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Trophic interactions in the microbial food web in a coastal upwelling system off central Chile (~36°S)

DISSERTATION

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Science cannot solve the ultimate mystery of nature. And that is because, in the last analysis, we ourselves are part of the mystery that we are trying to solve

Max Planck

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Contents

Summary

Coastal upwelling areas are highly productive systems and were initially characterized by having a short food chain, being ecologically efficient in the trophic transfer. Large micro-phytoplankton (>20 μm; mainly chain-forming diatoms), predominating under high availability of nutrients in the mixed layer, are grazed by large herbivorous zooplankton, and they, in turn, are consumed by planktivorous fishes. Under this scheme, little attention was paid to the role of micro-organisms (protists and metazoans <200 μm) in these areas. This thesis provides an assessment of the temporal variability in the structure of micro-organism assemblages and of the trophic interactions in microbial food webs in the Humboldt Current System (HCS) off Concepción, central Chile (~36°S), as a basis to understand the relevance of the carbon flow through the microbial food web in this coastal upwelling area.

Temporal changes in the structure (composition, abundance, and biomass) of nanoplanktonic assemblages, as well as the potential grazing impact of nano-heterotrophs on picoplanktonic prokaryotes (autotrophic and heterotrophic bacteria), were investigated on the shelf off Concepción (Sta. 18; 36°30'S, 73°08'W; 90 m depth) during contrasting seasonal periods (upwelling, non-upwelling) over two annual cycles (18 August 2004 - 28 July 2006). Most of the nanoplankton was concentrated in surface waters (<30 m) during all the samplings and no clear seasonal differences in abundance or biomass in this layer was observed. Changes in nanoplankton abundance were significantly but weakly correlated with changes in the hydrographic variables ($r < 0.4$). Nanoflagellates dominated the total integrated nanoplankton abundance (3 to 317 x 10^9 cells m^{-2}; 0 - 80 m) whereas nanodiatoms and nanodinoflagellates generally contributed to a lesser degree (<20%) though, sporadically, they were important components of the total integrated nanoplankton biomass (total: 0.02 - 10.6 g C m^{-2}). The potential grazing rates on prokaryotic prey ranged from 3 to 242 bacterioplankton cells predator^{-1} h^{-1} and from 0.1 to14 cyanobacteria predator^{-1} h^{-1}, the nanodinoflagellates having higher grazing rates than the nanoflagellates. The resulting grazing impact by nano-heterotrophs on the standing stock of prokaryotes ranged from 6 to 152% (mean: 59%), implying that they control the picoplankton assemblages in the upwelling area off Concepción.

Micro-heterotrophs have been shown to have a significant grazing impact on nano- and microphytoplankton abundances and to channel a large proportion of the primary production (PP) in a variety of marine systems. Micro-heterotrophic grazing rates were assessed with the seawater dilution method in Coliumo Bay (36°32´S, 72°56´W; 20 m depth) during the non-upwelling, autumn/winter period in 2003 and 2004. Chlorophyll a (Chl-*a*) and cell abundance

were estimated to assess the changes of prey and predators during the incubations. Mean instantaneous phytoplankton net growth rates (k) and microzooplankton grazing rates (g) ranged between 0.19 - 0.25 day^{-1} and 0.26 - 0.52 day^{-1}, respectively. These estimates were used to calculate the potential PP (6 - 17 mg C m^{-3} d^{-1}) and the percentage of PP that is removed by microzooplankton assemblages. In all experiments, the grazing impact represented a significant (>100%) fraction of the potential PP and most of the removal by the grazers corresponded to the <20 μm fraction (cyanobacteria and autotrophic nanoflagellates). These results suggest that microzooplankton grazing has an important impact on total PP during non-upwelling conditions in the coastal area.

In addition, the feeding behaviour and grazing rates of an abundant and persistent micro-heterotroph in the system under study, the naupliar phase of *Oithona spp*., were explored. Diet composition, ingestion rates, and food-type preferences were assessed through grazing experiments (bottle incubations) with: i) different size fractions of natural planktonic assemblages (<3, <20, <100 and <125 μm), and ii) cultures of the nanoflagellate *Isochrysis galbana*. When offered nano- and microplanktonic prey fraction, the nauplii ingested nanoflagellates, small-sized dinoflagellates, and diatoms in solitary form (range: 0.07 - 73 x 10^3 cells $nauplii^{-1}$ d^{-1}). Under a mixture of pico- and nanoplankton, the nauplii mainly fed on nanoflagellates (3 - 14 x 10^3 cells $nauplii^{-1}$ d^{-1}). Picoplankton was also ingested, but at higher rates when it was the solely food available (5 - 18 x 10^6 cells $nauplii^{-1}$ d^{-1}). Ingestion rates on *I. galban*a (28 - 31 x 10^3 cells $nauplii^{-1}$ d^{-1}) were in the range of those estimated for natural nanoflagellates as food. Carbon uptake by the *Oithona* nauplii was mainly derived from the nanoflagellates (mean = 350 ng C $nauplii^{-1}$ d^{-1}). At the highest abundances of the nauplii in the system under study (15 L^{-1}), the daily grazing impacts on the prey standing stocks ranged from <21% for picoplankton, <68% for nanoflagellates (mean = 34%), <24% for dinoflagellates, and <13% for diatoms. This suggests that *Oithona* spp. nauplii exert a significant control on the abundances of the nanoplankton assemblages in the coastal area.

Altogether, these findings indicate that the microbial food web is a fundamental and permanent element in the upwelling system off Concepción. Given the high productivity of this system, a need to revise the microbial food web being an inefficient carbon pathway, acting as a *sink* of biogenic carbon, is discussed. Microbial food webs do not strictly include several grazing steps to incorporate the photosynthetically fixed carbon into higher trophic levels. Instead, this carbon could be channelled through the microbial food web as efficiently as through the classical herbivorous food web, thus sustaining a high, year-round, productivity in the system.

Zusammenfassung

Küstenauftriebsgebiete gehören zu den produktivsten Systemen der Ozeane, die üblicherweise durch eine klassische, kurze Nahrungskette vom Mikrophytoplankton über große, herbivore Zooplankter zu planktivoren Fischen charakterisiert wurden, ökologisch effizient in Bezug auf den trophischen Transport. Dagegen wurde die Bedeutung und Funktion des mikrobiellen Nahrungsnetzes (Protisten und Metazooplankton <200 µm) in diesen Gebieten bisher nur unzureichend untersucht. Die vorliegende Dissertation liefert eine umfassende Beschreibung der zeitlichen Entwicklung in der Struktur von Mikroorganismen sowie der Rolle und Bedeutung trophischer Interaktionen im Humboldtstrom (HCS) in Zentral-, Südchile (Concepción ~36°S) um die Relevanz des Kohlenstoffflusses autotropher Biomasse und Produktion durch das mikrobielle Nahrungsnetz im Untersuchungsgebiet tiefgreifender zu verstehen.

Die zeitliche Entwicklung in der Struktur (Zusammensetzung, Abundanz und Biomasse) des Nanoplanktons sowie der potentielle Fraßdruck von Nanoheterotrophen auf Prokaryonten des Pikoplanktons (autotrophe und heterotrophe Bakterien) wurde über zwei Jahre (18. August 2004 bis 28. Juli 2006) zu unterschiedlichen hydrographischen Bedingungen (Auftrieb und Nicht-Auftrieb) am Kontinentalschelf vor Concepción (St. 18; 36°30'S, 73°08' W; 90 m Tiefe) untersucht. Maximale Abundanzen des Nanoplanktons zeigten sich stets im Oberflächenwasser (<30 m) und keine saisonalen Unterschiede bezüglich ihrer Abundanzen oder Biomasse wurden in dieser Schicht der Wassersäule beobachtet. Variationen in den Abundanzen des Nanoplanktons korrelierten signifikant, wenn auch nur schwach mit Variationen in den hydrographischen Variablen ($r < 0.4$). Nanoflagellaten dominierten die Abundanz des Gesamtnanoplanktons (3 bis 317 x 10^9 Zellen m^{-2}; 0-80 m), während Nanodiatomeen und Nanodinoflagellaten generell einen geringen Anteil hatten (<20%). Dagegen stellten Diatomeen und Dinoflagellaten gelegentlich einen wesentlichen Teil der Gesamtbiomasse des Nanoplanktons (0.02 bis 10.6 g C m^{-2}) dar. Fraßraten des Nanozooplanktons variierten zwischen 3 bis 242 Bakterien Räuber^{-1} h^{-1} bzw. von 0.1 bis 14 Cyanobakterien Räuber^{-1} h^{-1}, wobei die Raten der Nanodinoflagellaten höher waren als die der Nanoflagellaten. Der resultierende Fraßdruck auf die Pikoplanktonbestände („standing stocks“) reicht von 6-152% (Mittelwert 59%) und ist ein Hinweis auf das Potential des Nanozooplanktons, die Populationen des Pikoplanktons im Auftriebsgebiet vor Concepción zu kontrollieren.

Mikroheterotrophe werden als Haupkonsumenten des Nano-, und Mikrophytoplankton gesehen und ihre Wichtigkeit, Primärproduktion (PP) an höhere Trophiestufen zu schleusen,

wurde in vielen verschiedenen marinen Ökosystemen anerkannt. Fraßraten von Mikroheterotrophen wurden mit der „Verdünnungsmethode“ während Nicht-Auftriebszeiten im südlichen Herbst/Winter 2003 und 2004 in der Bucht von Coliumo bestimmt (36°32´S, 72°56´W; 20m Tiefe). Chlorophyll a (Chl-*a*) wurde als allgemeiner Indikator verwendet um Änderungen im autotrophen Beutebestand während der Inkubationszeit zu ermitteln, jedoch wurden zusätzlich Zellzählung (Mikroskopie) von Beute-, als auch Räuberorganismen durchgeführt. Die Verdünnungsexperimente zeigten das erwartete Muster von zunehmender Phytoplanktonsterblichkeit mit Abnahme des Verdünnungsfaktor. Die Mittelwerte der Phytoplanktonwachstumsrate (k) und Mikrozooplanktonfraßrate (g), erstreckten sich zwischen 0.19 - 0.25 Tag^{-1} und 0.26 - 0.52 Tag^{-1}, und wurde verwendet, um die potentielle PP (6 - 17 mg C $m^{-3}d^{-1}$) und deren Anteil zu berechnen, der durch das Mikrozooplankton entfernt wird. In allen Experimenten stellte der Fraßeinfluss einen bedeutenden Anteil (>100%) der PP dar und Zellzählungen zeigten, dass der größte entfernte Anteil aus der Fraktion < 20 µm (Cyanobakterien und autotrophe Nanoflagellaten) stammte. Dies zeigt, dass Mikrozooplankton einen bedeutenden Einfluss auf die Gesamtprimärproduktion während Nicht-Auftriebszeiten besitzt.

Des weiteren wurde das Fraßausmaß einer ganzjährig präsenten Mikroheterotrophen Komponente (kleine cyclopoide Copepoden Nauplien von *Oithona* spp. <200 µm) untersucht. Nahrungsspektrum, Fraßraten sowie Nahrungspräferenzen wurden in Experimenten (mit Flascheninkubationen) ermittelt, in denen i) unterschiedliche Fraktionen natürlicher Planktongemeinschaften (<3, <20, <100 und <125 µm) und ii) Kulturen des Nanoflagellaten *Isochrysis galbana* den Nauplien als Nahrung angeboten wurde. Bei einem Nahrungsangebot aus Nano,- und Mikroplankton konsumierten die Nauplien ausschließlich Nanoflagellaten, kleine Dinoflagellaten und einzellige Diatomeen (0.07 - 73 x 10^3 Zellen $Nauplius^{-1}$ d^{-1}). Bei einer gemischten Nahrung aus Piko,- und Nanoplankton, konsumierten die Nauplien überwiegend Nanoflagellaten (3 - 14 x 10^3 Zellen $Nauplius^{-1}$ d^{-1}) und nur vereinzelt Pikoplankton. Wird letzteres jedoch als alleiniges Futter angeboten, treten Frassraten häufiger auf und erstreckten sich zwischen 5 - 18 x 10^6 Zellen $Nauplius^{-1}$ d^{-1}. Fraßraten von *I. galbana* (28 - 31 x 10^3 Zellen $Nauplius^{-1}$ d^{-1}) lagen im Bereich des natürlichen Nanoflagellatenfutters. Höchste Kohlenstoffaufnahmen erzielten die *Oithona* Nauplien durch die Ernährung von Nanoflagellaten (Mittelwert von 350 ng C $Nauplius^{-1}$ d^{-1}). Bei einer maximalen Abundanz der Nauplien im Untersuchungsgebiet von 15 Nauplien L^{-1}, wurde der tägliche Fraßdruck auf die jeweiligen Beutebestände („standing stocks“) ermittelt: <21% für Pikoplankton, <68% für Nanoflagellaten (Mittelwert = 34%), <24% für Dinoflagellaten und <13% für die Diatomeen

beträgt. Daraus lässt sich schließen, dass *Oithona* spp. Nauplien in der Lage sind die Abundanzen des Nanoplanktons zu kontrollieren.

Die Ergebnisse der vorliegenden Arbeit zeigen, dass das mikrobielle Nahrungsnetz einen saisonal bedeutenden, ganzjährig wichtigen Bestandteil des pelagischen Nahrungsnetzes im Auftriebsgebiet von Concepción darstellt. In Bezug auf den Kohlenstofftransport wird das mikrobiellen Nahrungsnetz als ineffizient gesehen, was jedoch der ganzjährigen Producktivität des Küstenauftriebsgebietes von Concepción wiederspricht. Dieses Konzept wird evaluiert, da der photosynthetisch fixierte Kohlenstoff genauso effizient durch das mikrobielle wie das herbovire Nahrungsnetz geschleust werden kann, was die ganzjährige Produktivität des Küstenauftriebsgebiets von Concepción unterstützt.

