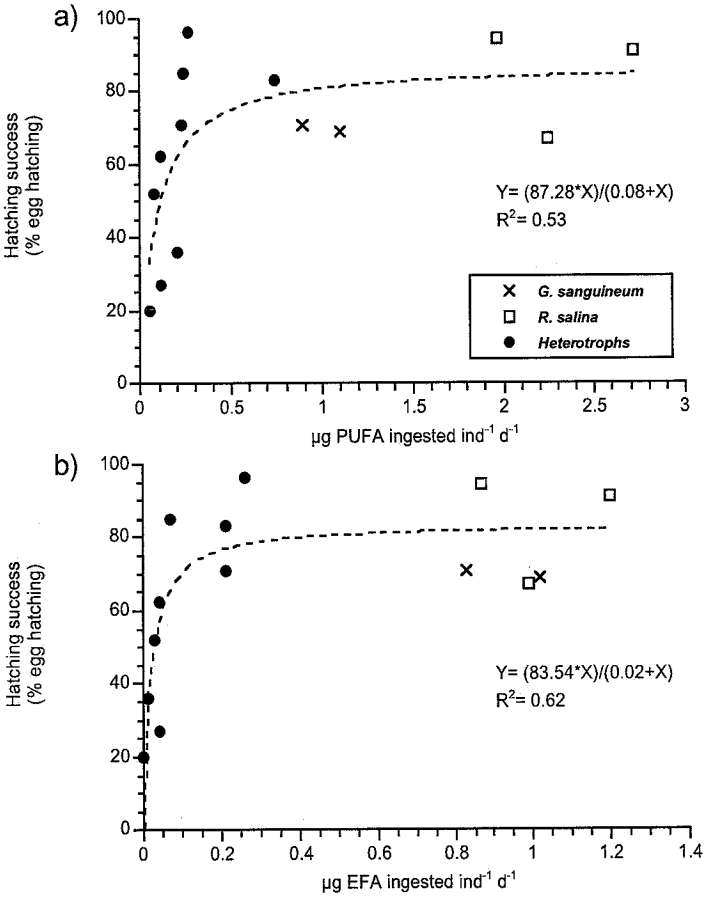

Figure 4. Relationship between hatching success (% of egg hatching) and ingestion rates of PUFA (a) or EFA (18: 3ω3 + 20:5ω3 + 22:6ω3) (b) by *A. tonsa*. Asymptotic curves are shown: Y= Hatching success; X= Ingestion of fatty acids.

However, although stoichiometric analysis can be indicative, there is evidence that C:N ratios can be inadequate as predictors of nutritional quality for crustaceans (Cahoon 1981, De Biase 1990) and recent approaches to obtain a better description of food quality have focused on different biochemical compounds, for example fatty acids (Ederington et al. 1997, Kleppel et al. 1998). Fatty acids are the principal form of stored energy in many organisms. Certain types of fatty acids are considered essential (EFA) as they cannot be easily synthesized by the organism and must be obtained in sufficient quantity from the food to maintain growth and survival (Sargent & Falk-Petersen 1988, Olsen 1998, Harrison 1990). EFA can be obtained directly from phytoplankton, but as our analysis and others show (e.g. Brown et al. 1998; Volkman 1998) the different protists vary greatly in their content of EFAs. Polyunsaturated fatty acids (PUFA) seem to play an important role in cell membrane activity as precursors of prostaglandins, the hormones regulating ionic fluxes, oocyte maturation and egg production in invertebrates (Harrison 1990).

The taxonomic differences in fatty acid composition have been well established in case of autotrophic phytoplankton (i.e. Volkman 1989). Our study and that of Klein Breteler et al. (1999) revealed that heterotrophic dinoflagellates are particularly rich in the fatty acid 22:6ω3 and that their fatty acid composition does not differ significantly from that of autotrophic dinoflagellates (Volkman 1989). In the case of ciliates, previous studies on marine ciliates reported very low contents of PUFA for bacterivorous ciliates (*Euplotes crassus*, < 25%, Zurkova et al. 1999; *Pleuronema* sp., < 15%, Ederington et al. 1997) and for the ciliate *Fabrea salina* fed on the prymnesiophyte *Isochrysis galbana* (Harvey et al. 1997). In contrast, Claustre et al. (1988) found very high PUFA contents for the herbivorous tintinnid *Stenosemella ventricosa* (59 %).

In our study we have dealt with heterotrophic ciliate genera commonly found in pelagic marine ecosystems (Vaqué et al. 1997, Pitta & Giannakourou 2000). Lipid profiles of ciliates have been reported to reflect the fatty acid composition of their diet (Harvey et al. 1997). In this regard, bacteria are particularly poor in PUFA, while in general algae tend to have higher contributions of PUFA (cited in Desvilettes et al. 1997). Our results show that heterotrophic prey also follow the trend in having similar profiles of fatty acid composition compared to their diets. However, the differences between heterotrophs fed with different diets were not always significant, which suggests that individual species may tend to maintain a certain stoichiometry in

their fatty acid composition, regardless of diet. Thus, *S. inclinatum* fed bacteria does not have the necessary precursor fatty acids in the bacterial diet to elongate to HUFA, and appears to be particularly poor in them; however, when feeding on *M. pelliculata*, *S. inclinatum* seems to have some capacity to elongate 18:3n3 to EPA and DHA (pathways shown in Castell 1982). A similar trend can be observed for *Gymnodinium dominans*.

Although the heterotrophs we studied appeared to be very different in terms of fatty acid content and composition both within the group and also compared to autotrophic species, our results show that egg production efficiency of *Acartia tonsa* was similar when fed on heterotrophs and on autotrophs and fell within the range of previous estimates for planktonic protozoans and metazoans (Straile 1997). In fact, the differences in EPE between the examined autotrophic prey were larger than those observed among the studied heterotrophic prey. Hence, the autotrophic dinoflagellate *Gymnodinium sanguineum*, which contained very high amounts of the 20:5ω3 and 22:6ω3 fatty acids but not higher PUFA contents than those of *Rhodomonas salina* or *Mesodinium pulex*, exhibited the highest EPE among the studied diets. Among studies investigating fatty acid composition there are some discrepancies regarding the importance of these components for the growth of copepods. For some copepods, the 20:5ω3 (EPA) and 22:6ω3 (DHA) fatty acids appear to be particularly important and have been correlated with copepod growth and development (Jónasdóttir 1994, Jónasdóttir & Kiørboe 1996, Pond et al. 1996, Støttrup et al. 1999). However, Støttrup & Jensen (1990) observed that the EPE of *Acartia tonsa* fed on *Isochrysis galbana* was higher than when fed *Thalassiosira weissflogii*, despite the fact that *T. weissflogii* was richer in 20:5ω3 and 22:6ω3 fatty acids. Lee et al. (1999) found no significant correlation between the egg production of the copepod *Pseudocalanus newmani* and either 20:5ω3 or 22:6ω3 concentrations in the diet.

Most studies until now have only attempted to find relationships between fecundity rates and fatty acid content of the diet, independently of actual fatty acid ingestion rates. Our correlation of specific fatty acid ingestion with egg production rate shows a possible dependence of egg production from these biochemical compounds. However, considering that fatty acids are only a fraction of the total carbon pool and that the carbon based ingestion also showed the same high correlation with EPR, it was difficult to discern if the effect on EPR was due to the carbon ingestion or to the lipid ingestion. The inconclusive result of the residual analysis could be either the consequence of a

species-specific difference in fatty acid requirement, or of a need for other essential nutritional components not measured here (e.g. sterols, amino acids and proteins) that have been shown to be implicated in regulating the egg production in copepods (for sterols e.g. Ederington et al. 1997, Klein Breteler et al. 1999, for amino acids e.g. Kleppel et al. 1998, Guisande et al. 1999, for proteins e.g. Jónasdóttir 1994, Kleppel & Hazzard 2000).

However, we cannot forget that recruitment (i.e. reproduction success) to copepod populations depends not only on fecundity rates but also on the viability of eggs. The literature is scarce on data on hatching success of copepods fed on heterotrophic diets to compare with. In our experiments, food quality seems to affect the viability of the eggs more than the egg production rate or the egg production efficiency. The hatching success of the eggs was found to be influenced by the ingestion of PUFA and the sum of the EFAs 18:3ω3, 20:5ω3 and 22:6ω3 (Fig 4). It seemed that a minimum amount of essential fatty acids was needed to reach > 60 % hatching success. Thus, fatty acid content and composition appear to be important factors in determining egg hatching success of *A. tonsa*. Our results are in agreement with general patterns of PUFA dependence of hatching success and embryonic development in crustaceans (Jónasdóttir & Kiørboe 1996, Tang et al. 2001). Similarly, the study of Ederington et al. (1997) showed equally high hatching success for the copepod *Acartia tonsa* fed either diatoms or bacterivorous ciliates. While these diets differed greatly in their fatty acid profile, they had the same relative concentration of PUFA, qualitatively agreeing with our study.

As concluding remarks, our result on fatty acid composition and its relationship with EPR does not support the notion that ciliates or heterotrophic dinoflagellates are nutritionally different from other prey, e.g. autotrophic ones, for copepods. However, further research is needed in order to complete the description of the biochemical composition of heterotrophs, because other biochemical compounds besides fatty acids can be also essential for copepod growth and egg viability. From an ecological point of view, these results imply that if the preference for ciliates and heterotrophic dinoflagellates exhibited by copepods in many field studies is not directly related to their higher nutritional quality, other factors like their size, shape, motility and patch behavior can very likely play a major role in favoring this predator-prey interaction.

CHAPTER IV

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Chapter IV

Small-scale processes in planktonic systems: effects of ciliate swimming behavior on predation risk

Introduction

During the last two decades, ciliates have been shown to be an important food source for zooplankton in both marine (Stoecker & Capuzzo 1990; Verity & Paffenhöfer 1996) and limnic systems (Adrian & Schneider-Olt 1999). Ciliates may here act as a significant link between microbial food webs and the classical diatom-copepod-fish food webs (Cushing 1990) with implications for the flow of matter and energy in the pelagial (Azam et al. 1983; Sherr and Sherr 1986). Although the interest in this subject has increased during the past years, an improved mechanistic approach is needed to understand the importance and magnitude of this link and to determine the factors involved in predator and prey dynamics.

Studies of the interactions between copepods and ciliates have mostly focused on measurements of copepod clearance rates based on the disappearance of ciliates in laboratory and field incubations. Only few works have considered the encounter and post-encounter processes at the individual scale. For instance, using videography, Jonsson & Tiselius (1990) described the raptorial feeding behavior of the copepod *Acartia tonsa* when encountering planktonic ciliates. These authors showed that the escape response of the ciliate *Mesodinium rubrum* reduced predation by *A. tonsa*. Similarly, in fresh water systems ciliates moving with jumps appeared to be less susceptible to predation by cladocerans (Jack & Gilbert 1993), rotifers (Gilbert & Jack 1993; Gilbert 1994) and copepods (Burns & Gilbert 1994). However, how changes in prey behavior can affect the outcome of both encounter and post-encounter processes is still scarcely known.

The main objective of this work is to investigate if differences in swimming behavior of two similarly sized ciliates can affect the predation risk from the copepod *Acartia clausi*. First we test for differences in ciliate swimming

patterns and possible behavioral changes in the vicinity of a predator. Second, copepod attacks on the two ciliates are recorded and the capture success measured. Finally, the behavioral studies are combined with incubation experiments where *A. clausi* is offered a 1:1 mixture of both ciliates. Our final aim was to determine to which extent small-scale processes cans shape trophic interaction within the pelagial and affect selectivity patterns.