Resumen

Las áreas de surgencia costera son sistemas altamente productivos y fueron inicialmente caracterizadas por tener una cadena alimentaria corta, siendo ecológicamente eficientes en la transferencia trófica. El micro-fitoplancton (>20 μm; principalmente diatomeas en cadena), predominantes en la capa de mezcla frente a una alta disponibilidad de nutrientes, son consumidas por zooplancton herbívoro de tamaño grande, y ellos a su vez son consumidos por peces planctívoros. Bajo este esquema, poca atención ha recibido el rol y la función de los micro-organismos (protistas y metazoa <200 μm) en áreas de surgencia. Esta tesis presenta una evaluación de la variabilidad temporal en la estructura de comunidades de micro-organismos y de las interacciones tróficas en la trama microbiana en el Sistema de Corrientes Humboldt (HCS) en la zona central de Chile frente a Concepción (~36° S), como base para entender la relevancia del flujo de carbono vía la trama microbiana en este sistema de surgencia costera.

Los cambios temporales en la estructura (composición, abundancia, y biomasa) de los componentes nanoplanctónicos, así como el impacto potencial de consumo de nano-heterótrofos sobre los procarióticas picoplanctónicos (bacterias autótrofos y heterótrofos) fueron investigados en un área de la plataforma continental frente a Concepción (Est. 18; 36°30'S, 73°08' W; profundidad de 90 m) en distintos períodos estacionales (surgencia y no surgencia), durante dos ciclos anuales (18 de agosto de 2004 - 28 de julio de 2006). La mayor parte del nanoplancton se concentró en las aguas superficiales (<30 m) durante todos los muestreos y no se observaron diferencias estacionales en abundancia o biomasa en esta capa. Los cambios en la abundancia del nanoplancton se correlacionaron débil pero significativamente con los cambios en las variables hidrográficas ($r < 0.4$). Los nanoflagelados fueron dominantes en la abundancia integrado total de nanoplancton (3 - 317 x 10^9 células m^{-2}; 0 - 80 m) mientras que las nanodiatomeas y los nanodinoflagelados fueron contribuyentes menores generalmente (<20%) aunque esporádicamente fueron componentes importantes en la biomasa integrada total de nanoplancton (total: 0.02 - 10.6 g C m^{-2}). Las tasas de ingestión potencial de los nano-heterótrofos sobre las presas procarióticas presentaron un rango entre 3 y 242 bacterioplancton depredador^{-1} h^{-1} y entre 0.1 y 14 cianobacterias depredador^{-1} h^{-1}, siendo las tasas de los nanodinoflagelados más altas que la de los nanoflagelados. Como resultado, el impacto de consumo por nano-heterótrofos sobre los "standing stocks" de procariontes se extendió entre 6 y 152% (promedio 59%), implicando que ellos controlan las comunidades de picoplancton en el área de surgencia frente a Concepción.

Los micro-heterótrofos han sido reconocidos por tener un impacto de consumo significativo sobre las abundancias de nano- y micro-fitoplancton y de canalizar una proporción importante de la producción primaria (PP) en una variedad de sistemas marinos. Las tasas de ingestión de micro-heterótrofos fueron determinadas con el método de dilución durante el período del otoño/invierno (no-surgencia) en 2003 y 2004, en la bahía de Coliumo (36°32´S, 72°56´W; profundidad de 20 m). La clorofila *a* (Chl-*a*) y la abundancia celular fueron estimadas para evaluar los cambios en las presas y los depredadores durante las incubaciones. El promedio de las tasas de neta crecimiento del fitoplancton (k) y de ingestión del microzooplancton (g) se extendieron entre 0.19 - 0.25 día^{-1} y 0.26 - 0.52 d^{-1}, respectivamente. Estas estimaciones fureon utilizadas para calcular la PP potencial (6 - 17 mg C m^{-3} d^{-1}) y el porcentaje de la PP que es removida por comunidades microzooplanctónicas. En todos los experimentos, el impacto de consumo representó una fracción significativa (>100%) de la PP potencial y la mayor parte de la remoción por micro-heterótrofos fue de la fracción <20 μm (cianobacterias y nanoflagelados autótrofos). Estos resultados sugieren que el consumo del microzooplancton tiene un impacto importante sobre la PP total durante condiciones de no-surgencia en el área costera.

Además, el comportamiento alimentario y la tasa de ingestión de un componente micro-heterótrofo abundante y persistente en el sistema en estudio, la fase naupliar de *Oithona* spp.) fueron explorados. La composición de la dieta, las tasas de ingestión, y las preferencias alimentarias fueron evaluadas en experimentos de consumo (incubaciones en botella) con: i) diversas fracciones planctónicos naturales (<3, <20, <100 y <125 μm), y ii) cultivos del nanoflagelado *Isochrysis galbana*. Cuando los nauplios tuvieron presas nano- y microplanctónicas, ellos consumieron nanoflagelados, nanodinoflagelados, y diatomeas solitarias (rango: 0.07 - 73 x 10^3 células nauplio^{-1} d^{-1}). Frente a una mezcla del pico- y nanoplancton, los nauplios se alimentaron principalmente de nanoflagelados (3 - 14 x 10^3 células nauplio^{-1} d^{-1}). El picoplancton también fue ingerido pero a tasas mayores cuando fue el único alimento disponible (5 - 18 x 10^6 células nauplio^{-1} d^{-1}). Las tasas de ingestión de células de *I. galbana* (28 - 31 x 10^3 células nauplio^{-1} d^{-1}) estuvieron en el rango de aquellas estimadas para los nanoflagelados naturales como alimento. La incorporación de carbono por los nauplios de *Oithona* fue derivado principalmente desde los nanoflagelados (promedio de 350 ng C nauplio^{-1} d^{-1}). A los niveles más altos en abundancia de nauplios en el sistema en estudio (15 L^{-1}), los impactos de consumo diario sobre los "standing stocks" fueron entre <21% para picoplancton, <68% para nanoflagelados (promedio = 34%), <24% para dinoflagelados, y

<13% para diatomeas. Esto sugiere que los nauplios de *Oithona* spp. ejercen un control significativo sobre las abundancias del nanoplancton en el área costera.

En conjunto, estos resultados indican que la trama microbiana es un elemento fundamental y permanente en el sistema de surgencia costera frente a Concepción. Dada la alta productividad de este sistema, se discute la necesidad de revisar que la trama microbiana sea una vía ineficiente en la transferencia de carbono. Las tramas tróficas microbianas no necesariamente incluyen varios pasos de consumo para la incorporación del carbono fijado fotosintéticamente hacia los niveles tróficos más altos. En cambio, este carbono se puede canalizar por la cadena microbiana en forma tan eficiente como por la cadena herbívora clásica y, por lo tanto, manteninedo una alta productividad durante todo el año en este sistema.

1. Introduction

Marine microbes form complex and dynamic communities within the water column and sea-floor of coastal and oceanic environments are now known to be responsible for about half of the Earth's primary productivity. They encompass a wide metabolic and physiological diversity and exhibit very fast growth rates; therefore they play a fundamental role in the transfer of matter and energy and in the cycling of biogeochemical important elements, such as carbon and nitrogen, through marine ecosystems. Technological advances, ranging from microbial genomics to satellite remote sensing, have improved the understanding of the structure and function of these microbial communities and their processes in the ocean. Still, there is much to be learned from more traditional whole-ecosystem approaches, such as those focused on pattern of species abundance and biomass, food webs and community structure, to understand the feedback mechanisms between marine ecosystems and the atmosphere.

1.1. Micro-organisms, the microbial food web and it's relevance in microbial ecology

Studies of the microbial food web and related interactions are relatively "new" in the discipline of microbial oceanography. Due to the small size of microbes, as well as the associated difficulties in their collection, preservation and identification, but also because of their great functional diversity, studies on these organisms had been mostly neglected. The recognition of the importance of heterotrophic microbes can be followed back to the very early studies of Beers & Stewart (1979) and Sorokin & Kogelschatz (1979). At about the same time, major progresses of methodological approaches in the field of microbial oceanography greatly improved the quantification of the abundance of marine bacteria (Hobbie *et al.*, 1977; Porter & Feigh, 1980) and protists (Davis & Sieburth, 1982; Caron, 1983), bacterial activity (Furhrmann & Azam, 1982; Kirchman *et al.*, 1985), and the microbial loop concept (Azam *et al.*, 1983; Ducklow 1983) that had been stimulated to a large extent by Pomeroy's seminal article (Pomeroy, 1974).

At the NATO Advanced Research Institute in Bombannes (France, 1982), members of the working group on bacteria and bacterivory brought together evolving information about microbial abundance and activity in the sea, resulting in the paper of Azam *et al.*, (1983) about the ecological role of water-column microbes in the sea. In this paper, the authors proposed that the microbial components of pelagic food webs formed a separate entity. They named this the "microbial loop" (heterotrophic bacteria, bacterivorous protists and larger protists) and distinguished it from the classical food chain (larger sized phytoplankton, herbivorous metazooplankton and planktivorous fish), as illustrated in Figure 1.

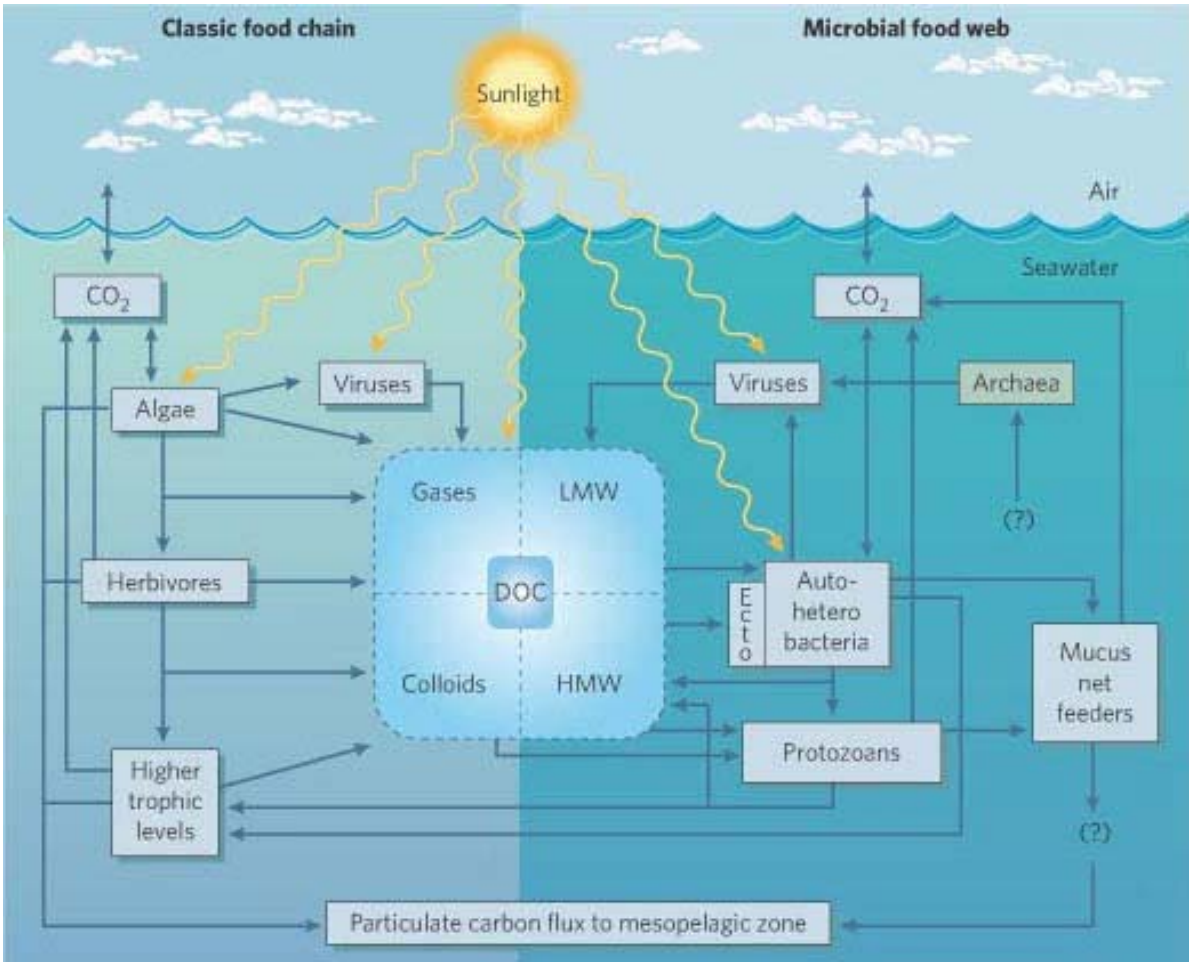

Figure 1. Marine microbial interactions in the upper ocean (DeLong & Karl, 2005)

Shown on the left in this figure is the classical pathway of the carbon and energy flow through algae, to metazoan herbivores and on to higher trophic levels (Ryther, 1969; Steele, 1974). On the right is the microbial food web, which uses energy stored in the non-living, detrital carbon pool to produce microbial biomass that can re-enter the classic pathway of carbon and energy flow. Also shown in the microbial food web are viral particles and Archaea. So far though, there is only rudimentary knowledge of the role of Archaea in the oceanic food web. The size structure and functional groups of the food web largely determine the downward flux of particulate carbon and energy (shown at the bottom of this diagram) and the rate, at which it is exported. The classical grazer pathway (on the left side) is regarded as important in this sense since large-sized photosynthetic Eukarya are thought to be either grazed by herbivores which produce rapidly sinking faecal pellets or to directly sink to the bottom. In contrast, the dominance of small-sized photoautotrophs (pico-to nanoplankton) favours lower production and increased recycling of carbon in the upper water column occurs since various grazing steps are necessary to incorporate the carbon fixed by the primary producers into higher trophic levels (Michaelis & Silver, 1988). In addition, the faecal aggregates produced by

small heterotrophs that graze on small-sized photoautotrophs are relatively small and light (Stoecker, 1984); they remain long in suspension and do not sink directly to the bottom, so export is low (*e.g*. Michaels & Silver, 1988; Rivkin *et al.*, 1996). The microbial and classical food webs coexist in all areas of the ocean, but their relative significance changes with region and season (*e.g.* Uye *et al.*, 1999).