Materials and methods

Culture and collection of organisms

The tintinnid *Metacylis* sp. (Fig. 1 A) and the strobilidid *Strobilidium* (*Lohmanniella*) *spiralis* (Leegaard 1915) (Fig. 1 B) were isolated from waters off Tjärnö Marine Biological Laboratory (TMBL), on the west coast of Sweden (58° 38' N: 11° 45' E). *Metacylis* sp. resembles *Metacylis jorgensenii* Cleve (1902) with a slightly pointed hyaline lorica. The ciliates were similar in size (measurements in 1% Lugol's acid solution preserved specimens; *S. spiralis*: body length 59±3.2 μm, width 55±4.4 μm; *Metacylis* sp.: lorica and body length 52±2.6 μm, width 49±2.1 μm), and abundant at the site of collection.

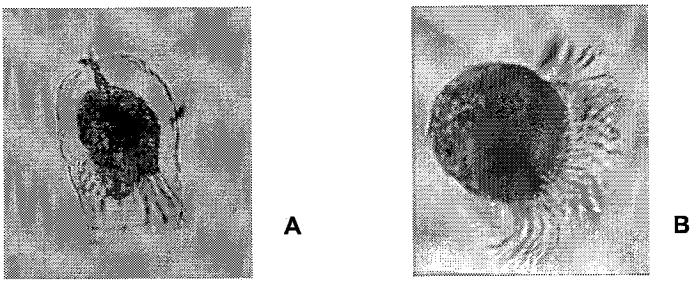

Figure1. *Metacylis* sp. (A) and *Strobilidium spiralis* (B). Photos of cells fixed in Lugol by E. Broglio and M. Johansson.

Ciliates were grown in a suspension of the microalgae *Isochrysis galbana* Parke 1949 (Prymnesiophycae) and *Pyramimonas disomata* Butcher 1959 (Prasinophycae) in filtered, autoclaved (120°C, 20 min) brackish seawater (15 ‰ S). Stocks of both ciliates were routinely kept in 50-ml culture flasks.

Larger quantities of ciliates required for the experiments were grown in glass bottles (620 ml, Pyrex) kept on a plankton wheel (speed: 0.25 rpm) on the same diet. Approximately 100 ml of the culture was renewed with fresh medium every day to ensure a supply of exponentially growing algae for the ciliates. All cultures were maintained at room temperature (20°C) under a natural light cycle (17h light: 7h dark). Adult females of the copepod *Acartia clausi* (Giesbrecht 1889) were picked out from net tows (mesh size 160 μm) in coastal waters off TMBL. This copepod was dominant in the field and co-occurred with the isolated ciliates. Prior to each filming copepods were starved for 30 min in filtered, autoclaved, seawater (17.5 ‰ S).

Video recording

Observations of the behavior of the copepod and the ciliate prey were obtained by video-recording the copepods in a suspension with a single prey species. For each recording, ciliates and approximately 20 adult females of *Acartia clausi* were added to an experimental aquarium (20x5x5 cm). The aquarium was placed inside a larger transparent tank (17x17x17 cm) filled with distilled water and with a cooling device at the bottom, in order to maintain the temperature as constant as possible (22 -23 °C) and to avoid convection currents. Observations were made using an horizontally mounted dissecting microscope (Wild M5A, 6 to 50 X) equipped with a video camera (Minitron MTV-1802CB, 795x596 pixels, light sensitivity 0.01 lux) and recorded on VHS tape, at 50 frames/sec. Field of view was 10 mm x 7.5 mm. Filming took place in a dark room and aquariums were illuminated by a back-transmitted red light (670 nm). The set up utilized is shown in Fig. 2.

The ciliates used for filming came directly from the cultures and consequently, they had ample supply of *Isochrysis galbana* and *Pyramimonas disomata*. Two cases were considered regarding the observations of ciliate behavior: sequences where no copepods appeared on the screen (called *undisturbed* hereafter), and those where an approaching copepod was present, sometimes leading to an attack against a ciliate. Frame-by-frame analysis of the video-recorded sequences was performed to quantify motility patterns of undisturbed ciliates and ciliates close to an approaching copepod. On the videotape, 10 to 15 individuals for each species were randomly selected and their positions traced onto acetate sheets frame by frame until out of focus. Ciliate positions on the acetate sheets were determined with a digitizing tablet and distances computed.

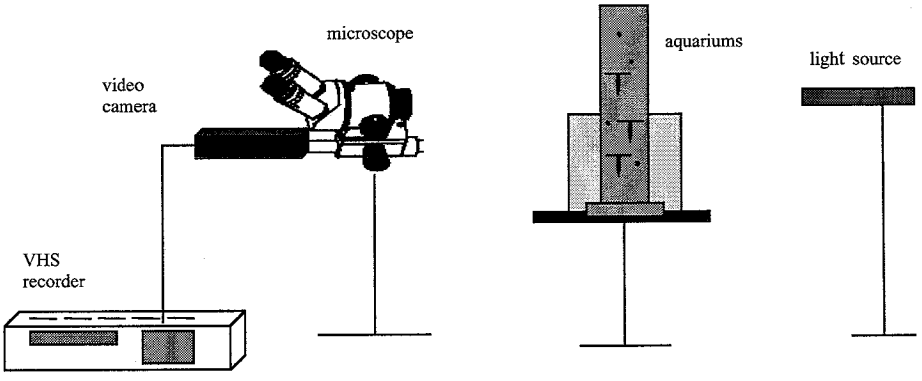

Figure 2. Set up utilized for the behavioral observations.

Because filming was in two dimensions (XZ), we selected ciliates that were swimming perpendicular to the camera axis hence displacement of the animals was measured in the XZ plane. Since measurements were based on 2-dimensional projections of 3-dimensional displacements, the estimates of swimming velocities and distances will be underestimated. However, we think that 2D analysis must not bias the comparison of swimming patterns between both ciliate species, as it does not seem likely that one ciliate species moves more in the XZ plane than in the YZ plane. Each sequence analyzed was ca 20 seconds for *Strobilidium spiralis* and 3 seconds for *Metacylis* sp.. The difference in observation time between the 2 ciliates reflects the difference in swimming behavior: more positions/sec had to be analyzed to describe the convoluted helical swimming path of *Metacylis* sp., while longer sequences were needed for *S. spiralis* in order to have appropriate resolution of its path (including sinking period and jumps).

The mean swimming speed was calculated as the total length of the swimming path divided by the duration of the path. The overall mean swimming speed was computed, by weighting the path means for each ciliate by the observation time. Both average jump length and jump speed were weighted by the number of events (jumps) and observation time recorded for each individual ciliate. When ciliates were approached by a copepod, if they performed more than one jump to escape from the copepod, only the first jump was used for calculations.

As a rough estimate of differences in diffusion rate between both ciliates, a 2-dimensional dispersal rate coefficient (XY plane; area t^{-1}) was determined by measuring the time required for a ciliate to move to the periphery of a given circle centered on a random position in its swimming path. We used two circle areas (of 4 and 16 mm^2 respectively) to account for a possible scale-dependence of the dispersal rates.

Aspects of the foraging behavior of *Acartia clausi* preying on the 2 ciliates were also measured. *Reaction distance* for the copepod was determined by measuring the shorter distance between the ciliate and the copepod head immediately prior to an attack. We defined *attack* as the jump (or the last jump in case of a chase) shown by the predator towards the ciliate, usually with a change in the orientation of the copepod. Attacks were divided into 3 groups depending on the final outcome: escaped, missed and captured. *Escaped* was the case when the ciliate jumped away from the predator; *Missed,* when the copepod failed to accurately locate the prey. The group *Captured* consisted of 2 subgroups: the attacks where the ciliate was eaten (*Eaten*) and the attacks where the prey was lost after being handled (*Lost*).

Incubation experiments

Prey selection by the copepod *Acartia clausi* was tested in incubation experiments where copepods were offered a 1:1 mixture of the ciliates *Strobilidium spiralis* and *Metacylis* sp. Two incubation experiments were run with identical protocol but different ciliate concentrations: for each species, 14 ciliates ml^{-1} and 5 ciliates ml^{-1} respectively. Ten glass bottles (620 ml, Pyrex) were filled with suspensions of the 1:1 ciliate species at desired concentrations. Two of the bottles were used to determine the initial ciliate concentration (100-ml aliquots preserved in 1 % Lugol's acid solution). Four bottles (without copepods) were used as control and four bottles were filled with 4-5 adult females of *A. clausi* each. All bottles were sealed with plastic film and capped, then incubated on a plankton wheel (speed: 0.25 rpm) at room temperature (20°C). The experiment was run for 24 hours and the contents of the bottles were gently filtered through a submerged mesh (180 µm) to collect the copepods and 100 ml of the screened water which were fixed in Lugol's acid solution. Ciliates concentration were determined in 50-ml aliquots of the preserved samples settled in Utermöhl chambers and counted under a microscope. A total of 300-600 ciliates were counted per sample. Ingestion and clearance rates were calculated according to the equations of Frost (1972).

Statistical analysis

All means are presented with the standard error of the mean (SE) and the sample size. Ciliate swimming speeds were analyzed in a 2-factor analysis of variance (ANOVA) with species and the presence/absence of a predator as fixed factors. Differences in ciliate jump length and velocity during spontaneous jumps and jumps performed when approached by a copepod and during copepod attack were tested with a 1-factor ANOVA and Student-Newman-Keuls (SNK) means-comparison test. The hypothesis of a difference in escape capability between the ciliate species was tested with a G-test (Sokal and Rohlf 1994) using the clearance rates in Table 3. The difference in predation mortality between the ciliate species was tested in a 2-factor ANOVA using the results on clearance rates in the feeding experiments (Tab. 4). The 2 experiments were analyzed jointly where each experiment was regarded as 2 levels in a random factor and the 2 ciliate species as a fixed factor. Since the interaction term was highly non-significant it was pooled with the residual term. The data analyzed with ANOVA were first tested for homoscedasticity using Cochran's test (Winer et al. 1991). In all tests a type-I error (α) of 0.05 was used.

Results

Prey swimming behavior in undisturbed situations

The ciliates *Strobilidium spiralis* and *Metacylis* sp. showed very different swimming patterns (Fig. 3). Like many other species in the Strobilidiidae family, *S. spiralis* moved forward slowly while generating a strong feeding current. When not disturbed by the raptorial copepod *Acartia clausi* (undisturbed ciliates), *S. spiralis* alternated between periods of slow upward swimming interrupted by sudden jumps involving very rapid swimming and periods of helical downward swimming. Mean swimming speed was 0.33 ± 0.02 mm s^{-1} (n=11). Jump frequency was 5.7 ± 1.6 jumps min^{-1} and mean jump length and velocity were 0.36 ± 0.05 mm and 3.93 ± 0.39 mm s^{-1}, respectively (Tab. 1). *Metacylis* sp. showed a swimming pattern with steep helical trajectories interrupted with occasional ciliary reversals (only 3 events observed). No jumps were observed for this species. When away from any

approaching copepod the tintinnid *Metacylis* sp. moved at a mean speed of 0.78 ± 0.06 mm s^{-1} (n=15).

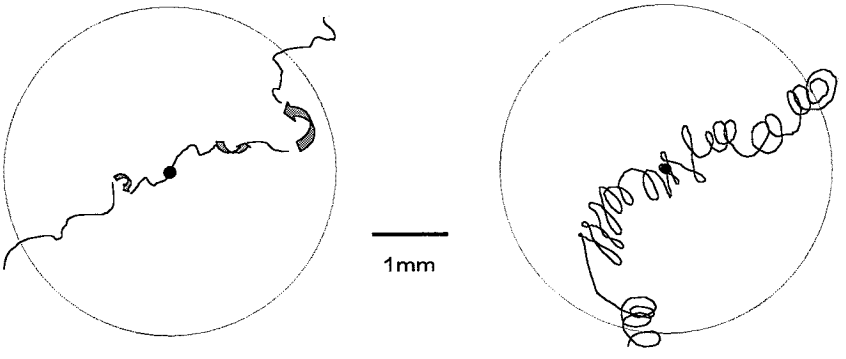

Figure 3. Swimming patterns of *S. spiralis* (A) and *Metacylis* sp. (B). Arrows indicate jumps. The 4 mm^2 circle used to calculate the dispersal rate is shown. The dispersal rate was estimated measuring the time required for the ciliate to move to the periphery of the circle, centered (black point) on a random position in the swimming path.