The size spectrum of the various planktonic components of Azam´s proposed food web (1983) was based on the terminology of Sieburth *et al.* (1978), still nowadays used to classify planktonic organisms into ecological groups on the basis of their size and trophic mode. Single-cell organisms, including autotrophic, heterotrophic, and mixotrophic prokaryotes (bacteria and cyanobacteria) and eukaryotes (algae and phagotrophic protists) and viruses are termed "microbes" (Table 1).

Table 1. Main groups of pelagic micro-organisms in the ocean modified after Sherr & Sherr, 2000. Microbial size categories are based on the terminology proposed by Sieburth *et al.* (1978).

Size category	Microbial group	Size Range (µm)
Femtoplankton	Viruses	0.01-0.2
Picoplankton	Prokaryotes	
	Bacteria	
	Photoautotrophic	0.5-1.0
	Prochlorophytes	0.5-1.0
	Coccoid cyanobacteria	1.0-2.0
	Chemautotrophic	0.-1.0
	Heterotrophic	0.3-1.0
	Eukaryotes	
	Picoalgae, picoheterotrophic flagellate	1.0-2.0
Nanoplankton	Diatoms, flagellates, dinoflagellates, ciliates	2-20
Microplankton	Diatoms, dinoflagellates, ciliates, crustacean nauplii	20-200

Autotrophic organisms achieve all requirements for life from inorganic compounds and chemical or light energy ("self-feeder"), whereas heterotrophs obtain their requirements from organic compounds. An organism, capable of being autotrophic and heterotrophic at the same time, is termed mixotroph (Caron, 2000). The mixotrophic feeding mode is diverse and can be distinguished as i) obligate mixotrophic (both light and particulate food is necessary for sustaining growth and maintenance), ii) obligate autotrophic and facultative heterotrophic (only photosynthesis is essential for growth and maintenance, heterotrophy can be used to backup the photosynthetic apparatus in times of low light intensity), iii) obligate heterotrophic and facultative autotrophic (only food is necessary for sustaining growth and maintenance, but photosynthesis can be used to backup heterotrophy in times of low food concentrations), as

well as iv) facultative mixotrophic (ability to grow exclusively by either photosynthesis or phagotrophy/ uptake of organic compounds).

The nanoplankton and microplankton comprise unicellular eukaryotic organisms ("Protists") ranging from 2 - 20 and 20 - 200 μm in size and are very diverse in their trophic modes with autotrophic, heterotrophic, and mixotrophic forms. In addition, small metazoans (mostly crustaecean nauplii) that are <200 μm in size are also part of the microzooplankton. The main microbial groups in these size categories are shown in Table 1. Flagellates (considering dinoflagellates as a different group) are mostly included in the nanoplankton (there are few known species of picoflagellates) and are the most abundant component of this fraction. Marine flagellates are an enormously diverse group (in terms of *e.g.* shape, size, and the number and position of the flagella) and they are spread among the two major algal divisions (Chromophyta and Chlorophyta) of the Eukarya, in nine out of ten algal classes, and in three zooflagellate orders. The differentiation according to their trophic modes (autotrophic, heterotrophic, mixotrophic) is more complex than previously thought since mixotrophy and/or symbiosis among flagellates (and other protists like *e.g*. dinoflagellates and ciliates) appear to be common in marine systems (Caron, 2000). There are many different feeding mechanisms involved in the bacterivory of heterotrophic flagellates (they are known as most important grazers of bacteria in many aquatic systems) which include filter-feeding, sedimentation, interception feeding, and raptorial feeding supported by a pharynx or pseudopods (Boenigk & Arndt, 2002).

Dinoflagellates are found in both, the nano- and microplankton size fraction. They are widely distributed in marine and freshwater habitats and are composed of two general groups, thecates (amoured) and athecates (non-amoured or naked); most of the around 2500 species are free living. Their nutritional modes include heterotrophic, autotrophic and mixotrophic forms, though nearly half of the known species are heterotrophic (Dodge & Lee, 2000). Dinoflagellates have evolved different feeding mechanisms (Jacobsen, 1999) which enable them to feed upon a wide range of food types, even on large spiny diatoms (Jacobsen & Anderson, 1986) and copepod eggs/nauplii (Jeong, 1994). "Gulp" feeding and peduncular feeding has been shown for both, athecate and thecate species, and in addition, thecates are additional known to feed via a pallium. Some dinoflagellates form red-tide patches in coastal, offshore and/or oceanic waters (Tyler & Seliger, 1978; Tester & Steidinger, 1997). Certain blooming genera have species that produce toxins which are fatal for fishes and invertebrates (Burkholder *et al.*, 1995); other are not-toxin producing but the very high biomass results in low oxygen waters and subsequent fish mortality (Kudela *et al.*, 2005).

Most ciliates are naked (aloricate), though some groups form a more or less robust lorica (loricate, like tintinnids). Aloricate ciliates make up the bulk of the ciliate community in the pelagial (Petz, 1999). Ciliates are characterized by having two kinds of nuclei: a micro- and a macronucleus (Cavalcanti *et al*., 2005). The macronucleus mediates the day-to-day functioning of the cell, and the micronucleus, of which there may be more than one, contains the chromosomes and is involved in the sexual processes (conjugation, autogamy, cytogamy) undergone by ciliates. They also have few to many cilia or compound ciliary organelles which are used for locomotion and for creating currents which bring food particles to their mouths. Among the ciliates, heterotrophic, autotrophic or mixotrophic forms can be found.

Small metazoans (developmental stages of calanoid and cyclopoid copepods) have been shown to feed on a variety of prey types, including bacterioplankton (Roff *et al*., 1995), small sized phytoplankton (Berggreen *et al.,* 1988) as well as protists (Merell & Stoecker, 1998; Lonsdale *et al.,* 2000), and detritus (Green *et al.,* 1992). While the developmental stages of cyclopoid copepods are strict ambush, raptorial feeders, relying on mechano-reception, those of calanoid copepods can also create a feeding current and, therefore, switch to suspension feeding (Svensen & Kiørboe 2000; Saiz *et al.*, 2003). In general, protists dominate the microplankton, although small metazoans can be the most abundant component in this fraction (Brownlee & Jacobs, 1987 *fid*e White & Roman, 1992).

Whereas micro-heterotrophs are effective consumers of prey from as small as bacteria to organisms larger than themselves, the diet of nano-heterotrophs is usually restricted to bacteria sized organisms. Both are important regulators of bacterial and phytoplankton production (McManus & Fuhrmann, 1988), as well as of the remineralization of organic matter and nutrients in the euphotic zone (Azam *et al.*, 1983; Sherr & Sherr, 2000). They are capable of responding quickly to changes in food supply (Verity *et al.*, 1992) and, therefore, maintain a close coupling between production and consumption in the euphotic zone. Furthermore, micro-heterotrophs represent a link in the transfer of matter and energy between the "microbial loop" and the higher trophic levels of the pelagic food web and their relevance in doing so is well accepted for a variety of marine ecosystems (*e.g.* Gifford, 1988; Paranjape, 1990; Azam *et al.*, 1991; Sherr & Sherr, 1992; Neuer & Cowles, 1994; Landry *et al.*, 1995; García-Pámanes & Lara Lara, 2001; Calbet & Landry, 2004; Strom *et al.*, 2007). Nevertheless, the microbial food web and the role of micro-grazers in coastal upwelling areas, especially in the HCS, has been poorly studied and understood until most recent (Calbet & Landry, 2004).

1.2. Background knowledge on the microbial food web in the coastal upwelling area off Concepción, central Chile

Coastal upwelling areas represent about 1% of the ocean's area, are extremely productive, and contribute remarkable (67%) to the global new production of the world's ocean (Chavez & Toggweiler, 1995) coastal regions of the Humboldt Current System (HCS) off Peru and Chile are well known for the upwelling of deep, high nutrient and CO_2, and low temperature and oxygen waters (Strub *et al.*, 1998), resulting in high autotrophic production (Montecino *et al.*, 2006). This production is either channelled through the food web or exported to the deep ocean and/or to adjacent oceanic areas. For a long time it was assumed that the autotrophic production in upwelling areas, largely dominated by chain-forming diatoms, was efficiently channelled onto higher trophic levels through a simple, herbivorous food chain (Ryther, 1969). Export from the euphotic zone as intact cells, faecal pellets, detritus, or marine snow was also thought to be important in terms of carbon flow (Legendre & Le Fèvre, 1995). Until recently, little attention was paid to the potential role of the microbial food web structure and functioning in these areas although earlier studies in the Peruvian upwelling system had documented high abundances of heterotrophic microbes in the water column (Beers *et al.*, 1971; Sorokin, 1978; Sorokin & Kogelschatz, 1979). The importance of small-sized autotrophs in primary production and in mediating carbon flux in coastal upwelling areas was also stressed earlier in the Benguela upwelling system (*e.g.* Probyn, 1992; Painting *et al.*, 1992; Brink *et al.*, 1995). Only recently, this has been the case for the HCS (Iriarte *et al.*, 2000; Vargas & González, 2004; Vargas *et al*., 2007).

The central-southern zone of the HCS off Concepción, central Chile (33 - 38°S; Figure 2), is characterized by an irregular coastline, including semi-enclosed coastal systems (bays of Coliumo, Concepción, San Vicente and Arauco Gulf). The continental margin off Concepción is the widest shelf along the HCS (up to 90km from the coast) and interrupted by a complex submarine topography (Sobarzo, 2002) associated with the Itata and Bio-Bio rivers. Consequently, river runoff is quite important in this area and low salinity waters can extend way offshore during the winter/early spring period (Strub *et al.*, 1998); mesoscale structures (*e.g.* filaments, eddies, upwelling plumes) are common features in the coastal transition zone (Montecino *et al.*, 2004). The area is recognized for its high biological productivity (Daneri *et al.*, 2000; Montecino *et al.*, 2006), which sustains one of the largest fisheries in the world (annual fish catch of over 7 million t); some of the highest primary production rates (PP; ~ 4 - 20 g C m^{-2} d^{-1}) in the world's oceans (Daneri *et al.*, 2000) have been estimated there, making it one of the most productive among all of them.

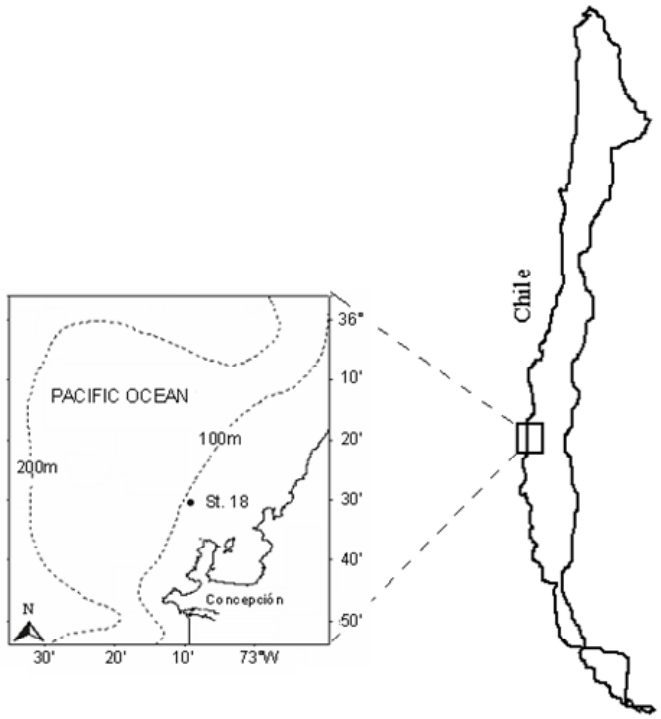

Figure 2. Study area of the coastal upwelling area off Concepción, central Chile

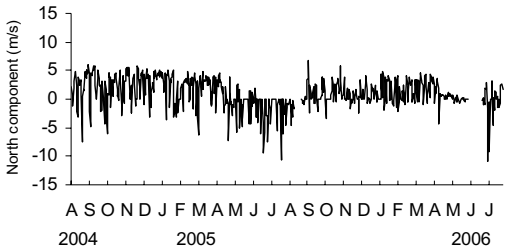

Figure 3. Northern component of wind velocity (equatorward) of the study area between August 2004 to July 2006)

This high productivity is mainly supported by the seasonal (spring and summer) dominance of S-SW winds during the austral spring-summer period (Figure 3) that force the upwelling of nutrient-repleted Equatorial Subsurface Waters (ESSW; Strub *et al.*, 1998), fertilizing the photic zone and enhancing new production. In winter (austral autumn/winter period), the weakening of the South Pacific anticyclone produces a wind pattern less favourable to upwelling, and this, coupled with a reduced light field, decreases the system's productivity in winter, although values of PP found during this period are relatively high anyways (530 - 1529 mg C d^{-2} d^{-1}; Montecino *et al*., 2006; Vargas *et al*., 2007).