Although *Strobilidium spiralis* showed a lower mean speed than *Metacylis* sp., the differences in swimming behavior (i.e. upward and downward swimming, and jumps for *S. spiralis*; continuous helical paths for *Metacylis* sp.) resulted in a 1.5 times higher dispersal rate for *S. spiralis* (small sample area: 1.18 ± 0.09 mm^2 s^{-1}; large sample area: 2.14 ± 0.18 mm^2 s^{-1}, n=23) than for *Metacylis* sp. (small sample area: 0.74 ± 0.08 mm^2 s^{-1}; large sample area: 1.50 ± 0.15 mm^2 s^{-1}, n=19).

Table 1. Length and velocity of jumps performed by *Strobilidium spiralis*. Jumps are classified into three scenarios: "undisturbed" (i.e. when ciliates were far from predators), "in danger" (i.e. when jumps were triggered by the predator presence) and "escape" (i.e. when ciliates were escaping from the predator). n = sample size. When more than one jump was performed as a response to the predator, only the first one was measured.

Scenarios	n	Jump length (mm)	Jump velocity (mm s^{-1})	Maximum jump velocity (mm s^{-1})
undisturbed	20	0.36 (±0.05)	3.93 (±0.39)	6.37
in danger	15	2.19 (±0.37)	6.48 (±0.50)	10.45
escape	7	2.80 (±0.40)	9.46 (±0.51)	11.85

Prey-predator interactions

When a sinking copepod detected a ciliate, it reoriented its body towards the prey and attacked. There was no need for physical contact between the copepod and a ciliate to trigger an attack. When the attack resulted in a successful capture, the ciliate was handled for a very short period of time before being ingested (<1 s).

Although true detection distances cannot be estimated from our observations, a minimum estimate can be obtained from the distance at which the prey triggered an attack from the copepod. Reaction distance differed for the two ciliates. Thus, the *Acartia clausi* average attack distance for *Strobilidium spiralis* was 0.69 ± 0.11 mm (n=16) measured from the head tip, while the distance was 0.40 ± 0.05 mm (n=21) for *Metacylis* sp.. Despite the higher reaction distance of the copepod for *S. spiralis*, the capture success was much lower than for *Metacylis* sp. (G-test, $p < 0.05$). In the case of *S. spiralis*, only 32% of the observed attacks resulted in successful captures and ingestion; the percentages of the attacks where the ciliate was lost, missed, or it jumped away escaping the predator are shown in Table 2. When *A. clausi* attacked *S. spiralis* the reaction distance was significantly greater (t-test, $p < 0.05$) for unsuccessful attacks (0.81 ± 0.12 mm) than for attacks resulting in eventual capture of the ciliate (0.52 ± 0.20 mm).

Although for *Metacylis* sp. a higher proportion of the attacks resulted in capture and ingestion (64 %, Tab. 2), the percentage of missed prey (32%) was also higher in comparison to *Strobilidium spiralis* (16%). Frequently, the

copepod slowly approached the tintinnid before lunging into attack, to assure the capture. After being captured, the capacity of *Metacylis* sp. to avoid ingestion was very low (4% of the observed attacks).

Table 2. Behavioural analysis of copepod-ciliate interactions. Frequencies (as %) of the different outcomes of the interaction. For *Strobilidium spiralis* and *Metacylis* sp., 19 and 22 attacks were analyzed, respectively.

Species	Escaped	Missed	Captured	
			Lost	Eaten
S. spiralis	36	16	16	32
***Metacylis* sp.**	0	32	4	64

Measurements of swimming behavior of *Strobilidium spiralis* and *Metacylis* sp. differed when in close vicinity of a copepod compared to observations well away from any copepod. When approached by a copepod the mean swimming speed of *S. spiralis* increased from 0.33 ± 0.2 mm s^{-1} to 0.51 ± 0.2 mm s^{-1} (n=5). Escape jumps were longer and faster than jumps performed by undisturbed ciliates (ANOVA, $F_{2,28} = 16.8$ $p < 0.05$) (Tab. 1) and the frequency of jumps increased after a copepod attack (18.5 ± 4.2 jumps min^{-1}). We also observed that in the proximity of the copepod, *S. spiralis* often initiated a jumping response at a mean distance of 0.53 ± 0.07 mm from the copepod head (n=10), indicating that the ciliate may detect the presence of the copepod in advance of an attack. Ciliate jumps initiated by an approaching copepod were also longer and more rapid compared to jumps in the absence of any copepod (1-factor ANOVA, $F_{2,28} = 16.7$ and SNK test, $p < 0.05$; Tab. 1).

Metacylis sp. did not show any escape (jump) response triggered by an approaching or attacking *Acartia clausi*. However, the swimming speed increased after an unsuccessful attack by the copepod (1.05 ± 0.18 mm s^{-1}, n=5) and the ciliate seemed to move in a steeper helical path. The increased swimming velocity may have contributed to the unsuccessful attacks performed by the copepod. Interestingly, we observed that some individuals of *Metacylis* sp., when lost after being handled by the copepod became motionless for a few seconds before resuming swimming again and were not pursued more by the predator after the first attack. This behavior was not observed for *Strobilidium spiralis*.

Feeding experiments

Both feeding experiments of *Acartia clausi* incubated in 1:1 mixture of ciliate suspension showed an unequal consumption of the 2 ciliate species (2-factor ANOVA, pooled $F_{1,13} = 12.0$, $p < 0.05$), with much higher clearance rates for *Metacylis* sp. in comparison with *Strobilidium spiralis*. This effect was most evident at the lowest ciliate concentration, where consumption rates on *S. spiralis* were sometimes below detection. Averaged over both experiments the copepod clearance rate for *Metacylis* sp. was almost 7 times higher than for *S. spiralis* (Tab. 3).

Table 3. Summary of the 2 feeding experiment where the copepod *Acartia clausi* was offered a 1:1 mixture of the 2 ciliates *Strobilidium spiralis* and *Metacylis* sp. Copepod clearance rates (mean ± SE, n=4) for the 2 ciliate species are based on 24-h incubations and corrected for ciliate growth. In the second experiment (*), two experimental bottles resulted in negative clearance rates due to very low consumption rates and they were rounded to zero.

Experiment	Ciliate prey	Copepod clearance (ml copepod^{-1} d^{-1})
Exp. 1 (28 cil. ml^{-1})	*S. spiralis*	12.2 ± 4.4
	Metacylis sp.	40.1 ± 20.6
Exp. 2 (10 cil. ml^{-1})	*S. spiralis*	6.6* ± 6.0
	Metacylis sp.	73.8 ± 15.9

Discussion

In coastal and oceanic environments the populations of marine planktonic ciliates are frequently limited by high predation rates by mesozooplankton (Stoecker and Capuzzo, 1990; Nielsen and Kiørboe, 1994). However, at small-scale predation and eventual capture depend on specific predator and prey behavior. Planktonic ciliates show a wide diversity of swimming patterns including different helical trajectories, rapid jumps, ciliary reversals and periods of inactivity and sinking (e.g. Buskey et al. 1993). The main hypothesis tested in the present paper is if differences in swimming behavior between two ciliates result in higher survival rates in the presence of the copepod *Acartia clausi*. The ciliates *Strobilidium spiralis* and *Metacylis sp.* were selected because they are of similar size but differ in their swimming paths and in their ability to react to fluid disturbances. *S. spiralis* has a richer repertoire of

swimming behavior with upward and downward swimming and jumps, while *Metacylis* sp. swims upwards in helical trajectories and lacks jumps, as is typical for many tintinnids. In our experiments, both ciliate species *S. spiralis* and *Metacylis* sp., were attacked and could be ingested by *A. clausi*.

Analysis of the encounter and post encounter processes can provide possible explanations of the mortality differences found for the two ciliate species. According to our estimated dispersal rates *Strobilidium spiralis* had a higher probability of encounter with the predator (1.5 times) than *Metacylis* sp.. Previous encounter models have proposed algorithms to calculate the encounter rate using some detection distance and assuming a random motility of predator and prey, e.g. Gerritsen & Strickler (1977) and Tiselius et al. (1997). Gerritsen & Strickler's model defines encounter rate as a function of the area over which the interaction occurs, the abundance of the prey and the relative velocities of predator and prey. Under the presence of a cruising predator, the swimming speed of the prey has little effect on encounter if the velocity of the predator is higher than that of the prey. However, when the predator is an ambush feeder, as in the case of *Acartia clausi* feeding on ciliates, the swimming velocity of the prey may have a strong effect on the encounter rate. Tiselius et al. (1997) incorporated into that model the hydrodynamic signaling produced by a jumping prey as a mechanism to increase the perceptive distance of the predator, and consequently the encounter rate. Using our data on swimming speed and jump behavior and applying the model of Tiselius et al. (1997), we find that the encounter rate of *Metacylis* sp. with the predator is expected to be higher (1.6 times) than for *S. spiralis*. This result contrasts with our observation (i.e higher dispersal of *S. spiralis* than *Metacylis* sp.) and is mainly a consequence that their model takes into account only the velocity of the prey (which in our case is higher for *Metacylis* sp.) but not the structure of the prey swimming path (more convoluted trajectories for the ciliate *Metacilys* sp. while displacement is higher for the ciliate *S. spiralis* mainly because of the jumping behavior).

On the other hand, although we would predict a higher encounter rate of *Acartia clausi* with *Strobilidium spiralis*, our behavioral observations conducted for each species separately indicate that *Metacylis* sp. could be the more eaten prey. Our feeding incubation experiments on a mixture of the two ciliates confirm this observation (Tab. 4). From our study it appears that the susceptibility to predation on ciliates is very dependent on their escape ability (either before being detected or while being attacked by the predator),

becoming more determinant than differences in encounter rates mediated by their swimming (cruising velocity, dispersal rate). In the present study, *Strobilidium spiralis* showed an effective jump response to copepod attacks. The escape jumps shown by *S. spiralis* reached velocities above 10 mm s^{-1} (c.a. 200 body lengths s^{-1}) with an average length of 2.8 mm. In contrast, the investigated tintinnid *Metacylis* sp. does not jump during swimming and does not show escape reactions to an approaching predator. Jump behavior in response to hydrodynamic signals have been described for some other ciliates, e.g. *Mesodinium rubrum* (Lindholm 1985; Jonsson & Tiselius 1990) and the freshwater ciliates *Strobilidium velox* (Jack & Gilbert 1993; Gilbert 1994) and *Halteria grandinella* (Tamar 1974; Gilbert 1994), but is probably more widespread as indicated in some descriptions of ciliates (e.g. Kahl 1932).

Table 4. Overview of relative susceptibilities of the 2 ciliate species as estimated from behavioral observations and from feeding incubation experiments. Relative susceptibility is defined as the ratio between the corresponding values (either concentrations, ingestion or clearance rates) for *Metacylis* sp. versus *Strobilidium spiralis*. (a) Nominal prey concentration, assuming an equal value of 100 (b) Effective prey concentration (i.e. encountered), considering that the dispersal of *S. spiralis* is 1.5 x that of *Metacylis* sp. (c) Attack success (as % of prey ingested after attack, see Table 2). (d) Expected ingestion rate calculated as the effective prey concentration times the attack success. (e, f) Clearance rates on both ciliates from experiments with mixtures (see Table 3).