Trophic relationships within the microbial food web in upwelling systems remain poorly understood and in this context, the Centre for Oceanographic Research in the eastern South Pacific (COPAS), University of Concepción, proposed a line of research involving several studies referred to the microbial food web and its impact on the carbon flow in the area off Concepción. So far, attempts have been made to evaluate the general structure of microbial assemblages (Anabalón *et al.*, *in press*; González *et al.*, *in press*; Morales *et al.*, *in press*) and of their impact upon carbon flux (Grünewald *et al.*, 2002; Troncoso *et al.*, 2003; Cuevas *et al.*, 2004; Vargas *et al.*, 2007; Montero *et al.*, *in press*).

The dominant diatom genera in the coastal zone (*Skeletonema*, *Thalassiosira*, and *Chaetoceros*; Figure 4*)* are well adapted to the highly turbulent, nutrient-replete environment and a clear seasonality near the surface in their abundance occurs, with maxima abundance

and biomass during the summer upwelling period (Vargas *et al.*, 2007; Anabalón *et al.*, *in press;* González *et al.*, *in press*) together with highest concentrations of Chl-*a* (Morales *et al.*, *in press*).

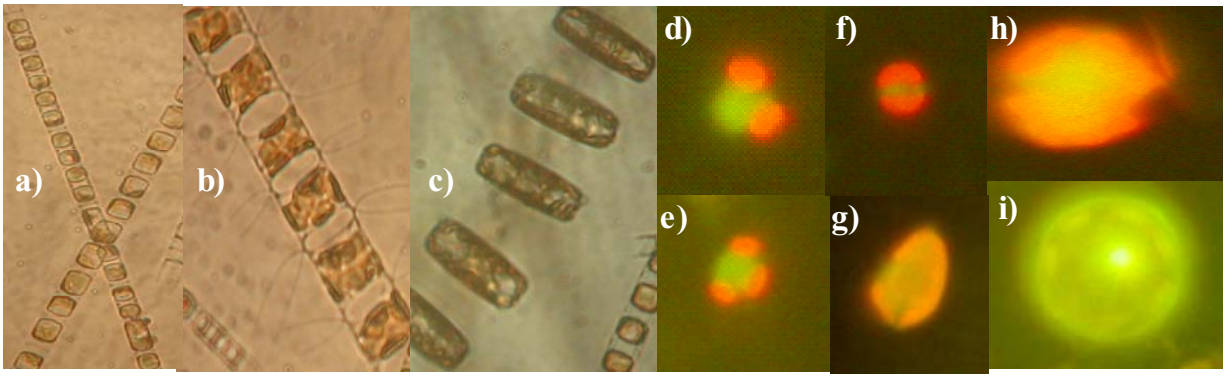

Figure 4. Digital photographs of some of the nano- and microplanktonic primary producers (a-c fixed with Lugol´s solution, d-f = stained with DAPI) found at the shelf off Concepción, Chile: a = *Skeletonema spp.*, b = *Chaetoceros spp*., c = *Thalassisoira spp*., d-f = unidentified autotrophic flagellates; g = *Cryptophyceae*; e = unidentified autotrophic dinoflagellate; i = solitary form of *Thalassiosira minsucula*.

Anabalón *et al.* (*in press*) combined the analysis of nano- and microplankton fractions (Figure 4) and stressed the co-occurrence in abundance maxima of both during the productive, upwelling period. This co-occurrence does not reflect the typical picture observed in coastal upwelling areas, with intense spring-summer blooms of diatoms followed by flagellates (*e.g.* Brink *et al.*, 1995; Tilstone *et al.*, 2000) but indicates the importance of the nanoplanktonic fraction as a dominant component in the coastal area of the upwelling region off Concepción, contributing to maintain the system's production. Among the micro-heterotrophs, Gonzalez *et al.* (*in press*) found *Protoperidinium* (Figure 5a), *Dinophysis* (Figure 5b) as well as *Ceratium* to be the dominant dinoflagellates. Tintinnids are the most common ciliates, including *Codonellopsis* (Figure 5c), *Helicostomella* (Figure 5d), and *Tintinnopsis*. Both tintinnids and dinoflagellates peak during the same period or shifted slightly after diatoms attained their maximum.

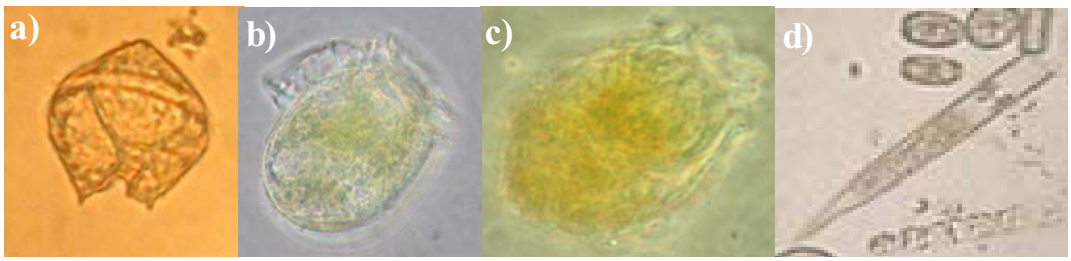

Figure 5. Digital photographs of some of the typical micro-heterotrophs in the upwelling area off Concepción: a = *Protoperdinium*, b = *Dinophysis*, c = *Codonellopsis*, d= *Helicostomella* (all fixed with Lugol's solution). Photographs b-d were provided by V. Anabalón (COPAS).

In terms of the carbon flux via microbial pathways, it has been shown that a significant proportion of the organic matter produced by phytoplankton is channelled through bacteria (Troncoso *et al.*, 2003; Cuevas *et al.*, 2004). Furthermore, Cuevas *et al*. (2004) predicted that

during upwelling period, the heterotrophic nanoflagellates incorporate only a small fraction (< 5%) of the bacterial production but that they are able to control it (>100%) during non-upwelling period. In addition, a recent study by Vargas *et al.* (2007), on the relative importance of microbial and classical pathway of carbon in the highly productive area off Concepción, indicated that a large part of the PP (13 - 84%) is channelled through the microbial food web and, in comparison, only a small fraction directly to copepods via the herbivorous food chain (1 - 6%). Carbon flux estimates for the coastal zone off Concepción during the upwelling (1040 mg C m^{-2} d^{-1}) and non-upwelling (230 mg C m^{-2} d^{-1}) seasons on the one hand point out that there is a significant vertical export of POC, representing 31 and 15% of the PP, respectively (Grünewald *et al.*, 2002). In addition, Gonzalez *et al.* (*in press*) reported recently that, on the average, 17% (range 2 - 67%) of the generated PP on the shelf of Concepción was exported below 50 m depth with *Thalassiosira* > *Chaetoceros* > *Skeletonema* appearing as the most important contributors of the sedimenting diatom-carbon on an annual basis (20%, 11%, 9%, respectively). Consequently, they play an essential role in the coupling between the productive upper layer and sediments in the system under study. Vargas *et al.* (2007), on the other hand, showed that during upwelling only 3 to 4% of the PP is sedimented; furthermore, Morales *et al.* (*in press*) stressed that autotrophic production might also be exported to adjacent oceanic areas via filaments and eddies.

Still there are various aspects, from the taxonomical to the ecological views, that require further research and many questions remain to be answered on the food webs in the upwelling area in the HCS. Is most of the photosynthetically fixed carbon effectively channelled through the microbial food web? Does grazing by micro-organisms play an important role in vertical carbon export? Is the micro-heterotrophic pathway an important trophic link in highly productive upwelling systems and how is the food-web structured in these areas? Overall, we need to improve the understanding of the role of micro-organisms in the highly productive waters of the HCS and their impact on primary production, nutrient recycling, as well as on secondary production in terms of interlinking bacterial production with higher trophic levels.

2. Thesis objectives and outline

This thesis attempts to understand the role and relevance of trophic interactions in the microbial food web of the highly productive coastal system off central Chile (~36°S). In this context, the following five questions were developed as an integral part of the investigation of the COPAS Center through the Research Program #3 on "Plankton communities: structure, trophic and metabolic processes":

I. What is the dominant structure of nanoplanktonic assemblages on the shelf off Concepción and how does it vary under upwelling and non-upwelling conditions?

II. To what extent nano-heterotrophic grazers control picoplanktonic prokaryotes?

III. How important is microzooplankton (2 - 200 μm) in channelling primary production during the non-upwelling period?

IV. What is the trophic role of microplanktonic metazoans in the system under study?

V. How important is the carbon flow from autotrophic and/or heterotrophic sources through the microbial food web?

The questions are addressed in the framework of three scientific contributions that have been already published, are *in press* or recently submitted to scientific journals. The first of the above addressed questions is explored in **Publication 1**. As part of a multidisciplinary, time series station at the shelf off Concepción, central Chile, the analysis of the composition, abundance and biomass of nanoplankton communities was of interest since the structure and functioning of nanoplanktonic assemblages in this coastal upwelling area had been overlooked in the assessments of the productivity of upwelling areas in general. A specific objective was to elucidate the temporal variability of these assemblages, as the system of study is exposed to different hydrographic conditions during an annual cycle; intense upwelling of equatorial subsurface water and increased solar radiation during the austral spring/summer period, and river influx and precipitation during the austral autumn/winter period. Another specific objective, alluded in the second question, was to investigate the grazing potential of nano-heterotrophic grazers (nanoflagellates and nanodinoflagellates; HNF and HND, respectively) on prokaryotic prey assemblages (autotrophic and heterotrophic bacteria). The aim was, on one hand, to evaluate the role of HND as bacterial-grazers, group to which little attention has been paid compared to the HNF. On the other hand, the grazing impact of both, HND and HNF, on prokaryotes was explored with respect to varying environmental conditions (upwelling and non-upwelling).

Micro-heterotrophs are assumed to have a significant grazing impact on nano- and microphytoplankton and, thereby, channel a large proportion of the PP to higher trophic levels, a theme addressed in the third question. **Publication 2** focuses on the grazing impact of micro-heterotrophs (2 - 200 μm) on PP during the autumn/winter, non-upwelling period off Concepción. The grazing of a micro-heterotrophic metazoan component (nauplii of *Oithona spp*.) in the upwelling area off Concepción was also investigated (4th question). The few reports available on the feeding of copepod nauplii indicate that they feed on a variety of prey types but grazing rates data are scarce. Results on the feeding and trophic role of *Oithona* spp. nauplii in the system under study is addressed in **Publication 3**, including diet composition, ingestion rates, food-type preferences, and an assessment of their potential in controlling prey populations of different size fraction is presented.

Each of the previous described publications (**Publication 1, 2, and 3**) discusses the importance of the carbon flow from autotrophic and/or heterotrophic sources through microbial pathways and, altogether, are included in the last question (question 5). In the coastal upwelling system of Concepción, carbon fixed by primary producers is transferred trough both, 'classical' and 'microbial' pathways but the proportion directed through each of these two pathways depends largely on the size of the primary producers, components that display a strong seasonality in their abundance and biomass in this area. The evidence provided in this thesis, and most recent studies in the area, implies that the microbial food web is a fundamental and, most probably, a permanent trophic pathway in this upwelling system (**Publication 1, 2, and 3**).

The three scientific contributions resulting from this thesis are either published, *in press* or submitted, and are listed below:

Publication 1

Böttjer D, Morales CE (*in press*) Nanoplanktonic assemblages in the upwelling area off Concepción (~36°S), Central Chile: abundance, biomass and grazing potential during the annual cycle. Progress in Oceanography, doi:10.1016/j.pocean.2007.08.024

• All nanoplankton analysis/data were contributed by D. Böttjer. Picoplankton, hydrographic and nitrate data were provided by Dr. O. Ulloa, Dr. W. Schneider and M.A. Varas. The manuscript was written by D. Böttjer under the supervision of C.E. Morales.

Publication 2

Böttjer D, Morales CE (2005) Microzooplankton grazing in a coastal embayment off Concepción, Chile, (~36°S) during non-upwelling conditions. *Journal of Plankton Research* 27(4): 383-391

• Experiments and data analysis were carried out by D. Böttjer. The manuscript was prepared by D. Böttjer and C.E. Morales.

Publication 3

Böttjer D, Morales CE, Bathmann U (submitted) Are small cyclopoid copepod nauplii (*Oithona* spp.) important grazers in the highly productive, upwelling system off central Chile? *Limnology and Oceanography*

• Experiments were carried out by D. Böttjer and the data were evaluated in conjunction with C.E. Morales. The manuscript was prepared by D. Böttjer in collaboration with the co-authors.

In the next section (section 3) the methodological approaches applied in the framework of this thesis are briefly introduced, including a theoretical background and practical application. Following that (section 4), the three scientific contributions are provided before coming to the general discussion of this dissertation (section 5), where the most important findings of this thesis are linked into a broader spectrum of knowledge and the understanding of the carbon flow in the coastal upwelling area off central Chile is re-evaluated.

3. Methods

3.1. Structure of nanoplanktonic assemblages

Theory

Seasonal variations (upwelling and non-upwelling) in the abundance and biomass of nanoplanktonic assemblages are assessed by epifluorescence microscopy (Davis & Sieburth, 1982; Caron, 1983). The basic function of a fluorescence microscope is to irradiate the specimen with a specific wavelength band (excitation), and to assess the emitted fluorescence. In a properly configured microscope, only the emission light should reach the eye or detector so that the resulting fluorescent structures are superimposed with high contrast against a very dark (or black) background. The light seen is the fluorescence from the specimen that has been stained with a specific fluorochrome and in some cases is also derived from autofluorochrome from phototrophic pigments. The fluorochromes (*e.g.* DAPI, Proflavin, Syber-Green) are stains that attach themselves to visible or sub-visible structures, and are often highly specific in their attachment target.

Practical application

In order to enumerate the different taxonomic groups of the nanoplanktonic assemblages (flagellates, dinoflagellates, diatoms, and ciliates) for subsequent abundance calculation, 20 mL of collected samples are stained (DAPI= 4',6-diamidino-2-phenylindole at a final concentration of 0.01%; Porter & Feigh, 1980) and filtered onto black polycarbonate membrane filters (0.8 μm pore size). Samples are frozen and stored at -20°C in the dark until analysis. Filters are examined with a Nikon® TE2000S (T-FL Epi-Fl) microscope, equipped with a digital camera (Nikon® Coolpix 4500), using UV, blue, or multiple excitation (NIKON Filter Blocks DAPI UV-2E/C, NB-2A, and DAPI/FITC/TRITC) at a magnification of 1000x. Total counts vary depending on sampling time and depth but at least 75 nanoplanktonic organisms are enumerated in each sample. Heterotrophic and autotrophic forms (flagellates, dinoflagellates and ciliates) are counted separately, assuming that those displaying autofluorescence were autotrophic or mixotrophic cells. Mean cell sizes of the most common specimens representing the different taxonomic groups are measured using the software Image Pro Plus® (Version 4.5). Carbon biomass estimates are derived from measured cell dimensions, calculated cell volumes using appropriate geometric formulae (Chrzanowski & Simek, 1990; Sun & Lui, 2003), and by applying literature-derived carbon to volume ratios for different taxonomic groups. Flagellate cell volumes are converted to carbon biomass using

a factor of 220 fg C μm^{-3} (Børsheim & Bratbak, 1987), whereas the remaining nanoplanktonic cell volumes are converted using the carbon to volume relationships given by Menden-Deuer & Lessard (2000): for diatoms, $\log_{10}$ pg C $cell^{-1}$ = -0.541 + 0.811 x $\log_{10}$ volume (μm^3); for dinoflagellates, $\log_{10}$ pg C $cell^{-1}$ = -0.353 + 0.864 x $\log_{10}$ volume (μm^3); and for ciliates, $\log_{10}$ pg C $cell^{-1}$ = -0.639 + 0.984 x $\log_{10}$ volume (μm^3).

3.2. Grazing rate estimates

A variety of approaches has been developed for the estimation of micro- and nanoheterotrophic grazing, all including different advantages and disadvantages (Båmstedt *et al.*, 2000). Grazing rate estimates are either expressed at the level of individual organisms or as entire assemblage depending on the method used and the analysis performed.

3.2.1. Microheterotrophic grazing – community estimates using the dilution method

Theory

Microzooplankton grazing rates can be estimated with the seawater dilution method (Landry & Hasset, 1982), which originally only used chlorophyll-a as a tracer, of food consumed by herbivores but later extended to estimate grazing on bacteria and cyanobacteria (Cambpell & Carpenter, 1986). This technique is based on the experimental decrease of the encounter rate of predators and prey by diluting natural seawater with filtered seawater from the same source. Grazing rates are expected to be lower in the most diluted treatment compared with less diluted and undiluted treatments. Changes in prey density after the incubation are usually expressed by an exponential growth model:

$P_t = P_o e^{(k-g)}$

or

$1/t \ ln \ (P_t/P_o) = k - g = \mu$

with P_o and P_t = phytoplankton concentrations at the beginning and at the end of the experiment (mg Chl-*a* m^{-3}), t= incubation time (h^{-1}), k= instantaneous algae growth coefficient (d^{-1}) and g= instantaneous grazing coefficient (d^{-1}). The growth and grazing coefficients are calculated from a linear regression of the apparent growth rate (μ) plotted against the different dilution factors. The slope of this relationship represents g, and the y-intercept k. The net rate of change in the phytoplankton density is expected to be linearly and negatively related to the dilution factor.

Practical application

Sample water, collected in the coastal zone off Concepción, is gently sieved by <200 µm or 125 µm in order to remove large grazers. One part of this water is filtered through a 0.8 µm prefilter followed by a 0.2 µm filter to obtain particle-free seawater; the remaining part is kept as unfiltered seawater. Subsequently, dilutions of filtered and unfiltered seawater at different proportions are prepared (e.g. 15, 30, 45, 60, and 100%), enriched with nitrate (final concentration of 5 µM) and phosphate (final concentration of 1 µM) and distributed in experimental bottles (polycarbonate). Triplicates are incubated for 48 h on a plankton rotation wheel (0.5 r.p.m.) at 12 h light: dark cycles. Measurements of chlorophyll-*a* are used to estimate P_o and P_t in all bottles in order to estimate the prey density changes during the incubations. For this purpose, subsamples of 100 ml are collected for subsequent Chl-*a* determination by fluorometry (Holm-Hansen *et al.*, 1965), and in addition, aliquots of 50 mL are sedimentated in Utermöhl chambers (Utermöhl, 1958) for microscopic analysis.

3.2.2. Microheterotrophic grazing – species-specific estimates using the traditional bottle incubation method

Theory

Species-specific grazing rates are carried out following a standard protocol for bottle incubations (Gifford, 1993) including sampling of the offered food at the beginning (t_1) and end (t_2) of the incubation period. Clearance and ingestion rates are assessed from changes in Chl-*a* concentrations (as total) and/or cell abundance per prey type at the beginning and end of the incubations without (control) and with grazers (experimental), following Frost (1972). The instantaneous growth coefficient of the prey (k, d^{-1}) is obtained from the changes in prey concentration (C, in mg Chl-*a* m^{-3} or cells mL^{-1}) in the control treatments at time t_1 (C_1) and t_2 (C_2) of the incubation:

$$C_2 = C_1 \cdot e^{k(t_2 - t_1)}$$

The instantaneous grazing coefficient (g, d^{-1}) is calculated from:

$$C_2^* = C_1 \cdot e^{(k-g)(t_2 - t_1)}$$

where C_2^* is the prey concentration at t_2 in the treatment containing the grazers. A mean food concentration $\left(\langle C \rangle\right)$, expressed in terms of Chl-*a* concentration, cell numbers or biomass, is calculated from:

$$\langle C \rangle = C_1 (e^{(k-g)(t_2 - t_1)} - 1) / (t_2 - t_1)(k - g)$$

The clearance rate (F = volume cleared copepod^{-1} time^{-1}) is obtained from the volume of the incubation bottle (V, in mL) and the copepod density in each bottle (N):

$$F = V \cdot g / N$$

The ingestion rate (I = food concentration or biomass copepod^{-1} time^{-1}) is calculated from:

$$I = F \cdot \langle C \rangle$$

Practical realization

Adult *Oithona* spp. (*O. nana* and *O. similis*) females with egg sacs are collected by gentle vertical hauls from 0 - 10 m depth in the coastal area off Concepción and incubated with food (natural seawater screened through 100 µm) until they produce a sufficient number of naupliar stages. The freshly hatched nauplii are separated from the females and kept in water plus the food type offered in the subsequent experiment until they reached naupliar stages NIII - NV (120 - 165 µm length). A sufficient number (21 - 60) is then incubated with different natural food assemblages (<3, <20, <100 and <125 µm) or cultured *Isochrysis galbana* for 24 h in a rotating wheel, and under a 12:12 h light: dark cycle. Three replicate bottles (500 mL) per condition are used in each experiment. The initial food samples in the controls are collected after 1 h incubation for the analyses of micro- nano- and/or picoplankton abundances, as well as for total Chl-*a* concentration. At the end of the incubations, samples from the control and grazing bottles are collected and treated as described above. Chl-*a* is determined by fluorometry (Holm-Hansen *et al.*, 1965), micro-, nano- and/or picoplankton samples are analysed by inverted and epifluorescence microscopy (Utermöhl, 1958; Davis & Sieburth, 1982).

3.2.3. Nanoheterotrophic grazing- estimates using a generic model

Theory

Nanoheterotrophic grazing rates are assessed using a generic model approach that predicts protistan grazing (Peters, 1994). Potential grazing rates (GR, number of prey predator^{-1} h^{-1}) are estimated from coefficients which are derived from a large data set covering freshwater and marine environments. The model includes the variables temperature (T, ºC), cell volumes (V, µm^3) and abundances (C, cells mL^{-1}) of both the prey (PY) and predators (PD):

$$ln\ GR = -2.701 - 0.344\ ln(V_{PY}) + 0.477\ ln(V_{PD}) + 0.489\ ln(C_{PY}) - 0.270\ ln(C_{PD}) + 0.033T$$

Practical realization

Predator abundances (heterotrophic nanoflagellates and nanodinoflagellates) are obtained from epiflourescence microscopy analysis (Davies & Sieburth, 1982). For this purpose, 20 mL of collected sample (unsieved seawater from selected depths) are stained with DAPI (4',6-diamidino-2-phenylindole) at a final concentration of 0.01% (Porter & Feigh, 1980), and are filtered onto black polycarbonate membrane filters of 0.8 µm pore size (filters can be stored at -20°C in the dark until microscopic analysis). Filters are examined with the same microscope and magnification as described in section 3.1. Mean cell sizes of the heterotrophic nanoflagellate and dinoflagellate are measured using the software Image Pro Plus® (Version 4.5) for subsequent estimation of the cell volumes using appropriate geometric formulae (Sun & Lui, 2003).

Samples for prey (bacterioplankton and cyanobacteria) abundance estimation are fixed immediately after collection with freshly prepared para-formaldehyde (0.1% final concentration), stored frozen and subsequently analysed by flow cytometry (Becton-Dickinson® FACScalibur flow cytometer; flow rate: 28-32 µL min^{-1}; >10,000 events counted) using SYBR-Green I for bacterial counts and forward scatter, side scatter, and orange (phycoerythrin) and red fluorescence (chlorophyll) for cyanobacterial counts. Cell volumes of bacterioplankton are based on those reported for samples taken in the area off Concepción (Cuevas *et al.*, 2004) and cyanobacteria cell volumes are derived from the same samples and in the same way as described for predators.

4. Scientific contributions

4.1.

Böttjer D, Morales CE (*in press*)

Nanoplanktonic assemblages in the upwelling area off Concepción (~36°S), Central Chile: abundance, biomass and grazing potential during the annual cycle.

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Nanoplanktonic assemblages in the upwelling area off Concepción (~36°S), central Chile: Abundance, biomass, and grazing potential during the annual cycle

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Abstract

The structure and functioning of nanoplanktonic assemblages in coastal upwelling areas have usually been overlooked in explorations of the productivity of these areas. As part of a multidisciplinary, time-series station in the coastal area off Concepción, seasonal variations (upwelling and non-upwelling) in the abundance and biomass of these assemblages were investigated. Hydrographic measurements and biological samples were taken monthly over a 2-year period (18 August 2004–28 July 2006). Nanoflagellates dominated the total integrated abundance ($3–317 \times 10^9$ cells m^{-2}; 0–80 m). Diatoms and dinoflagellates usually contributed to a lesser degree (<20%) but sporadically made important contributions to the total integrated nanoplankton biomass (0.0003–10.6 g C m^{-2}). Most of the nanoplankton was concentrated in surface waters (<30 m) during all the samplings and no seasonal differences in abundance or biomass were found in this layer, although the mean values and dispersions around them were highest during the upwelling period along with maximum integrated (0–80 m) chlorophyll-*a* values, as total or in the <20 μm fraction. Changes in nanoplankton abundance were significantly but weakly ($r < 0.4$) correlated with changes in the hydrographic variables; the highest correlation values were positive for temperature and oxygen, factors that varied with depth and date. The potential grazing rates of heterotrophic nano-predators (flagellates and dinoflagellates) on prokaryotic prey, estimated with a generic model, ranged from 3 to 242 bacterioplankton predator^{-1} h^{-1} and from 0.1 to 14 cyanobacteria predator^{-1} h^{-1}. Our results imply a small impact of seasonal hydrographic variability on the abundance and biomass of nanoplanktonic assemblages and suggest that grazing by small dinoflagellates might control the prokaryotic picoplankton populations in the upwelling area off Concepción.

Regional index terms: SE Pacific; Central Chile; Bio–Bio Region; Concepción

Keywords: Nanoplankton; Temporal variability; Grazing; Coastal upwelling; Humboldt current system

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1. Introduction

Nanoplankton, comprising the unicellular organisms ranging from 2 to 20 μm in size (Sieburth et al., 1978), is an integral part of the pelagic food web in marine systems (Azam et al., 1983) and very diverse in its trophic modes with autotrophic, heterotrophic, and mixotrophic forms (Lenz, 2000). In general, it consists primarily of flagellated protists, but other components (*e.g.*, dinoflagellates, diatoms) can also be important (Stoecker et al., 1994; Garrison et al., 1998). Heterotrophic and mixotrophic nanoflagellates are primary consumers of bacterioplankton and, therefore, capable of regulating bacterial populations in a variety of marine systems (Sieburth et al., 1978; Fenchel, 1982; McManus and Fuhrmann, 1988; Sanders et al., 1992). In turn, nanoflagellates are an important food source for larger protists (Edwards et al., 1999) as well as for metazoans (Turner and Granéli, 1992).

In terms of the role of nanoplankton in carbon production and flux/export, it is usually thought that, when it is a dominant component together with the smaller picoplankton fraction, lower production and increased recycling of carbon in the upper water column occurs and export is lower (*e.g.*, Michaels and Silver, 1988; Rivkin et al., 1996). The importance of nanoplankton in primary production and in mediating carbon flux in coastal upwelling areas was stressed earlier in the case of the Benguela system (*e.g.*, Probyn, 1992; Painting et al., 1992; Brink et al., 1995) and, more recently, in the case of the Humboldt Current System (Iriarte et al., 2000; Vargas and González, 2004). Little other information on the relative importance of these components has accumulated for upwelling systems, perhaps because of the classical view that large diatoms predominate in productive systems where there is a higher availability of nutrients in the mixed layer (Hutchings et al., 1995; Kudela et al., 2005).