	PARAMETERS	*S. spiralis*	*Metacylis* sp	Ratio
From behavioral observation				
a	Nominal prey concentration	100	100	1
b	Effective prey concentration	150	100	0.7
c	Attack success (%)	32	64	-
d	Expected ingestion rate	48	64	1.3
From feeding experiments				
e	Clearances rates from Exp. 1	12	40	3.3
f	Clearances rates from Exp. 2	7	74	11

Therefore, although jumps could act as a mechanism to avoid capture by a predator, it has been suggested that the strong hydrodynamical signals generated by jumps could alert the predator and facilitate the detection and eventual attack by the predator (Tiselius et al. 1997). Actually, using our data and applying the encounter model presented in Tiselius et al. (1997) that assumes a random motion, about 40% of the encounters with a predator like

Acartia clausi should occur during jumps despite that the ciliates only spend ca 1 % of the time jumping. It can only be speculated that the jump behavior also serves other functions, maybe to efficiently shed the envelope of water around the ciliate that may be depleted of food particles and repleted with excretion products. However, we need to remark that although previous results and our experiments suggest that escape can be considered an efficient way to avoid capture by a potential predator, rates of copepod predation on ciliates can vary considerably for different combinations of copepod and ciliate species (Wickham 1995). For instance, Burns & Gilbert (1993) found that copepods efficiently preyed on the jumping ciliate *Strobilidium velox*, indicating that the escape response may only give protection against slower predators. Also, Jonsson & Tiselius (1990) found high clearance rates for *Acartia tonsa* when feeding on *Strobilidium spiralis* despite its jumping behavior, suggesting that *A. tonsa* is a more efficient predator on fast-moving prey than *A. clausi*. The lack of escape responses and the presence of a dense lorica around the cell body suggest a different defense strategy for the tintinnid *Metacylis* sp. It can be speculated that the lorica may protect motionless tintinnids against some predators, causing rapid sinking during the attack and making capture and handling more difficult. The lorica may also shield the diffusion of chemical substances that may be required by some predators to complete ingestion (Capriulo et al. 1981). However, tintinnids have been found in the gut contents and fecal pellets of copepods, and many others mesozooplankters (Gilmer & Harbison 1991), indicating that a lorica does not protect against many predators.

Interestingly, the differences in encounter probability and escape ability between *S.spiralis* and *Metacylis* sp. do not explain the ca 7-fold difference in clearance rate found in the feeding experiments. First of all, our behavioral results can underestimate the advantage of the escape response since the analysis did not include events where *Strobilidium spiralis* detects the approaching copepod first and escapes without triggering a copepod attack. Secondly, our prey-predator behavioral interaction was analyzed following the predator with a single prey at a time while feeding experiments were made in mixture of both ciliates.

A prey switching behavior might be an explanation of the high predation pressure on *Metacylis* sp. and it can be supposed that the more easily caught *Metacylis* sp. may have induced a switch to selective feeding by *Acartia clausi* on the tintinnid. There is previous evidence of prey switching in copepods in

relation to prey size and velocity and also to turbulence (Landry & Fagerness 1988; Kiørboe et al. 1996). In natural systems, predators encounter a diversity of prey types, differing not only in abundance but also in size, motility, taste and nutritional quality. In this diverse prey environment, switching should represent a strategy to optimize the predator intake of energy in presence of an alternative prey (Pyke 1984). In effect, switching strategies may have a considerable effect on prey composition in pelagic systems with copepods controlling ciliate abundance due to higher selectivity for ciliates over algae (Gismervik & Andersen 1997).

As concluding remarks, we would like to emphasize the importance of small-scale processes in predator-prey interactions. Not only swimming speed but also the shape of swimming paths can affect dispersal rates and consequently encounter rates. Moreover, we have shown that post-encounter processes, like the ability to escape, can significantly change the outcome of the encounter. Further research should focus on other mechanisms, like switching behavior, which might greatly outweigh escape behavior and predator perceptive abilities considered in isolation.

GENERAL DISCUSSION

GENERAL DISCUSSION

Understanding the role played by zooplankton in the upper ocean and how it can influence the flux of material out of this zone are key topics in marine biological oceanography (Banse 1995; Kiørboe 1997; Wassmann 1998). During the last years, the need for top-down approaches and investigations at the small-scale level has been emphasized on these topics (Verity & Smetacek 1996; Thingstad 1998). With respect to zooplankton, its trophic interaction with ciliates is one of the less understood pathways, despite its potentially great importance for the carbon cycle (i.e. Wassmann 1998). In this thesis, some relevant aspects related to the study of this trophic relationship have been dealt with using different approaches in order to provide a more general picture of it.

The major aim of this thesis has been to study the importance of ciliates in the diet of crustacean zooplankton, in comparison to autotrophic prey. **Chapters I** and **II** analyze the actual contribution of ciliates to the diet of mesozooplankton at the community and species levels, respectively and respond to this objective. The general result that emerges from these studies is that ciliates are indeed an important food source for zooplankton, since they may represent a significant part in the diet of many species. In fact, despite the fact that on average phytoplankton contributed the most to the daily food ration of zooplankton, ciliates can contribute up to 50, 92 and 93 % of the total carbon ingested by the zooplankton community and the studied copepod or cladoceran species, respectively (Fig. 9, **Chapter I**; Fig. 3b, **Chapter II**). Furthermore, if food rations are computed in terms of nitrogen, ciliates appears to supply, on median, the 51% and 34% of the total nitrogen intake for copepods and cladocerans, respectively (**Chapter II**). Their overall contribution to zooplankton diet, however, is not always so high, showing to be < 5% in some situations.

The variability in the importance of ciliates in the diet of zooplankton with respect to phytoplankton reflects in part the diversity of feeding behaviors, characteristic of zooplankton. The species of marine zooplankton considered in this thesis, six copepods and three cladocerans species, were among the ones most abundantly found in the area at the time of the study and were in fact different in feeding behaviors. The second objective of this thesis was to determine if a major part of this fluctuation in the contribution of ciliates to zooplankton diet could be related with the presence and availability of alternative prey, as suggested by previous works conducted with other copepod

species and in different areas (i.e. Gifford & Dagg, 1990; Fessenden & Cowles, 1994; Levinsen et al., 2000). The study presented in **Chapter II** furnishes data along the seasonal succession. The natural assemblages utilized as prey background for the studied predators provided scenarios characterized by diverse potential prey composition (e.g. relative abundance of autotrophic and heterotrophic prey in bloom and no bloom situations). The experiments conducted during the cruise in the Alboran Sea (**Chapter I**) were also designed in order to compare the zooplankton feeding response in contrasting environments (coastal upwelling versus oceanic environments). Unfortunately, the high temporal variability found during the study masked any inshore-offshore water patterns and the repeated visits to stations had to be considered individually. In terms of biomass availability, in these studies ciliate biomass was generally lower than that of phytoplankton (Tab. 2, **Chapter I**; Fig. 3a **Chapter II**). However, prey availability is not only a question of absolute biomass concentration, since zooplankton does not ingest "biomass", but individuals. In this regard, cell size is important. In fact, most copepods seem to effectively feed only on prey > 5 μm, with optimum prey sizes being relatively large (e.g. *Acartia tonsa* 14-70 μm, Berggreen et al. 1988). Information about prey size preference for marine cladocerans however, is very scarce. Some are thought to feed preferentially on small particles (i.e. *Penilia* sp.: Paffenofer & Orcutt, 1986; Turner 1998), others prey on a size range of 20-170 μm (*Podon* sp. and *Evadne* sp.: Egloff et al. 1997). Marine ciliates are typically larger than 5 μm (Finlay & Fenchel, 1996) and in the studies reported here about 50% of the ciliate community was composed of cells larger than 20μm (Fig. 1b, **Chapter II**), this size spectrum making them specifically well suited as potential prey for copepods. On the contrary, the size distribution of phytoplankton was different and varied depending on the group of experiments considered. In the case of the Alboran Sea, at the depth of the Chl*a* maximum, phytoplankton was dominated by cells > 5μm (except for station C2, Fig. 3, **Chapter I**), whereas in the experiments conducted in the Catalan Sea, the > 5μm phytoplankton fraction was only dominant during the seasonal blooms (Fig. 1a, **Chapter II**).

We expected the maximum ciliate contribution to the diet of zooplankton to occur during post bloom situations, or in more oceanic stations, when small (< 5μm) phytoplankton dominated. The results showed in **Chapter II** (Fig. 3) however, do not clearly confirm such pattern. What more clearly comes out from the analysis of these data is that the importance of ciliates in the zooplankton diet is species specific and depends also on the abundance of

ciliates in the field. Thus for instance, when a phytoplankton bloom occurred with an accompanying high ciliate concentration, ciliates were a significant part in the diet of the studied predators. In **Chapter I**, a similar evidence appeared with the experiment in station B1, were ciliates represented almost the 50% of the zooplankton diet despite the phytoplanktonic bloom. At this station, the high contribution of ciliates to daily food ration could be due to the considerable abundance of *Centropages* spp. in the zooplankton community (Tab. 4, **Chapter I**), a copepod with apparent strong tendencies toward carnivory (Bundy & Paffenhöefer 1996).

Higher clearance rates on ciliates than on phytoplankton have been repeatedly reported in the literature as indicative of positive selection. The experiments conducted during this thesis also show copepod clearance rates for ciliates being higher than those found for phytoplankton, suggesting such selectivity. However, clearance rates depend on prey concentration. For this reason, this preference for ciliates was further tested by the use of the Chesson's selectivity index, which is independent on prey density. Results from **Chapter II** indicated a positive selection for ciliates in most of the experiments conducted, i.e. ciliates were ingested in a higher proportion than expected from their field abundance. However, clear prey switching mechanisms (thus e.g. a switch from carnivory to herbivory as a consequence of food availability), as we expected, do not clearly emerge from the experiments (Fig. 5, **Chapter II**). What do these results suggest? Switching responses are not necessarily on-off responses, which imply the complete exclusion of certain prey type from the diet. In this sense, it seems reasonable that when available, even when alternative prey (i.e. phytoplankton) were abundant, ciliates were actively selected (but not exclusively) and ingested. The analysis of the data in **Chapter II** also suggest that this kind of feeding responses (e.g. feeding on ciliates in spite of high phytoplankton concentration) could be highly species-specific.

But if ciliates seem to be positive selected even under the presence of abundant alternative prey, why do copepods select ciliates? Certainly, prey availability in terms of abundance and adequate size determines the conditions the predators must cope with. However, other qualities of the prey could also play a significant role in determining the preference by a predator. In the case of ciliates, higher nutritional quality has often been suggested as possible causes of preference, despite very few studies were conducted on this subject. Field studies showed certain correlation between copepod egg production rates

(EPR) and ciliate biomass (Kleppel et al. 1991) and in the laboratory the inclusion of ciliates in the diet of copepods rendered higher EPR (Stoecher & Egloff 1987). However, this evidence was sometimes relatively weak (e.g. poor correlation, inadequate non-ciliate diets in laboratory experiments). The first attempt to study nutritional quality of ciliates (Ederington et al. 1995) showed lower EPR of the copepod *Acartia tonsa* when fed on a ciliate in comparison with when fed on a diatom, and related it to a poor content of suitable fatty acids of the ciliate for copepod egg production. However, two main limitations can be found in this work that made evident the need for further studies. The use of a bacterivorous ciliate (*Pleuronema* sp.) not very representative of the truly planktonic marine ciliate community, and the prey concentrations used in the experiment (1700 cells ml^{-1}), really far from much lower ciliate concentrations typically found in the oceans.