In the Humboldt Current System (HCS), the central region off Chile (30–40°S) presents a marked seasonality in wind-driven upwelling with upwelling-favourable equatorward winds dominating during the austral spring–summer (October–March), and a shift to predominantly northerly to north-westerly directions in autumn–winter (May–August), with transitional periods of variable winds (Shaffer et al., 1999; Sobarzo et al., this issue). The area off Concepción (~36°S) displays some of the highest primary production rates (~4–20 g C m^{-2} d^{-1}) in the world's oceans and has a potentially significant impact on the global carbon budget in terms of organic matter export (Daneri et al., 2000). Large micro-phytoplankton (>20 μm) blooms are frequent during the upwelling period (Anabalón et al., this issue and González et al., this issue) but also the smaller fraction (<20 μm) appears to make significant contributions (15–100% of total chlorophyll-*a*) during upwelling/relaxation events (Peterson et al., 1988). There is, however, little knowledge about the temporal variability of the structure of autotrophic assemblages in this area and on how much they contribute to the total production of the system.

The carbon flux via microbial pathways in the upwelling area off Concepción is also poorly understood at present. There have been two studies dealing with this aspect for the shelf and oceanic areas during the austral spring (October) of 1998 and winter (July) of 1999. Their results indicated that an important fraction of the primary production (10–24%) is channelled through the bacterioplankton, suggesting the microbial loop to be an important recycling pathway in the upper water column (Troncoso et al., 2003). Results based on a grazing model predict that, in spring, the heterotrophic nanoflagellates might incorporate only a small fraction (<5%) of the bacterial production but that they are able to control it (>100%) during the winter (Cuevas et al., 2004). In contrast, Böttjer and Morales (2005) found that, during non-upwelling winter conditions, an important fraction of the primary production, mainly in the <20 μm fraction and nanoflagellates in general, is channelled directly through the microzooplankton; no observations were provided for the upwelling period in that study.

Overall, the few data on the structure and dynamics of microbial (<200 μm) assemblages in the upwelling region off Chile suggest that, at times, they can be important agents in the cycling of carbon in the coastal upwelling system and adjacent oceanic waters. In this context, the present study deals with the temporal changes in the structure (composition, abundance, biomass) of the nanoplanktonic assemblages in the upwelling area off Concepción during contrasting seasonal periods (upwelling, non-upwelling) over two annual cycles. This information is also used to derive the potential predation of heterotrophic nano-predators upon prokaryotic prey so as to assess the importance of microbial carbon cycling pathways in this region. This study is complemented by two other studies on planktonic components presented in this issue, with data from the same time series station (Anabalón et al., this issue; González et al., this issue).

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2. Materials and methods

2.1. Field sampling and environmental variability

Sampling took place between August 2004 and July 2006 (22 samplings) aboard RV *Kay-Kay* at a fixed sampling station on the shelf off Concepción (Station 18; 36°30′S, 73°08′W; 90 m depth), located 18 nautical miles from the coast (Fig. 1). This station was selected for a time series study in August 2002, with the support of the FONDAP-COPAS Center (www.copas.cl); the time series dataset also contains hourly wind data from a coastal meteorological station on the headland off Concepción (36°38'S). Water column samples for nanoplankton (2–20 μm) abundance and biomass were collected monthly during the daytime at nine depths (0, 5,

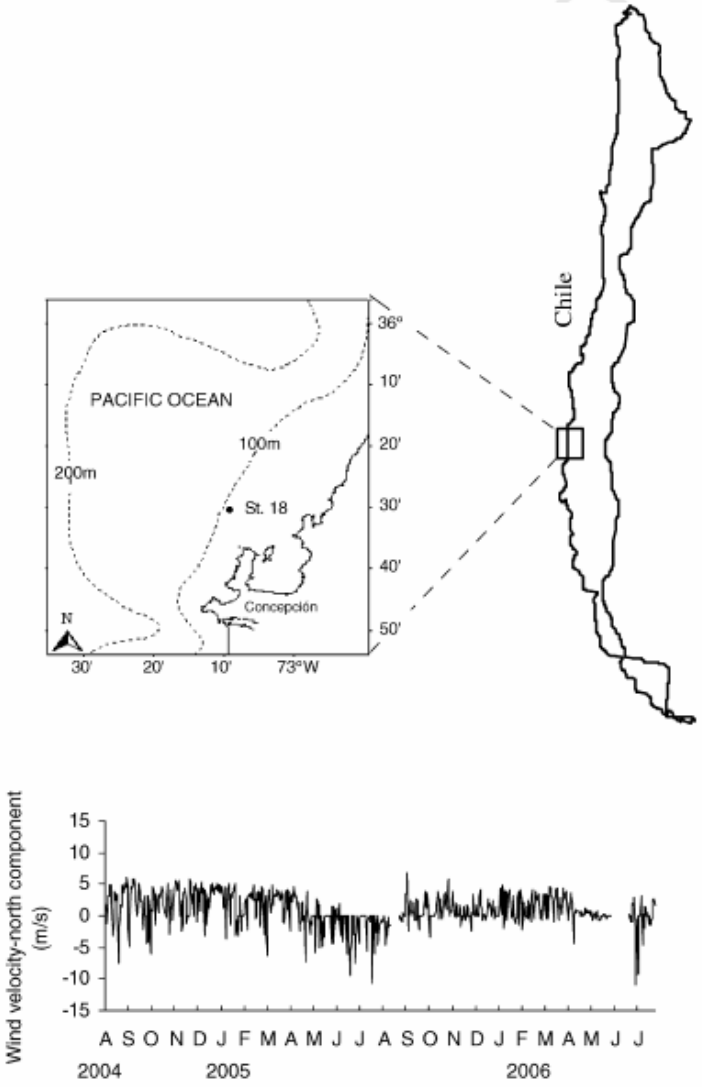

Fig. 1. Study area and geographic location of the fixed sampling Station 18 on the shelf (Itata terrace) off Concepción, central Chile (upper panel), and the north-south component of wind velocity (positive equatorward) for the study period (August 2004–July 2006) (lower panel).

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10, 15, 20, 30, 40, 50, 80 m) using a General Oceanic rosette equipped with Niskin bottles (10 L). Vertical profiles of temperature, salinity, and oxygen (0–80 m) were obtained from a continuous CTD register (Sea Bird SBE-25); these data were provided by Dr. W. Schneider (U. Concepción) and are available on the COPAS webpage. In addition, discrete samples for dissolved oxygen (Winkler titration), nutrients (autoanalyzer), and chlorophyll-*a* (fluorometric analysis; total and size fractionated: filters of 3 μm and meshes of 20 μm for pico- and nanoplanktonic size ranges) were taken at the same depths as the nanoplankton samples. The oxygen and nitrate data were provided by M.A. Varas (U. Concepción) and are also available on the COPAS webpage. For nanoplankton analysis, three replicate samples (50 mL centrifuge tubes) were collected directly from the Niskin bottles, preserved with glutaraldehyde (2% final concentration), and stored in the dark and cold (5 °C) until subsequent analysis within 2 weeks of sampling.

2.2. Nanoplankton abundance and size distribution

Duplicate sub-samples of 20 mL were extracted from each of two collected tubes for nanoplankton (the third tube was stored as a backup), stained with DAPI (4′,6-diamidino-2-phenylindole) at a final concentration of 0.01% (Porter and Feig, 1980), and filtered onto black polycarbonate membrane filters (0.8 μm pore size), supported underneath by 0.45 μm membrane filters. The filters with samples were frozen and stored at −20 °C in the dark for subsequent analysis using epifluorescence microscopy (Porter and Feig, 1980).

Filters were examined with a Nikon® TE2000S (T-FL Epi-Fl) microscope, equipped with a digital camera (Nikon® Coolpix 4500), using UV, blue, or multiple excitation (NIKON Filter Blocks DAPI UV-2E/C, NB-2A, and DAPI/FITC/TRITC). Samples were counted at a magnification of 1000×. Total counts varied depending on sampling time and depth but at least 75 nanoplanktonic organisms were enumerated in each sample. Nanoplanktonic components were separated into the following taxonomic groups: flagellates, dinoflagellates, diatoms, and ciliates. Heterotrophic and autotrophic flagellates (HNF and ANF, respectively) and dinoflagellates (HND and AND, respectively) were counted separately, assuming that those displaying autofluorescence were autotrophic or mixotrophic cells. In addition, mean cell sizes of the most common specimens representing the different taxonomic groups during each sampling were measured (30–50 cells for nanoflagellates; 15 for other groups) using the software Image Pro Plus® (Version 4.5).

2.3. Nanoplankton biomass estimates

Carbon estimates were derived from measured cell dimensions, calculated cell volumes using appropriate geometric formulae (Chrzanowski and Simek, 1990; Sun and Lui, 2003), and then by applying literature-derived carbon to volume ratios for different taxonomic groups. Flagellate cell volumes (heterotroph + autotroph) were converted to carbon biomass using a factor of 220 fg C μm^{-3} (Børsheim and Bratbak, 1987). Other nanoplanktonic cell volumes were converted using the carbon to volume relationships given by Menden-Deuer and Lessard (2000): for diatoms, $\log_{10}$pg C $cell^{-1} = -0.541 + 0.811 \times \log_{10}$volume ($\mu m^3$); for dinoflagellates, $\log_{10}$pg C $cell^{-1} = -0.353 + 0.864 \times \log_{10}$volume ($\mu m^3$); and for ciliates, $\log_{10}$pg C $cell^{-1} = -0.639 + 0.984 \times \log_{10}$volume ($\mu m^3$).

2.4. Nanoplankton variability and environmental conditions

An analysis of the variability in the abundance and biomass of nanoplanktonic assemblages was performed using sampling dates and depths as factors and applying a non-parametric, two-way analysis of variance (Friedman test; Sokal and Rohlf, 1998), since the data did not display normality or homogeneity of variance (Kolmogorov–Smirnov and Browne–Forsythe, respectively; Zar, 1984). Differences between depths (three strata: 0–10, 15–30, 40–80 m) and hydrographic periods (upwelling *vs.* non-upwelling) were further tested with non-parametric, multi-comparison tests with or without ties depending on each data set (Tukey test for equal sample size and Dunn test for unequal sample size, respectively; Zar, 1984). The data were grouped into stratum or periods using criteria related to the observed hydrographic variability. Water column (0–80 m) stratification (Φ, J m^{-3}) was derived from the potential energy anomaly after Bowden (1983):

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$$\Phi = \frac{1}{H}\int_{-H}^{0}(\rho m - \rho)gz\,dz$$

where H is the height of the water column (m), ρm is the mean density of the water column (kg m^{-3}), ρ is the density at depth z, and g is the acceleration due to gravity (9.8 m s^{-2}). Additionally, coastal wind data from the study period were used (www.copas.cl; Fig. 1). The association between oceanographic variability and nanoplankton abundance and biomass was explored using the non-parametric correlation test, the Kendall Rank correlation coefficient (Sokal and Rohlf, 1998).

2.5. Potential grazing by nano-heterotrophs on picoplanktonic assemblages

HNF and HND grazing rates on cyanobacteria and bacterioplankton were estimated using a model approach for protistan heterotrophs (Peters, 1994), where potential grazing rates (GR, number of prey predator^{-1} h^{-1}) are estimated from coefficients derived from a large data set obtained from a wide range of freshwater and marine environments. The estimate includes the variables temperature (T, °C), cell volumes (V, μm^3) and abundances (C, cells mL^{-1}) of both the prey (PY) and predators (PD):

$$\ln GR = -2.701 - 0.344\ln(V_{PY}) + 0.477\ln(V_{PD}) + 0.489\ln(C_{PY}) - 0.270\ln(C_{PD}) + 0.033T$$

HNF and HND predator abundance and volume were obtained as detailed previously (Sections 2.2,2.3). The abundances of bacterioplankton and cyanobacteria were obtained from parallel samplings at the same station (fixed with freshly prepared *para*-formaldehyde 0.1% final concentration and stored frozen). These samples were analyzed by flow cytometry (Becton–Dickinson® FACScalibur flow cytometer; flow rate: 28–32 μL min^{-1}; >10,000 events counted) using SYBR-Green I for bacterial counts and forward scatter, side scatter, and orange (phycoerythrin) and red fluorescence (chlorophyll) for cyanobacterial counts; these data were provided by Dr. O. Ulloa (U. Concepción). Estimates of bacterioplankton cell volumes were based on those reported for samples taken in the area off Concepción (Cuevas et al., 2004). Cyanobacteria cell volumes (mean = 1.5 μm^3) were derived from the same samples as those from which nanoplankton volumes were obtained. The variability in grazing rates, using dates and depths as factors, was tested with Friedman's two-way analysis of variance. Differences between (a) depth layers (0–30 and 40–80 m) and (b) seasonal periods (upwelling and non-upwelling) were analysed by applying non-parametric multiple comparison for unequal sample size (Dunn test; Zar, 1984).

3. Results

3.1. Oceanographic variability and chlorophyll-a distribution during the annual cycle

A higher degree of variability in temperature (Fig. 2a) in the water column appeared during the spring–summer months as compared with the autumn–winter months. In contrast, the highest degree of variability in salinity (Fig. 2b) was observed during the latter period, the surface values being higher (>34) during the spring–summer months (Table 1). Surface temperature variation at the fixed station (Table 1) was relatively low during the 2-year period (range: 11–14 °C) with the exception of the sampling in February 2006 (18 °C). Seasonal differences in the stratification of the water column were detected, with lower values during the spring–summer samplings compared with the autumn–winter samplings (Table 1). This, together with the surface salinity, was used as criteria to distinguish between "upwelling" (<50 J m^{-3} and salinity >34) and "non-upwelling" seasonal periods in Table 1; the wind data during the period of study (Fig. 1) also sustain this seasonality. The presence of oxygen deficient waters (<2 ml O_2 L^{-1}) below the surface layer (Fig. 2c), together with salinities >34, during the spring–summer samplings denoted the presence of Equatorial Subsurface Waters (ESSW) in the study area. This was accompanied by increases in nitrate concentrations in the upper layer (<30 m), although the values were relatively high (>5 μM) during the whole year (Fig. 2d; Table 1).

The integrated (0–80 m) chlorophyll-*a* (Chl-*a*) values, total and size fractionated (<20 and <3 μm), are presented in Table 1. Total integrated values were lowest (16–66 mg m^{-2}) during the non-upwelling period and variable but frequently higher (>100 mg m^{-2}) during the upwelling period. The integrated <20 μm Chl-*a* size

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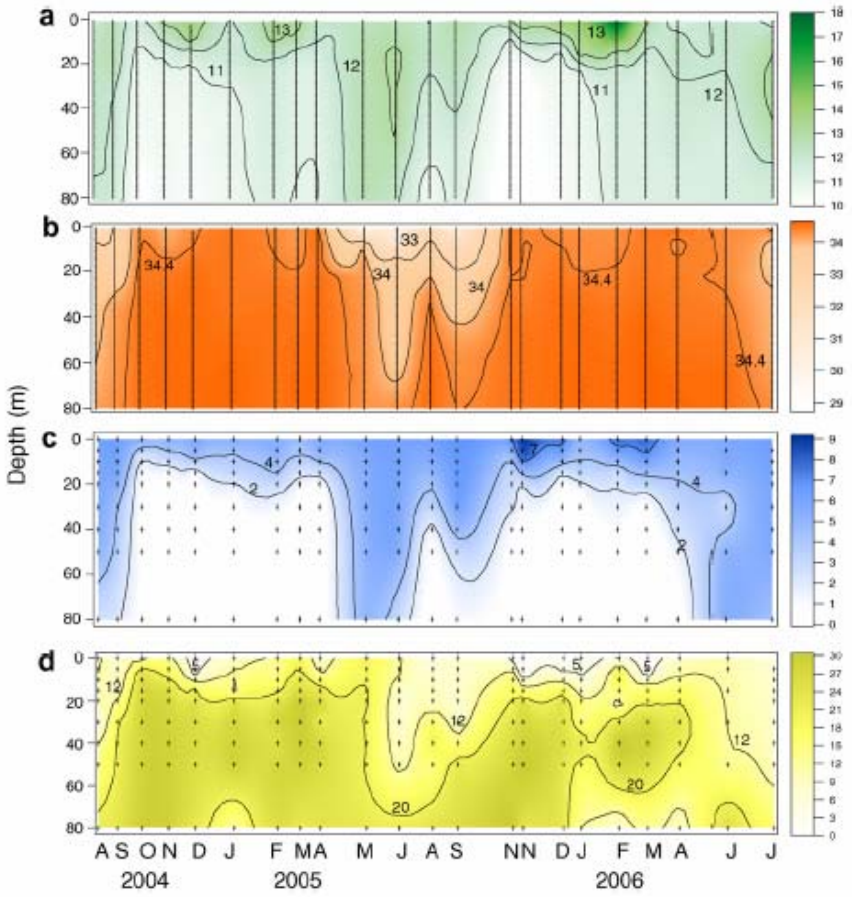

Fig. 2. Temporal distribution of the hydrographic conditions at Station 18 during the study period (August 2004–July 2006): (a) temperature (°C), (b) salinity, (c) dissolved oxygen (mL L^{-1}), and (d) nitrate (μM).

fraction (11–86 mg m^{-2}), corresponding to the nano- and picoplanktonic autotrophs, was highly variable throughout the annual cycle and contributed much (>60%) of the total Chl-*a* whenever the latter values were lower (<60 mg m^{-2}). That is, the microplankton (>20 μm) was the dominant fraction when Chl-*a* concentrations were highest, mostly during the upwelling period; however, the maximum values in the <20 μm fraction (>60 mg m^{-2}) were also observed during the upwelling period. The smallest Chl-*a* fraction (<3 μm; picoplankton), usually contributed only a small proportion (<20%) of the total Chl-*a* (2–10 mg m^{-2}), except in the March 2005 sampling, when both the nano- and picoplankton fractions similarly contributed to total Chl-*a* and were dominant over the microplankton fraction.

3.2. Variability in the structure of the nanoplanktonic assemblages

The nanoplankton collected at Station 18 was composed of flagellates, dinoflagellates, diatoms, and ciliates; except for a few identifications, the detailed taxonomic composition in each of these groups remains to be determined but some of the most common species in each group are presented in Fig. 3. The assemblages were numerically dominated by nanoflagellates throughout the sampling period, as revealed by the integrated nanoplankton abundance data (Fig. 4), separated into a surface layer, with higher hydrographic variability (0–30 m), and a bottom layer (40–80 m). Nanoflagellates, mostly in the <10 μm size range, usually contributed

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Table 1
Oceanographic conditions at Station 18, on the shelf off Concepción (central-south Chile), between August 2004 and July 2006

Date (dd/mm/yy)	State	SST	SSS	Φ_{80}	Integrated nitrate		Integrated Chl-*a*		
					0–30 m	40–80 m	Total	<20 μm	<3 μm
18/08/2004	NUPW	12.0	31.2	72	313	762	24	18	3
09/09/2004	NUPW	12.5	32.8	88	337	1064	66	37	4
05/10/2004	UPW	11.6	34.3	23	761	1439	120	19	2
03/11/2004	UPW	13.1	34.0	47	689	1434	38	31	5
01/12/2004	UPW	13.9	34.3	42	463	1247	246	29	8
11/01/2005	UPW	11.9	34.5	27	536	1182	379	26	9
26/02/2005	UPW	13.4	34.3	34	593	1334	217	86	6
22/03/2005	UPW	13.1	34.2	45	764	1346	24	20	10
12/04/2005	UPW	12.1	34.5	22	576	1271	289	23	3
31/05/2005	NUPW	12.2	28.7	62	590	1028	36	34	4
05/07/2005	NUPW	12.6	30.4	138	282	637	33	27	2
09/08/2005	NUPW	11.9	32.2	86	284	936	16	11	2
07/09/2005	NUPW	12.8	30.1	143	237	1032	24	20	4
03/11/2005	UPW	12.8	34.4	6	506	1407	300	81	10
15/11/2005	UPW	13.6	34.4	9	421	1386	207	47	4
27/12/2005	UPW	14.3	34.3	34	463	1207	183	46	5
14/01/2006	UPW	14.5	34.3	14	281	829	281	43	5
21/02/2006	UPW	18.0	34.3	6	462	1167	146	68	9
22/03/2006	UPW	13.1	34.5	10	405	1149	698	82	6
26/04/2006	UPW	12.6	34.1	15	ND	ND	124	30	4
14/06/2006	NUPW	12.9	33.7	30	340	818	37	32	6
28/07/2006	NUPW	11.3	25.9	123	259	565	51	44	9

State: upwelling favourable (UPW) or non-upwelling (NUPW) periods (see Section 3.1); SST: surface temperature (°C), SSS: surface salinity; Φ_{80}: stratification index (J m^{-3}), top 80 m; integrated nitrate concentrations (mmol m^{-2}); integrated (0–80 m) chlorophyll-*a* (Chl-*a*) concentration (mg m^{-2}) for the total, and the <20 and <3 μm size fractions. ND = not determined.

>80% of the total nanoplankton integrated abundance, whereas nanodiatoms (mostly *Navicula* spp. and *Thalassiosira minuscula*) and nanodinoflagellates (mostly *Gymnodinium* spp. and *Gyrodinium* spp.) represented the rest. In the April 2005 sampling, however, the nanodiatoms (*T. minuscula*) made a similar contribution to that of the flagellates. Ciliates only appeared on a few occasions and in very low numbers (<12 cells mL^{-1}); they were not considered in further analyses because the fixative used was not the most appropriate for this taxonomic group (Choi and Stoecker, 1989; Stoecker and Gifford, 1994). The integrated (0–80 m) abundance of total nanoplankton during the whole period ranged between 3 and 317×10^9 cells m^{-2}, with most of it corresponding to the flagellates ($2–315 \times 10^9$ cells m^{-2}).

In terms of average cell biovolumes, the nanoflagellates were the smallest components (Fig. 5). Amongst the ANF, the Cryptophyceae (clearly distinguished by their accessory photosynthetic pigment phycoerythrin) presented the largest volumes (56–347 μm^3), the highest values (>90 μm^3) generally appearing during the second half of the upwelling months (January–April 2005; January–March 2006), whereas the smaller representatives of the ANF (14–60 μm^3) did not display a clear pattern of change between upwelling and non-upwelling periods. The HNF were also in the small range of biovolumes (10–51 μm^3) and no clear pattern of variation between the two periods was observed. The nanodiatoms showed a wide range of biovolumes (70–2370 μm^3), the highest values being mostly found during the spring–summer samplings (Fig. 5) and attributable to *T. minuscula*. The cell volumes of the HND (range: 170–546 μm^3) and the AND (range: 142–563 μm^3) were, in comparison, intermediate with respect to those of flagellates and diatoms and did not display a clear pattern of variation between upwelling and non-upwelling periods.

The temporal and vertical distribution (0–80 m) of the nanoplankton biomass in the different taxonomic and trophic groups during the whole of the study period is presented in Fig. 6. In general terms, the nanoplankton was concentrated in the upper 30 m of the water column. The biomass of the ANF (Fig. 6a) ranged between 0 and 139 μg C L^{-1} and was highest in February 2005, coinciding with a maximum value in abundance (23×10^3 cells mL^{-1}) in the top 30 m layer, and contrasting with lower abundance during the rest of

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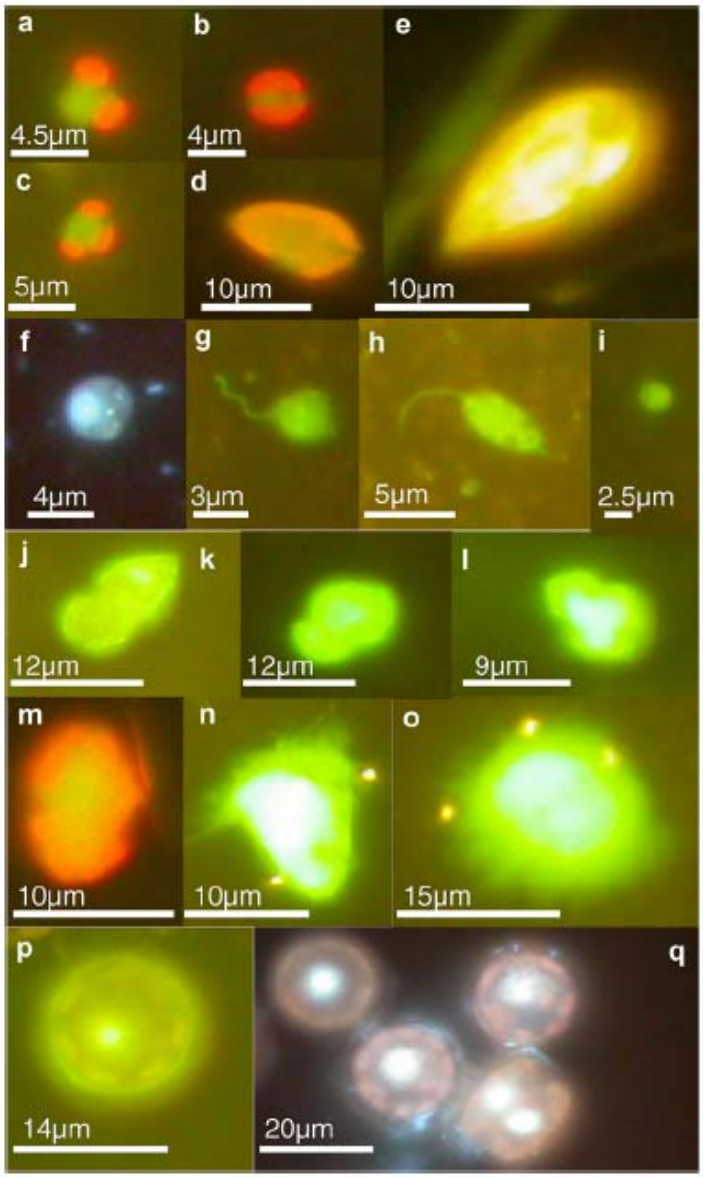

Fig. 3. Digital photographs of some of the most common nanoplankton forms (stained with DAPI) found at the shelf off Concepción, Chile: a–c = unidentified autotrophic flagellates; d–e = *Cryptophyceae*; f–i = unidentified heterotrophic flagellates; j–l = unidentified heterotrophic dinoflagellates; m = unidentified autotrophic dinoflagellate; n–o = unidentified ciliates; p = solitary form of *Thalassiosira minuscula*, q = cluster of *T. minuscula*. A multiple excitation filter was used in images f and q, and a blue excitation filter for the remaining images.

the samplings ($<1 \times 10^3$ cells mL^{-1}). The biomass of HNF (Fig. 6b) was more evenly distributed in the water column (range: 0–13.5 µg C L^{-1}); their abundance was in general lower ($<0.5 \times 10^3$ cells mL^{-1}) than that of the ANF, with occasional maxima (February and March 2006: up to 1.5×10^3 cells mL^{-1}). The biomasses of the AND (Fig. 6c) and HND (Fig. 6d) were of the same order of magnitude as those of the HNF (0–12.3 and 0–14.3 µg C L^{-1}, respectively) but their maximum values did not co-occur, that of the AND appear-