In **Chapter III** the biochemical composition of two common genera of marine ciliates (*Strombidium* and *Mesodinium*) was analyzed, for comparison purposes, together with one other heterotrophic species (the dinoflagellate *Gyrodinium dominans*), and with two autotrophic prey (*Rhodomonas salina* and *Gymnodinium sanguineum*). Stoichiometry based on C:N ratios (estimated by this study or by conversion factors from the literature), did not evidence important differences among the ciliates species and the other prey types, either heterotrophs or autotrophs, contrary to previously assumed (Stoecker & Capuzzo 1990). The studied species however, showed different fatty acid profiles, with dissimilar concentrations of some fatty acid considered essential for copepod reproduction (Sargent & Falk-Petersen 1988; Harrison 1990) among species. Thus the concentration of the fatty acid 22:6ω3 was particularly high in *Mesodinium pulex*, whereas *Strombidium inclinatum* appeared very poor in polyunsaturated fatty acids (PUFA) contents (Tab. 2, **Chapter III**). The effect of these diets, different nutritionally, on the reproduction of the copepod *Acartia tonsa* was tested feeding the copepod with prey concentrations not exceeding reasonable values found in coastal areas (see Tab. 4, **Chapter III**). Due to differences in the biomass offered as food source to the copepods, egg production efficiency ratios (EPE = egg production rate/ingestion rate) and not directly egg production rates were compared. EPE with the heterotrophic prey diet (two ciliate spp. + two dinoflagellate spp.) did not differ from that obtained with the autotrophic diet (except for *Gymnodinium sanguineum*, Fig. 1, **Chapter III**), and fell within the range of estimated EPE for metazoans (Straile, 1997). This result contrasts, in part, to prior expectations of high egg production rates due to ciliate ingestion. Reproduction success however, is not

only a question of the lying of eggs (i.e. the number of egg laid) but also of the viability of these eggs (egg hatching success). Egg hatching success was well related to the ingestion of essential fatty acids, indicating that these components might be more limiting for egg viability than for egg production. (See Fig. 4, **Chapter III**). Nutritional quality however, is difficult to study because of the diversity of components that can be essential for any vital process. For instance, components such as sterols, amino acids, proteins or vitamins, not considered in this study may be more essential for copepod egg production and hatching success than the traditionally considered fatty acids. When interpreting these results and attempting to extrapolate them to the field, one must be aware that in the experiments reported here, prey types were offered to the copepod as mono diets, to directly measure the ciliate effect on copepod reproduction. In the field copepods encounter and certainly ingest more than one prey type. It can be assumed that, even if as a single prey some ciliate species may lack some essential fatty acids for copepods, their nutritional deficiencies could be compensated by the ingestion of other prey types.

The deeper we go into the trophic relationship between zooplankton and ciliates, the clearer it becomes that mechanistic approaches at the small-scale level can be useful tools for the study of trophic interactions. Detailed behavioral studies of zooplankters were scarce in the literature until two decades ago, when due to the use of high-speed cinematography swimming behaviors, predation strategies and different predator-prey interactions started to be described. In relation to the trophic interaction ciliates-copepods, previous studies showed that copepod eat ciliates through ambush feeding behaviors (Jonsson & Tiselius 1990; Saiz & Kiørboe 1995). Ciliates have shown a broad diversity of swimming patterns, including different trajectories, rapid jumps or periods of inactivity and sinking (e.g. Buskey et al. 1993). The presence of escape reactions to avoid predator attacks was demonstrated in some naked ciliate in both marine (Jonsson & Tiselius 1990) and freshwater systems (e.g. Gilbert 1994). Other ciliates (Tintinnids) possess a solid protection around the body (the *lorica*) which was traditionally considered a possible strategy to avoid capture (Capriulo et al. 1981; Fenchel 1987). Predator and prey abilities to detect the presence of each other, to trigger either attack or escape, widely depend on the predator-prey pair. **Chapter IV** focused on the study of some of the different behavioral components that play a role in the copepod-ciliate interaction, and also on how they might translate into prey selectivity patterns by a predator and, in last instance, shape the transfer of energy and matter within planktonic food webs.

In this regard, a question to address was to determine how different swimming behaviors of prey (ciliates) could affect the probability of being encountered ad captured by a predator (i.e. copepod). To simplify the study, two common ciliate species, an aloricate ciliate and a tintinnid similar in size, were chosen and their swimming behavior described (**Chapter IV**). This comparison revealed differences in the shape of the trajectory, average velocities and presence/absence of jump events. As a consequence of these diversities, the swimming trajectories differed also in the dispersal rates (time required by the ciliate to move off to the periphery of a determined area), with important implications for the encounter rate with the predator, the copepod *Acartia clausi*. Indeed, the ciliate that was estimated to be encountered more often by the predator was actually the less captured (as shown from behavioral observations) and less ingested when offered in a mixture with the other ciliate species (as observed from grazing experiments). Post-encounter processes (as presence of escape reactions) resulted significant for the fate of this trophic relationship, the *lorica* of the tintinnid appearing a poorly effective mechanism of defense against the studied predator. The results from the behavioral observations were a useful complement to the grazing rates obtained from incubation experiments since they helped understanding the mechanisms involved in pre encounter and post encounter processes. The high effectiveness of escape reactions in avoiding predator attack was confirmed. Furthermore, a need for new models to determine the effective prey concentration perceived by the predator, with the inclusion of the characteristic of the swimming path is emphasized.

The last objective of this thesis was to determine the effect of zooplankton predation on the ciliate communities. The experiments carried out with both, the whole zooplankton community and the different copepods and cladocerans studied species showed that, despite the high feeding rates on ciliates, zooplankton predation had a low impact on the ciliate populations (< 2.5 % of the ciliate standing stock grazed per day, **Chapter I** and **II**). Although higher impacts can be found in the literature (Dolan 1991; Fessenden & Cowles 1994), the estimates reported here fell within the range of values found in most previous studies (Atkinson et al. 1996; Atkinson 1996; Londsale et al. 2000; Batten et al. 2001; Zeldis et al. 2002; Calbet et al. 2002). Within the values found in this study, the relatively highest impacts (up to 7%) were shown in period of very scarce ciliate abundance or when alternative prey was scarce, as well as when predator abundance was especially high. The overall low impacts

observed indicate that mesozooplankton was not able to control ciliate populations in the studied systems, suggesting a bottom-up control of them.

To end this discussion section, special mention is due to two factors, not considered in this study that may affect some of the conclusions drawn, strengthening even more the importance of the ciliate-copepod interaction. First of all, turbulence has not been considered in the experiments. Turbulence is an ubiquitous characteristic of the oceans, which can affect encounter rates between predator and prey and also, at high levels, disrupt feeding mechanisms or impair prey detection. Feeding experiments run under different turbulence intensities have shown that moderate turbulence levels may enhance up to three times the ingestion rate of ciliates by copepods (Saiz & Kiørboe 1995; Caparroy et al.1998) and induce a change in selectivity patterns by shifting copepod feeding behavior from herbivory into a ciliate diet (Saiz & Kiørboe 1995; Kiørboe et al. 1996). All the estimates of predation impact on ciliate communities by zooplankton have been done under the assumption of absence of turbulence (i.e. incubations were performed in calm waters). Similarly, the rates presented here could be underestimated because field experiments were conducted with water from the deep chlorophyll maximum (**Chapter I**) or from ca 1 m below surface (**Chapter II**), and assuming homogeneous distribution of ciliate concentrations throughout the water column. It is known, however, that ciliates are not homogeneously distributed in the vertical and horizontal (Montagnes et al. 1999; Jonsson 1989), and furthermore that copepods are able to found and remain in even small-sized patches of prey (Tiselius 1992; Saiz et al. 1993). As clearly pointed out by Mullin & Brooks (1976) heterogeneous prey distribution can result in much higher feeding rates for copepods that homogeneous ones, depending on the shape of the functional response. Consequently, one might expect that both, the contribution of ciliates in the diet of zooplankters and the predation pressure on ciliates, could be much higher than reported here, if the patchy distribution of plankton is taken into account.

This thesis has been an attempt to characterize the transfer of matter and energy between micro and mesozooplankton, with special attention to the factors implicated in this trophic relationship. What emerged is that the micro-mesozooplankton link is a significant one, ciliates being important contributors to the diet of zooplankton and usually selected preferentially over phytoplankton. Factors like the presence of alternative prey, prey nutritional quality and behavioral interactions may largely influence this trophic

relationship. At it has been shown both broad and detailed analyses (at the community and species levels) have proved to be useful tools to understanding the importance of this trophic link.

CONCLUSIONS

CONCLUSIONS

The study conducted along this thesis lead to the following main conclusions:

1. Ciliates represent an important part (both, in carbon and nitrogen) of the diet of copepods and cladocerans, as well as of the whole zooplankton community. Thus, the contribution of the trophic link between ciliates and mesozooplankton cannot be ignored when global balances of the energy and matter fluxes are made.

2. Ciliate contribution to the diet of copepod and cladocerans was variable, being species-specific and dependent on ciliate availability. Maximum contributions were detected when ciliate concentrations in the field were high (>12 cells ml^{-1}).

3. Copepod clearance rates on ciliates were higher than those on phytoplankton, confirming a tendency already observed in previous studies. The results from the selectivity index revealed that ciliates were positively selected over phytoplankton by most of the studied species.

4. The presence of alternative prey (phytoplankton > 5μm) at higher concentrations (bloom situations) did not clearly induce a switching to a more herbivorous diet in most of the studied species. It appeared however, that a change from exclusive selection for ciliates towards shared selection with phytoplankton > 5μm can occur in some species.

5. Contrarily to expectations, the copepod egg production efficiency (egg production rate/ingestion rate) derived from ciliate ingestion did not appear to be different from that derived from autotrophic species.

6. The studied ciliates showed to be different in their fatty acids content and composition. *Mesodinium pulex* had a higher total fatty acid content and was also richer in PUFA than *Strombidium inclinatum*.

7. The ingestion of certain fatty acids (18:3ω3, 20:5ω3, 22:6 ω3 and other polyunsaturated fatty acids) by copepod appeared to be more limiting for egg hatching success than for egg production rates.

8. Behavioral studies on predator-prey relationship can help to understand the mechanisms behind the feeding processes in zooplankton. Ciliate behaviors (both swimming speed and the geometry of the swimming path) had large effects on the encounter rates with a potential predator. Post-encounter processes (e.g. ability to detect a potential predator or immediate attack, and escape successfully) had also important implications for the fate of the trophic interaction.

9. Despite the important predation rates on ciliates exhibited by zooplankton in the studied areas, zooplankton feeding did not significantly affect the ciliate populations, being on median the predation impact on the ciliate standing stock < 2.5%.

REFERENCES

REFERENCES

Adrian R, Schneider-Olt B (1999) Top-down effects of crustacean zooplankton on pelagic microorganisms in a mesotrophic lake. J Plankton Res 21:2175-2190

Alcaraz M, Paffenhöfer GA, Strickler JR (1980) Catching the algae: a first account of visual observations on filter-feeding calanoids. In: Evolution and Ecology of Zooplankton Communities (ed. W.C. Kerfoot) New England Univ. Press pp 241-248

Alcaraz M (1985) Vertical distribution of zooplankton biomass during summer stratification in the Western Mediterranean. In: GIBBS PE (ed) Proceedings of the 19th EMBS. Cambridge Univ. Press pp 135-143

Alldredge AL, Silver MW (1988) Characteristics, dynamics and significance of marine snow. Prog Oceanogr 20: 41-82

Arin L, Morán XAG, Estrada M (2002) Phytoplankton size distribution and growth rates in the Alboran Sea (SW Mediterranean): Short-term variability related to mesoscale hydrodynamics. J Plankton Res 24:1019-1033

Atkinson A (1996) Subantarctic copepods in an oceanic, low chlorophyll environment: ciliate predation, food selectivity and impact on prey populations. Mar Ecol Prog Ser 130: 85-96

Azam F, Fenchel F, Field T, Gray JG, Meyer-Reil JS, Thingstad LA (1983) The ecological role of water-column microbes in the sea. Mar Ecol Prog Ser 10: 257-263

Bagøien E, Miranda A, Reguera B, Franco JM (1996) Effects of two paralytic shellfish toxin producing dinoflagellates on the pelagic harpacticoid copepod *Euterpina acutifrons*. Mar Biol 126:361-369

Banse K (1995) Zooplankton: pivotal role in the control of ocean production. ICES J Mar Sci 52:265-277

Batten SD, Fileman ES, Halvorsen E (2001) The contribution of microzooplankton to the diet of mesozooplankton in an upwelling filament off the north west coast of Spain. Progress in Oceanography 51: 385-398

Berggreen U, Hansen B, Kiørboe T (1988) Food size spectra ingestion and growth of the copepod *Acartia tonsa* during development: implications for determination of copepod production. Mar Biol 99:341-352

Brown MR, Jeffrey SW, Volkman JK, Dunstan GA (1998) Nutritional properties of microalgae for mariculture. Aquaculture 151: 315-331

Bundy MH, Paffenhöefer GA (1993) Innervation of copepod antennules investigated using laser scanning confocal microscopy. Mar Ecol Prog Ser 102: 1-14

Bundy MH, Paffenhoefer, GA (1996) Analysis of flow fields associated with freely swimming calanoid copepods. Mar Ecol Prog Ser 133: 99-113

Burns CW, Gilbert JJ (1993) Predation on ciliates by freshwater calanoid copepods: rates of predation and relative vulnerabilities of prey. Fresh Biol 30: 377-393

Burns CW, Shallemberg M (2001) Short-term impacts of nutrients, *Daphnia*, and copepods on microbial food-webs of an oligotrophic and eutrophic lake of New Zealand. J Mar and Fresh Res 35: 695-710

Buskey EJ, Coulter C, Strom S (1993) Locomotory patterns of microzooplankton: potential effects on food selectivity of larval fish. Bull of Mar Sci 53 (1): 29-43

Cahoon LB (1981) Reproductive response of *Acartia tonsa* to variations in food ration and quality. Deep-Sea Res 28:1215-1221

Calbet A, Alcaraz M (1996) Effects of constant and fluctuating food supply on egg production rates of *Acartia grani* (Copepoda: Calanoida). Mar Ecol Prog Ser 140:33-39

Calbet A, Landry MR (1999) Mesozooplankton influences on the microbial food web: direct and indirect trophic interactions in the oligotrophic open ocean. Limnol Oceanogr 44:1370-1380

Calbet A, Landry MR, Scheinberg RD (2000a) Copepod grazing in a subtropical bay: species-specific responses to a midsummer increase in nanoplankton standing stock. Mar Ecol Prog Ser 193:75-84

Calbet A Trepat I, Arin L (2000b) Naupliar growth versus egg production in the calanoid copepod *Centropages typicus*. J Plankton Res 22:1393-1402

Calbet A, Garrido S, Saiz E, Alcaraz M, Duarte CM (2001) Annual zooplankton succession in coastal NW Mediterranean waters: the importance of the smaller size fractions. J Plank Res 23: 319-331

Calbet A (2001) Mesozooplankton grazing effect on primary production: A global comparative analysis in marine ecosystems. Limnol Oceanogr 46:1824-1830

Caparroy P, Perez MT, Carlotti F (1998) Feeding behaviour of *Centropages typicus* in calm and turbulent conditions. Mar Ecol Progr Ser 168: 109-118

Capriulo GM, Gold M, Okubo A (1981). Evolution of the lorica in tintinnids: a possible selective advantage. Ann Inst Oceanogr, Paris 58 (S): 319-324

Carrick HJ, Fahnenstiel GL, Stoemer EF, Wetzel RG (1991) The importance of zooplankton-protozoan trophic couplings in Lake Michigan. Limnol Oceanogr 36:1335-1345

Castell JD (1982) Fatty acid metabolism in crustaceans. In: Pruder GD, Langdon C, Conklin D (eds) Proceedings of the second international conference on

aquaculture nutrition: biochemical and physiological approaches to shellfish nutrition. Lousisiana State University, Baton Rouge, pp 124-145

Chesson J (1983) The estimation and analysis of preference and its relationship to foraging models. Ecology 64: 1297-1304

Chisholm LA, Roff JC (1990) Size-weight relationships and biomass of tropical neritic copepods off Kingston, Jamaica. Mar Biol 106: 71-77

Christie WW (1989) Gas Chromatography and lipids: a practical guide. The Oily Press, Ayr, Scotland

Claustre H, Marty JC, Cassiani L, Dagaut J (1988) Fatty acid dynamics in phytoplankton and microzooplankton communities during a spring bloom in the coastal Ligurian Sea: ecological implications. Mar Microb Food Webs 3:51-66

Conover RJ (1968) Zooplankton life in a nutritional dilute environment. Am Zoolog 8: 107-118

Coste B, Le Corre P, Minas HJ (1988) Re-evaluation of the nutrient exchanges in the Strait of Gibraltar. Deep-Sea Res 35:765-775

Cowles TJ (1979) The feeding response of copepods from the Peru upwelling system: food size selection. J Mar Res 37:601-622

Cowles TJ, Olson RJ, Chisholm SW (1988) Food selection by copepods: discrimination on the basis of food quality. Mar Biol 100: 41-49

Cushing DH (1989) A difference in structure between ecosystems in strongly stratified waters and in those that are only weakly stratified. J Plankton Res 11: 1-13

Dam, HG, Peterson W, Bellantoni DC (1994) Seasonal feeding and fecundity of the calanoid copepod *Acartia tonsa* in Long Island Sound: is omnivory important to egg production? Ecology and morphology of copepods. Hydrobiol 292-293: 191-199

Davis CS, Flierl GR, Wiebe PH, Franks PJS (1991) Micropatchiness, turbulence and recruitment in plankton. J Mar Res 49:109-151

Davis CS, Alatalo P (1992) Effects of constant and intermittent food supply on life-history parameters in a marine copepod. Limnol Oceanog 37: 1618-1639

DeBiase AE, Sanders RW, Porter KG (1990) Relative nutritional value of ciliate protozoa and algae as food for *Daphnia*. Microb Ecol 19: 199-210

Delgado M (1990) Phytoplankton distribution along the Spanish coast of the Alboran Sea. Scient Mar 54:169-178

Delgado M, Alcaraz M (1999) Interactions between red tide microalgae and herbivorous zooplankton: the noxious effects of *Gyrodinium corsicum* (Dinophyceae) on *Acartia grani* (Copepoda: Calanoida). J Plankton Res 21:2361-2371

Desvilettes C, Bourdier G, Amblard C, Barth B (1997) Use of fatty acids for the assessment of zooplankton grazing on bacteria, protozoans and microalgae. Freshw Biol 38:629-637

Dolan JR (1991) Microphagous ciliates in mesohaline Chesapeake Bay waters: estimates of growth rates and consumption by copepods. Mar Biol 111: 303-309

Ederington MC, McManus GB, Harvey HR (1995) Trophic transfer of fatty acids, sterols, and a triterpenoid alcohol between bacteria, a ciliate, and the copepod *Acartia tonsa*. Limnol Oceanogr 40:860-867

Egloff DA, Fofonoff PW, Onbé T (1997) Reproductive biology of marine cladocerans. Adv Mar Biol 31:79-168

Fenchel T (1980) Suspension feeding in ciliated protozoa: feeding rates and their ecological significance. Microb Ecol 6: 13-25

Fessenden L, Cowles TJ (1994) Copepod predation on phagotrophic ciliates in Oregon coastal waters. Mar Ecol Prog Ser 107: 103-111

Finlay BJ, Fenchel T (1996) Ecology: role of ciliates in the natural environment. In Ciliates as organisms. Klaus Hausmann, Phyllis C. Bradbury (Eds). Gustav Fischer Stuttgart Jena Lubec Ulm

Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226: 497-509

Froneman PW, Pakhomov EA, Perissinotto R, McQuaid CD (1996) Role of microplankton in the diet and daily ration of Antarctic zooplankton species during austral summer. Mar Ecol Prog Ser 143: 15-23

Frost BW (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnol Oceanogr 17: 805-815

Gallienne CP, Robins DB (2001) Is *Oithona* the most important copepod in the world's oceans? J Plank Res 23: 1421-1432

García A, Camiñas JA (1985) Coastal variability of zooplankton biomass in the Northwestern sector of the Albora Sea. Rapp Comm Int Mer Médit 29:309-310

Gasol JM, Del Giorgio PA, Duarte CM (1997) Biomass distribution in marine planktonic communities. Limnol Oceanogr 42:1353-1363

Gerritsen J, Strickler J R (1977) Encounter probabilities and community structure in zooplankton: a mathematical model. J Fish Res Can 34: 73-82

Gifford DJ, Dagg MJ (1988) Feeding of the estuarine copepod *Acartia tonsa* Dana: Carnivory vs herbivory in natural microplankton assemblages. Bull Mar Sci 43:458-468

Gifford DJ (1991) The protozoan-metazoan trophic link in pelagic ecosystems. J Protozool 38: 81-86

Gifford DJ, Dagg MJ (1991) The microzooplankton-mesozooplankton link: consumption of planktonic protozoa by the calanoid copepods *Acartia tonsa* Dana and *Neocalanus plumchrus* Murukawa. Mar Microb Food Webs 5: 161-177

Gilbert JJ, Jack JD (1993) Rotifers as predators on small ciliates. Hydrobiologia 255/256: 247-253

Gilbert JJ (1994) Jumping behavior in the oligotrich ciliate *Strombilidium velox* and *Halteria grandinella*, and its significance as a defence against rotifer predators. Microb Ecol 27, 189-200

Gilmer RW, Harbison GR (1991) Diet of *Limacina helicina* (Gastropoda Thecosomata) in arctic waters in midsummer. Mar Ecol Prog Ser 77: 125-134

Gismervik I, Andersen T (1997) Prey switching by *Acartia clausi*: experimental evidence and implications of intraguild predation assessed by a model. Mar Ecol Prog Ser 157:247-259

Guillard RRL, Hargraves PE (1993) *Stichochrysis immobilis* is a diatom, not a chrysophyte. Phycol 32: 234-236

Guisande C, Riveiro I, Maneiro I (2000) Comparisons among the amino acid composition of females, eggs and food to determine the relative importance of food quantity and food quality to copepod reproduction. Mar Ecol Prog Ser 202:135-142

Hansen B, Bjoernsen PK, Hansen PJ (1994) The size ratio between planktonic predators and their prey. Limnol Oceanogr 39:395-403

Harrison KE (1990) The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans: a review. J Shellifish Res 9: 1-28

Harvey HR, Ederington MC, McManus GB (1997) Lipid composition of marine ciliates *Pleuronema* sp. and *Fabrea salina*: shifts in response to changes in diet. J Euk Microbiol 44: 189-193

Heinle DR, Harris RP, Ustach JF, Flemer DA (1977) Detritus as food for estuarine copepods. Mar Biol 40:341-353

Houde SEL, Roman MR (1987) Effects of food quality on the functional ingestion response of the copepod *Acartia tonsa*. Mar Ecol Prog Ser 40: 69-77

Ikeda T (1985) Metabolic rates of epipelagic marine zooplankton as a function of body mass and temperature. Mar Biol 85:1-11

Irigoien X, Head R, Klenke U, MeyerHarris B, Harbour D, Niehoff B, Hirche HJ (1998) A high frequency time series at weathership M, Norwegian Sea, during the 1997 spring bloom: feeding of adult female *Calanus finmarchicus*. Mar Ecol Prog Ser 172: 127-137

Jack JD, Gilbert JJ (1993) Susceptibilities of different-sized ciliates to direct suppression by small and large cladocerans. Fresh Biol 29: 19-29

Jakobsen HH (2002) Escape of protists in predator-generated feeding currents. Aquat Microb Ecol 26: 271-281

Jones RH, Flynn KJ, Anderson TR (2002) Effect of food quality on carbon and nitrogen growth efficiency in the copepod *Acartia tonsa*. Mar Ecol Prog Ser 235: 147-156

Jiménez F, Rodríguez J, Bautista B, Rodríguez V (1987) Relations between chlorophyll, phytoplankton cell abundance and biovolume during a winter bloom in Mediterranean coastal waters. J Exp Mar Biol Ecol 105:161-173

Jónasdóttir SH (1994) Effect of food quality on the reproductive success of *Acartia tonsa* and *Acartia hudsonica*: laboratory observations. Mar Biol 101:67-81

Jónasdóttir SH, Kiørboe T (1996) Copepod recruitment and food composition: Do diatoms affect hatching success? Mar Biol 125: 743-750

Jonsson, PR (1989) Vertical distribution of planktonic ciliates: an experimental analysis of swimming behaviour. Mar Ecol Prog Ser 52:39-53

Jonsson PR, Tiselius P (1990) Feeding behavior, prey detection and capture efficiency of the copepod Acartia tonsa feeding on planktonic ciliates. Mar Ecol Prog Ser 60: 35-44

Kahl A (1932) Urtiere oder Protozoa. I Wimpertiere oder Ciliata (Infusoria). 3 Spirotricha. In: Dahl F (ed) Die Tierwelt Deutschland und der angrenzenden Meeresteile. Gustav Fischer Verlag, Jena

Karl DM, Hebel DV, Bjoerkman K, Letelier RM (1998) The role of dissolved organic matter release in the productivity of the oligotrophic North Pacific Ocean Limnol Oceanogr 43: 1270-1286

Kiørboe T, Saiz E, Viitasalo M (1996) Prey switching behavior in the planktonic copepod *Acartia tonsa*. Mar Ecol Prog Ser 143: 65-75

Kiørboe T, Visser A (1999) Predator and prey perception in copepods due to hydromechanical signals. Mar Ecol Prog Ser 179: 81-95

Kiørboe T, Saiz E, Visser A (1999) Hydrodynamic signal perception in the copepod *Acartia tonsa*. Mar Ecol Prog Ser 179: 97-111

Kiørboe T, Møhlenberg F, Riisgård HU (1985) In situ feeding rates of planktonic copepods: a comparison of four methods. J Exp Mar Biol Ecol 88: 67-81

Kiørboe T, Møhlenberg F, Hamburger K (1985) Bioenergetics of the planktonic copepod *Acartia tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action. Mar Ecol Prog Ser 26: 85-97

Kiørboe T (1996) Material flux in the water column. In Eutrophication in coastal marine ecosystems. American Geophysical Union. Coastal and Estuarine Studies 52: 67-94

Kiørboe T, Saiz E, Viitasalo M (1996) Prey switching behavior in the planktonic copepod *Acartia tonsa*. Mar Ecol Prog Ser 143: 65-75

Kiørboe T (1997) Population regulation and role of mesozooplankton in shaping marine pelagic food webs. Hydrobiol 363: 13-27

Kirk KL (1985) Water flows produced by *Daphnia* and *Diaptomus*: implications for prey selection by mechanosensory predators. Limnol Oceanogr 30: 679-686

Klein Breteler WCM, Schogt N, González SR (1990) On the role of food quality in grazing and development of life stages and genetic change of body size during cultivation of pelagic copepods. J Exp Mar Biol Ecol 135: 177-189

Klein Breteler WCM, Schogt N, Baas M, Schouten S, Kraay GW (1999) Trophic upgrading of food quality by protozoans enhancing copepod growth: role of essential lipids. Mar Biol 135:191-198

Kleppel GS, Frazel D, Pieper RE, Holliday DV(1988) Natural diets of zooplankton off southern California. Mar Ecol Prog Ser 49: 231-241

Kleppel GS, Holliday DV, Pieper RE (1991) Trophic interactions between copepods and microzooplankton: a question about the role of diatoms. Limnol Oceanogr 36:172-178

Kleppel GS (1993) On the diets of calanoid copepods. Mar Ecol Prog Ser 99: 183-195

Kleppel GS, Burkart CA (1995) Egg production and the nutritional environment of *Acartia tonsa*: the role of food quality in copepod nutrition. ICES J Mar Sci 52:297-304

Kleppel GS, Burkart CA, Houchin L (1998) Nutrition and the regulation of egg production in the calanoid copepod *Acartia tonsa*. Limnol Oceanogr 43: 1000-1007

Kleppel GS, Hazzard SE (2000) Diet and egg production of the copepod *Acartia tonsa* in Florida Bay. II. Role of the nutritional environment. Mar Biol 137: 111-121

Koski M, Breteler WK, Schogt N (1993) Effect of food quality on rate of growth and development of the pelagic copepod *Pseudocalanus elongatus* (Copepoda, Calanoida). Mar Ecol Prog Ser 170: 169-187

Koski M, Breteler WK, Schogt N (1998) Effect of food quality on rate of growth and development of the pelagic copepod *Pseudocalanus elongatus* (Copepoda, Calanoida). Mar Ecol Prog Ser 170: 169-187

Krebs JR, Davies NB (1993) An introduction to behavioural ecology. Blackwell, Oxford

Landry MR (1980) Detection of prey by *Calanus pacificus*: implications of the first antennae. Limnol Oceanogr 25: 545-549

Landry MR (1981) Switching between herbivory and carnivory by the planktonic marine copepod *Calanus pacificus*. Mar Biol 65: 77-82

Landry MR, Fagerness VL (1988) Behavioral and morphological influences on predatory interactions among marine copepods. Bull Mar Sci 43: 509-529

Leakey RJG, Burkill PH, Sleigh MA (1994) A comparison of fixatives for the estimation of abundance and biovolume of planktonic ciliate populations. J Plankton Res 16:375-389

Lee HW, Ban S, Ando Y, Ota T, Ikeda T (1999) Deleterious effect of diatoms diets on egg production and hatching success in the marine copepod *Pseudocalanus newmani*. Plank Biol Ecol 46: 104-112

Legendre L, Rassoulzadegan F (1995) Plankton and nutrient dynamics in marine waters. Ophelia 41: 153-172

Legendre L, Rivkin RB (2002) Pelagic food webs: Responses to environmental processes and effects on the environment. Ecol Res 17: 143-149

Levinsen H, Turner JT, Nielsen TG, Hansen BW (2000) On the trophic coupling between protists and copepods in arctic marine ecosystems. Mar Ecol Prog Ser 204: 65-77

Lima SL, Dill LM (1990) Behavioral decision made under the risk of predation: a review and prospectus. Can J Zool 68: 619-640

Lindholm T (1985) *Mesodinium rubrum*: a unique photosynthetic ciliate. Adv Aquat Microbiol 3: 1-48

Longhurst AR (1991) Role of the marine biosphere in the global carbon cycle. Limnol Oceanogr 36: 1507-1526

Lonsdale D, Caron DA, Dennett MR, Schaffner R (2000) Predation by *Oithona* spp on protozooplankton in the Ross Sea, Antarctica. Deep-Sea Res II 47:3273-3283

Mackas DL, Denman KL, Abbott MR (1985) Plankton patchiness: biology in the physical vernacular. Bull Mar Sci 37: 652-674

Matsuyama Y, Miyamoto M, Kotani Y (1999) Grazing impacts of the heterotrophic dinoflagellate *Polykrikos kofoidii* on a bloom of *Gymnodinium catenatum*. Aquat Microb Ecol 17:91-98

Menden-Deuer S, Lessard EJ (2000) Carbon to volume relationships for

dinoflagellates, diatoms, and other protist plankton. Limnol Oceanogr 45:569-579

Merrell JR, Stoecker DK (1998) Differential grazing on protozoan microplankton by developmental stages of the calanoid copepod *Eurytemora affinis* Poppe. J Plank Res 20: 289-304

Minas HJ, Coste B, Le Corre P, Minas M, Raimbault P (1991) Biological and geochemical signatures associated with the water circulation through the Strait of Gibraltar and in the western Alboran Sea. J Geophys Res, 96:8755-8771

Modigh M (2001) Seasonal variations of photosynthetic ciliates at a Mediterranean coastal site. Aquat Microb Ecol 23: 163-175

Montagnes DJS, Poulton AJ, Shammon TM (1999) Mesoscale, finescale and microscale distribution of micro- and nanoplankton in the Irish Sea, with emphasis on ciliates and their prey. Mar Biol 134: 167-179

Morán XAG, Estrada M (2001) Short-term variability of photosynthetic parameters and particulate and dissolved primary production in the Alboran Sea, SW Mediterranean. Mar Ecol Prog Ser 212:53-67

Mullin MM, Brooks ER (1976) Some consequences of distributional heterogeneity of phytoplankton and zooplankton. Limnol Oceanogr: 21: 784-796

Nakamura Y, Turner JT (1997) Predation and respiration by the small cyclopoid copepod *Oithona similis*: How important is feeding on ciliates and heterotrophic flagellates? J Plankton Res 19:1275-1288

Nielsen TG, Kiørboe T (1994) Regulation of zooplankton biomass and production in a temperate, coastal ecosystem. II: Ciliates. Limnol Oceanogr 39 (3): 508-519

Nival P, Nival S (1973) Particle-filtering ability of planktonic copepods. Ann. Inst Oceanogr Monaco 49: 135-144

Ohman M, Runge JA (1994) Sustained fecundity when phytoplankton resources are in short supply: omnivory by *Calanus finmarchicus* in the Gulf of St. Lawrence. Limnol Oceanogr 39: 21-36

Olsen Y (1998) Lipids and essential fatty acids in aquatic food webs: what can freshwater ecologists learn from mariculture? In: Arts MT, Wainman BC (eds.) Lipids in freshwater ecosystems. Springer-Verlag, New York pp 161-202

Omori M, Ikeda T (1984) Methods in marine zooplankton ecology. New York

Paffenhöfer G-A, Orcutt JD Jr (1986) Feeding growth and food conversion of the marine cladoceran *Penilia avirostris*. J Plankton Res 8:741-754

Parsons TR, Maita Y, Lalli CM (1984a) A manual of chemical and biological

methods for seawater analysis. Pergamon Press, Oxford
Parsons TR, Takahashi M, Hargrave B (1984b) Biological Oceanographic Processes, 3rd ed. Pergamon Press, Oxford
Pérez MT, Dolan JR, Fukai E (1997) Planktonic oligotrich ciliates in the NW Mediterranean: growth rates and consumption by copepods. Mar Ecol Prog Ser 155: 89-101
Perkins H, Kinder T, La Violette P (1990) The Atlantic inflow in the western Alboran Sea. J Phys Oceanogr 20:242-263
Pierce RW, Turner JT (1992) Ecology of planktonic ciliates in marine food webs. Aquatic Sciences 6: 139-181
Pitta P, Giannakourou A (2000) Planktonic ciliates in the oligotrophic Eastern Mediterranean: vertical, spatial distribution and mixotrophy. Mar Ecol Prog Ser 194: 269-282
Pomeroy LR (1974) The ocean's food web: A changing paradigm. BioScience 24:499-504
Pond D, Harris R, Head R, Harbour D (1996) Environmental and nutritional factors determining seasonal variability in the fecundity and egg viability of *Calanus helgolandicus* in coastal waters off Plymouth, UK. Mar Ecol Progr Ser 143:45-63
Price HJ, Paffenhoefer GA (1984) Effects of feeding experience in the copepod *Eucalanus pileatus*: A cinematographic study. Mar Biol 84: 35-40
Putt M, Stoecker DK (1989) An experimentally determined carbon: volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. Limnol Oceanogr 34:1097-1103
Pyke GH (1984) Optimal foraging theory: a critical review. Ann Rev Ecol Syst 15: 523-575
Rassoulzadegan F, Laval-Peuto M, RW Sheldon (1988) Partitioning of the food ratio of marine ciliates between pico and nanoplankton. Hydrobiol 159: 75-88
Razouls S, Razouls C, Huntley M (1991) Development and expression of sexual maturity in female *Calanus pacificus* (Copepoda: Calanoida) in relation to food quality. Mar Biol 110: 65-74
Rivkin RB, Legendre L (2002) Roles of food web and heterotrophic microbial processes in upper ocean biogeochemistry: Global patterns and processes. Ecol Res 17: 151-159
Ryther JH (1969) Photosynthesis and fish production in the sea. Science 166: 72-76
Rodríguez J, Blasco JM, Jiménez-Gómez F, Echevarría F, Gil J, Rodríguez V, Ruiz J, Bautista B, Guerrero F (1998) Patterns in the size structure of the

phytoplankton community in the deep fluorescence maximum of the Alboran Sea (southwestern Mediterranean). Deep-Sea Res I 45:1577-1593

Rudstam LG, Danielsson K, Hansson S, Johansson S (1989) Diel vertical migration and feeding patterns of *Mysis mixta* (Crustacea, Mysidacea) in the Baltic Sea. Mar Biol 101:43-52

Saiz E, Tiselius P, Jonsson PR, Verity P, Paffenhoefer GA (1993) Experimental records of the effects of food patchiness and predation on egg production of *Acartia tonsa*. Limnol Oceanogr 38: 280-289

Saiz E, Kiørboe T (1995) Predatory and suspension feeding of the copepod *Acartia tonsa* in turbulent environments. Mar Ecol prog Ser 122: 147-158

Saiz E, Calbet A, Irigoien X, Alcaraz M (1999) Copepod egg production in the western Mediterranean: response to food availability in oligotrophic environments. Mar Ecol Prog Ser 187: 179-189

Sanders RW, Wickham SA (1993) Planktonic protozoa and metazoa: predation, food quality and population control. Mar Microb Food Webs 7:197-223

Sargent JR, Falk-Petersen S (1988) The lipid biochemistry of calanoid copepods. Hydrobiol 167/168: 101-114

Satapoomin S (1999) Carbon content of some common tropical Andaman Sea copepods. J Plank Res 21: 2117-2123

Sherr E, Sherr B (1988) Role of microbes in pelagic food webs: a revised concept. Limnol Oceanogr 33:1225-1227

Sherr EB, Sherr BF, Paffenhöfer GA (1986) Phagotrophic protozoa as food for metazoans: A "missing" trophic link in marine pelagic food webs? Mar Microb Food Webs 1:61-80

Sherr EB, Sherr BF, Albright JL (1987) Bacteria: link or sink? Science 235: 88-89

Siegenthaler U, Sarmiento JL (1993) Atmospheric carbon dioxide and the ocean. Nature 365: 119-125

Smetacek V, Pollehne F (1986) Nutrient cycling in pelagic systems: a reappraisal of the conceptual framework. Ophelia 26: 401-428

Sokal R, Rohlf J (1994) Biometry. Freeman, New York

Steele JH (1974) The structure of marine ecosystems. Harvard University Press, Cambridge

Stoecker DK, Sanders NK (1985) Differential grazing by *Acartia tonsa* on a dinoflagellate and a tintinnid. J Plankton Res 7:85-100

Stoecker DK, Evans GT (1985) Effects of protozoan herbivory and carnivory in a microzooplankton food web. Mar Ecol Prog Ser 25: 159-167

Stoecker DK, Egloff DA (1987) Predation by *Acartia tonsa* Dana on planktonic ciliates and rotifers. J Exp Mar Biol Ecol 110:53-68

Stoecker DK, Capuzzo MCJ (1990) Predation on Protozoa: its importance to zooplankton. J Plankt Res 12(5): 891-908

Stoecker DK, Gifford DJ, Putt M (1994) Preservation of marine planktonic ciliates: losses and cell shrinkage during fixation. Mar Ecol Prog Ser 110: 293-299

Schmidt K, Jónasdóttir SH (1997) Nutritional quality of two cyanobacteria: How rich is 'poor' food? Mar Ecol Prog Ser 15: 1-10

Støttrup JG, Jensen J (1990) Influence of algal diet on feeding and egg-production of the calanoid copepod *Acartia tonsa* Dana. J Exp Mar Biol Ecol 141:87-105

Støttrup JG, Bell JG, JR Sargent (1999) The fate of lipids during development and cold-storage of eggs in the laboratory-reared calanoid copepod, *Acartia tonsa* Dana, and in response to different algal diets. Aquacult 173: 257-269

Straile D (1997) Gross growth efficiencies of protozoan and metazoan zooplankton and their dependence on food concentration, predator-prey weight ratio, and taxonomic group. Limnol Oceanogr 42: 1375-1385

Strickler JR, Bal AK (1973) Setae of the first antennae of the copepod *Cyclops scutifer* (Sars): their structure and importance. Proc Nat Acad Sci USA 70: 2656-2659

Strickler JR (1984) Sticky water: A selective force in copepod evolution. In: Trophic Interactions Within Aquatic Ecosystems SEL SYMP SER vol. 85 pg. 187-242

Suzuki K, NakamuraY, Hiromi J (1999) Feeding by the small calanoid copepod *Paracalanus* sp on heterotrophic dinoflagellates and ciliates. Aquat Microb Ecol 17: 99-103

Takahashi M, Hori T (1984) Abundance of picophytoplankton in the subsurface chlorophyll maximum layer in subtrophical and tropical waters. Mar Biol 79: 177-186

Tamar H (1974) Further studies on *Halteria*. Acta Protozool 13: 177-190

Tang KW, Jakobsen HH, Visser AW (2001) *Phaeocystis globosa* (Prymnesiophyceae) and the planktonic food web: feeding, growth, and trophic interactions among grazers. Limnol Oceanogr 46: 1860-1870

Teegarden GJ (1999) Copepod grazing selection and particle discrimination on the basis of PSP toxin content. Mar Ecol Prog Ser 181:163-176

Thibault D, Gaudy R, Le Fevre J (1994) Zooplankton biomass, feeding and metabolism in a geostrophic frontal area (Almeria-Oran Front, western Mediterranean): significance to pelagic food webs. J Mar Syst 5:297-311

Thingstad TF (1998) A theoretical approach to structuring mechanisms in the pelagic food web. Hydrobiol 363: 59-72

Thouvenot A, Debroas D, Richardot M, Devaux J (1999) Impact of natural metazooplankton assemblage on planktonic microbial communities in a newly flooded reservoir. J Plankton Res 21:179-199

Tintoré J, Gomis D, Alonso S, Parrilla G (1991) Mesoscale dynamics and vertical motion in the Alborán Sea. J Phys Oceanogr, 21, 811-823

Tiselius P (1989) Contribution of aloricate ciliates to the diet of *Acartia clausi* and *Centropages hamatus* in coastal waters. Mar Ecol Prog Ser 56:49-56

Tiselius P, Jonsson PR (1990) Foraging behaviour of six calanoid copepods: Observations and hydrodynamic analysis. Mar Ecol Prog Ser 66: 23-33

Tiselius P (1992) Behavior of *Acartia tonsa* in patchy food environments. Limnol Oceanogr 37: 1640-1651

Tiselius P, Jonsson PR, Kaartvedt S, Olsen E M, Jørstad T (1997) Effects of copepod foraging behavior on predation risk: an experimental study of the predatory copepod *Pareuchaeta norvegica* feeding on *Acartia clausi* and *A. tonsa* (Copepoda). Limnol Oceanogr 42: 164-170

Turner JT, Anderson DM (1983) Zooplankton grazing during dinoflagellate blooms in a Cape Cod Embayment with observation of predation upon tintinnids by copepods. PSZNI Mar Ecol 4: 359-374

Turner JT (1988) The marine cladoceran *Penilia avirostris* and the "microbial loop" of pelagic food webs. Limnol Oceanogr 32:245-255

Turner JT, Graneli E (1992) Zooplankton feeding ecology: grazing during enclosure studies of phytoplankton blooms from the west-coast of Sweden. J Exp Mar Biol Ecol 157: 19-31

Turner JT, Tester PA (1997) Toxic marine phytoplankton, zooplankton grazers, and pelagic food webs. Limnol Oceanogr 42

Turriff N, Runge JA, Cembella AD (1995) Toxin accumulation and feeding behaviour of the planktonic copepod *Calanus finmarchicus* exposed to the red-tide dinoflagellate *Alexandrium excavatum*. Mar Biol 123:55-64

Uchima M, Hirano R (1988) Swimming behaviour of the marine copepod *Oithona davisae* : Internal control and search for environment. Mar Biol 99: 47-56

Uye S (1982) Population dynamics and production of *Acartia clausi* Giesbrecht (Copepoda: Calanoida) in inlet waters. J Exp Mar Biol Ecol 57: 55-83

Uye S, Sano K (1998) Seasonal variations in biomass, growth rate and production rate of the small cyclopoid copepod *Oithona davisae* in a temperate eutrophic inlet. Mar Ecol Prog Ser 163: 37-44

Uye S (1991) Temperature-dependent development and growth of the planktonic copepod *Paracalanus* sp. in the laboratory. Bull Plankton Soc Japan. Special Volume 627-636

Vanderploeg HA, Paffenhöfer GA (1985) Modes of algal capture by the freshwater copepod *Diaptomus sicilis* and their relation to food-size selection. Limnol Oceanogr 30: 871-885

Vaqué D, Blough HA, Duarte CM (1997) Dynamics of ciliate abundance, biomass and community composition in a oligotrophic coastal environment (NW Mediterranean). Aquat Microb Ecol 12: 71-83

Véra A, Desvilettes C, Bec A, Bourdier G (2001) Fatty acid composition of freshwater heterotrophic flagellates: an experimental study. Aquat Microb Ecol 25: 271-279

Verity PG, Paffenhöfer GA (1996) On assessment of prey ingestion by copepods. J Plankt Res 18: 1767-1779

Verity PG, Smetacek V (1996) Organism life cycles, predation, and the structure of marine pelagic ecosystems. Mar Ecol Prog Ser 130: 277-293

Videau C, Sournia A, Prieur L, Fiala M (1994) Phytoplankton and primary production characteristics at selected sites in the geostrophic Almeria-Oran front system (SW Mediterranean Sea). J Mar Syst 5:235-250

Vincent D, Hartmann HJ (2001) Contribution of ciliated microprotozoans and dinoflagellates to the diet of three copepod species in the Bay of Biscay. Hydrobiol 443:193-204

Volkman JK (1989) Fatty acids of microalgae used as feedstocks in aquaculture. In: Cambie RC, Horwood E (eds) Fats for the future, Chichester, pp 263-283

Von Elert E, Wolffrom T (2001) Supplementation of cyanobacterial food with polyunsaturated fatty acids does not improve growth of *Daphnia*. Limnol Oceanog 46: 1552-1558

Walve J, Larsson U (1999) Carbon, nitrogen and phosphorus stoichiometry of crustacean zooplankton in the Baltic Sea: implications for nutrient recycling. J Plankton Res 21: 2309-2321

Wassmann P (1998) Retention versus export food chains: process controlling sinking loss from marine pelagic systems. Hydrobiologia 363: 29-57

Watanabe T, Katajima CS, S Fujita (1983) Nutritional values of live organisms used in Japan for mass propagation of fish: a review. Aquacul 34: 115-134

White JR, MR Roman (1992) Egg production by the calanoid copepod *Acartia tonsa* in the mesohaline Chesapeake Bay: the importance of food resources and temperature. Mar Ecol Prog Ser 86: 239-249

Wiackowski K, Brett M.T, Goldman CR (1994) Differential effects of zooplancton species on ciliates community structure. Limnol Oceanogr 39: 486-492

Wiadnyana NN, Rassoulzadegan F (1989) Selective feeding of *Acartia clausi* and *Centropages typicus* on microzooplankton. Mar Ecol Prog Ser 53:37-45

Wickham SA (1995) Cyclops predation on ciliates: species-specific differences and functional responses. J Plankt Res 17: 1633-1646

Williamson CE, Sanders R, Moeller RE, Stutzman P (1996) Utilization of subsurface food resources for zooplankton reproduction: Implications for diel vertical migrations. Limnol Oceanogr 41:224-233

Winer B J, Brown D R, Michels KM (1991) Statistical principles in experimental design, McGraw-Hill, New York

Yentsch CS, Menzel DW (1963) A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep-Sea Res 10: 221-231

Zeldis J, James MR, Grieve J, Richards L (2002) Omnivory by copepods in the New Zealand Subtropical Frontal Zone. J Plankton Res 24: 9-23

Zhukova NV, Kharlamenko VI (1999) Sources of essential fatty acids in the marine microbial loop. Aquat Micr Ecol 17: 153-157