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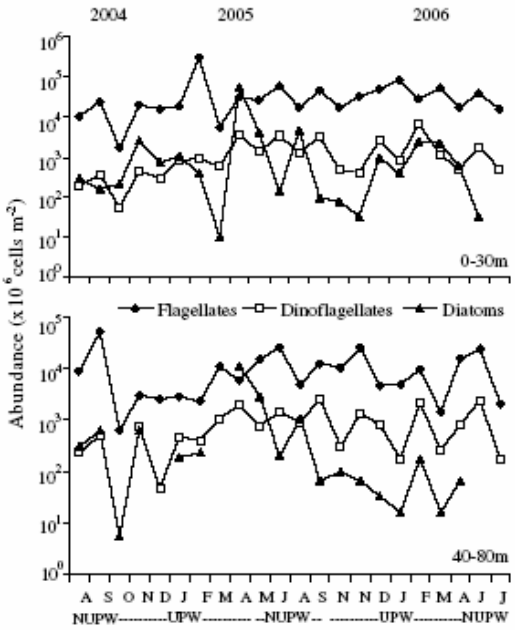

Fig. 4. Temporal distribution of the integrated abundance of the nanoplankton (cells $\times 10^6$ m^{-2}) at Station 18 during the study period (August 2004–July 2006) and in two depth layers: upper panel = 0–30 m, lower panel = 40–80 m. Symbols: diamonds = flagellates, triangles = diatoms, squares = dinoflagellates.

ing in February 2006 (12.3 μg C L^{-1}) and that of HND in April 2005 (14.4 μg C L^{-1}). The latter did coincide with the maximum in nanodiatom biomass (456 μg C L^{-1}; Fig. 6e), however, that of the diatoms was one order of magnitude higher (range: 0–456 μg C L^{-1}). The integrated (0–80 m) biomass of the total nanoplankton during the entire study period ranged from 0.02 to 10.6 g C m^{-2}, the flagellates contributed from 0.01 to 1.9 g C m^{-2}.

3.3. Association between nanoplanktonic structure and physical–biological variability

The abundance and biomass of total nanoplankton and nanoflagellates displayed highly significant differences with respect to sampling dates and depth (two-way Friedman's, $n = 195$, $p < 0.001$). Non-parametric multiple comparison analyses were used to search for these differences with the data grouped into (a) three depth ranges (0–10, 15–30, 40–80 m) and (b) two contrasting periods (upwelling *vs.* non-upwelling; Table 1). The results of these analyses revealed that the variations in the abundance and biomass of ANF, HNF, and total nanoplankton were not significantly different between the two upper layers but both were so with respect to the deeper layer (40–80 m), especially in the case of ANF and total nanoplankton (Table 2). A trend of decreasing nanoplankton mean abundances and biomasses from the surface to the bottom layer was observed but the dispersion of the values was high.

In the subsequent comparison between the two seasonal periods (upwelling *vs.* non-upwelling), only two depth ranges were analysed (0–30 m and 40–80 m), following the results from the above test. The results (Table 3) indicate that the abundance and biomass of ANF, HNF, and total nanoplankton were similar during the two contrasting periods in the 0–30 m range but not in the 40–80 m layer (except for the HNF biomass, as confirmed in Fig. 6b). A trend of higher mean values was observed for the samplings during the upwelling period in the 0–30 m layer, with high dispersion of the values; in the 40–80 m layer, however, the mean values for ANF and total nanoplankton were considerably lower than in the upper layer.

The results of the correlation analyses (Kendall test, τ values) between hydrographic and biological variables (total and size fractionated Chl-*a* concentration, and nanoplankton abundance) are presented in

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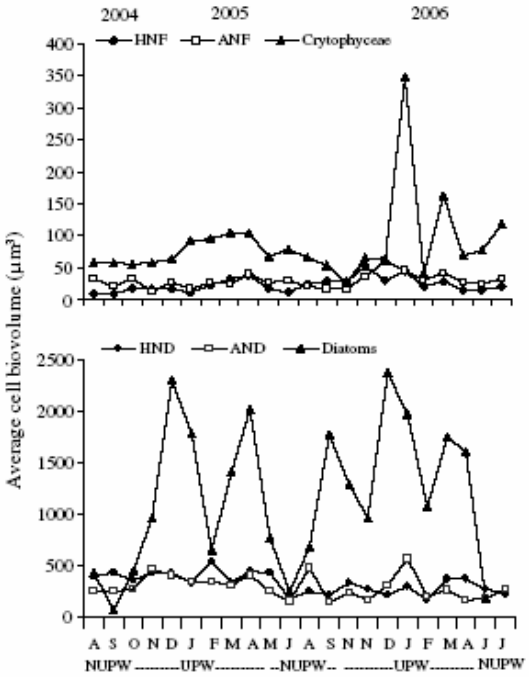

Fig. 5. Temporal distribution of the average cell biovolume of the nanoplankton (μm^{-3}) at Station 18 during the study period (August 2004–July 2006): upper panel = flagellates (Cryptophyceae: dotted line with triangles; autotrophic flagellates: dashed line with squares; heterotrophic flagellates: solid line with circles); lower panel = diatoms (solid line with circles), autotrophic dinoflagellates (dotted line with triangles), and heterotrophic dinoflagellates (solid line with squares).

Table 4 (correlations with nanoplankton biomass are not shown but displayed the same result as the abundance data). First, the hydrographic variables (temperature, salinity, oxygen, nitrate) were all found to be significantly correlated with each other in the water column (0–80 m; $\tau > 0.38$, $p < 0.001$); temperature was positively correlated with oxygen and negatively with salinity and nitrate (data not shown). The biological variables were significantly but weakly associated with hydrographic variation ($\tau < 0.44$). Among the highest correlations, temperature and oxygen were positively correlated with higher abundances of total nanoplankton and ANF. To a lesser degree, nitrate and salinity were negatively correlated with these biological variables. Also, the <20 µm Chl-*a* fraction was positively correlated with the abundance of ANF and total nanoplankton (to which the ANF made the largest contribution). On the other hand, there was a lack of correlation between the abundance of nanoplankton and the degree of stratification of the water column.

3.4. Grazing rates by nano-heterotrophs on picoplanktonic assemblages

In calculating the potential grazing rates (GR), a mean cell volume of 0.11 μm^3 (Cuevas et al., 2004) was used for bacterial prey and 1.5 μm^3 for cyanobacterial cells. The mean GR values of HNF on bacterioplankton ranged between 3 and 27 cells $HNF^{-1}\,h^{-1}$ and between 0.1 and 4 cells $HNF^{-1}\,h^{-1}$ for cyanobacteria (Fig. 7). The HND were also potentially important as grazers, with mean GR values on bacterioplankton of 27 to 242 cells $HND^{-1}\,h^{-1}$ and of 0.3 to 14 cells $HND^{-1}\,h^{-1}$ on cyanobacteria (Fig. 7). In the search for differences between depth strata and seasonal conditions, significant differences were found (Friedman test, $n = 195$; $p < 0.001$). In terms of changes in GR with depth, the values for HNF and HND grazing on cyanobacteria were significantly higher in the upper layer (means ± SD: 0.7 ± 1.1 cells $HNF^{-1}\,h^{-1}$ and 3.8 ± 4.6 cells $HND^{-1}\,h^{-1}$; $n = 130$) compared with the lower layer (0.2 ± 0.3 cells $HNF^{-1}\,h^{-1}$ and 1.2 ± 1.6 cells $HND^{-1}\,h^{-1}$; $n = 65$) (Dunn test, $p < 0.001$). GR on bacterioplankton by HNF were similar

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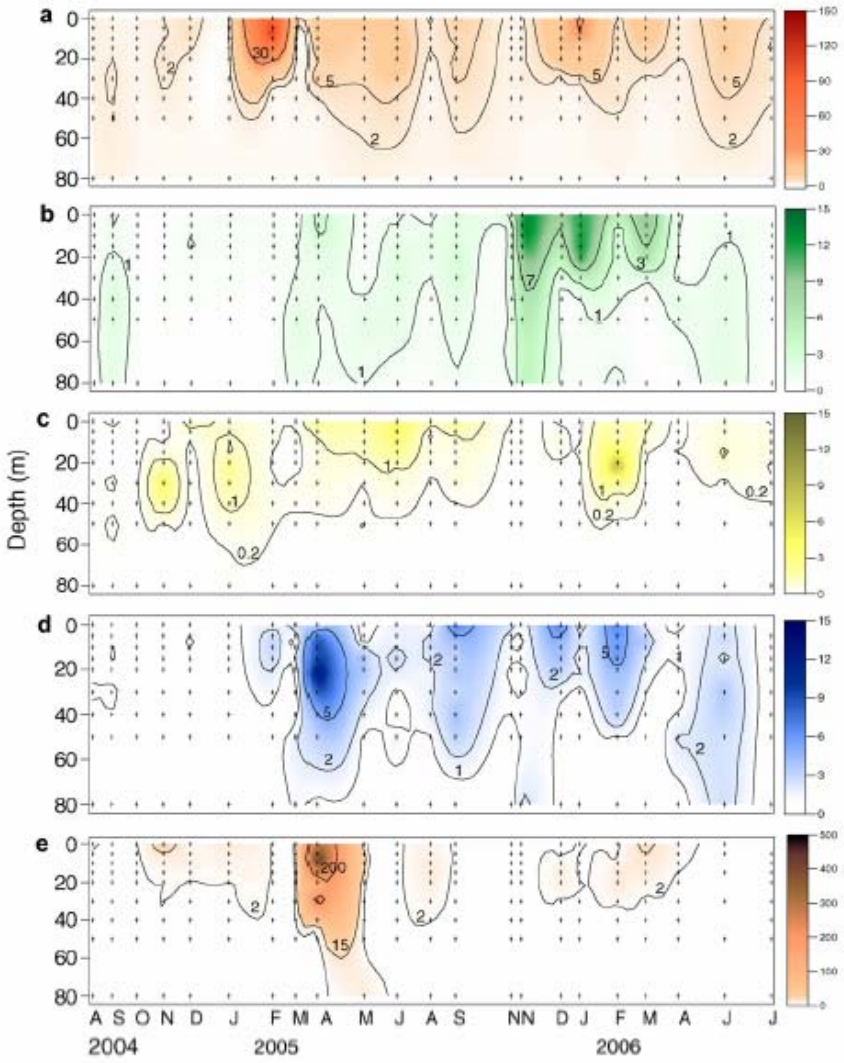

Fig. 6. Temporal distribution of the biomass of the nanoplankton (μg C L^{-1}) at Station 18 during the study period (August 2004–July 2006): (a) autotrophic flagellates, (b) heterotrophic flagellates, (c) autotrophic dinoflagellates, (d) heterotrophic dinoflagellates, and (e) diatoms.

in both strata (means: 12 ± 7 cells $HNF^{-1}\,h^{-1}$; $p < 0.05$) and not very different in the case of the HND (means: 73 ± 44 and 63 ± 60 cells $HND^{-1}\,h^{-1}$ for the upper and lower strata, respectively; $p < 0.05$). With respect to the seasonal conditions, GR on bacterioplankton in the water column (0–80 m) were only slightly higher (Dunn test, $p < 0.05$) during upwelling (13 ± 8 cells $HNF^{-1}\,h^{-1}$ and 71 ± 50 cells $HND^{-1}\,h^{-1}$) compared to non-upwelling conditions (9 ± 6 cells $HNF^{-1}\,h^{-1}$ and 68 ± 57 cells $HND^{-1}\,h^{-1}$). Mean GR on cyanobacteria were similar under the two different conditions for HNF but with a higher dispersion in the former (UPW $= 0.5 \pm 1$ vs. NUPW $= 0.5 \pm 0.4$; $p < 0.001$); the HND displayed slightly different mean values with a similar dispersion (UPW $= 2.4 \pm 4$ vs. NUPW $= 4.0 \pm 4$; $p < 0.05$). In summary, most of the GR on cyanobacteria were higher in the surface layer and during the upwelling condition, whereas GR on bacterioplankton displayed lesser differences in these two factors.

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Table 2
Mean ± standard deviation values for the abundance (AB × 10^3 cells mL^{-1}) and biomass (BM: µg C L^{-1}) of nanoplankton assemblages in three depths ranges (0–10, 15–30, 40–80 m) at the fixed station off Concepción

Depth (m)	ANF		HNF		NANO	
	AB	BM	AB	BM	AB	BM
1: 0–10	1.4 ± 3.7	10.7 ± 22.5	0.4 ± 0.4	2.8 ± 3.8	2.0 ± 3.7	37.2 ± 86.4
2: 15–30	0.7 ± 1.3	5.2 ± 8.5	0.4 ± 0.3	2.2 ± 3.0	1.2 ± 1.4	20.8 ± 52.3
3: 40–80	0.1 ± 0.2	0.8 ± 1.2	0.2 ± 0.3	1.0 ± 1.2	0.4 ± 4.2	3.2 ± 3.1
Comparison between depth ranges						
1 vs. 2	2.0 ns	2.0 ns	0.8 ns	0.2 ns	2.2 ns	3.0 ns
1 vs. 3	10.6***	10.7***	4.9**	3.7*	9.7***	10.7***
2 vs. 3	8.6***	8.8***	4.1*	3.5*	7.5***	7.8***

ANF = autotrophic nanoflagellates, HNF = heterotrophic nanoflagellates, and NANO = total nanoplankton (flagellates, diatoms, dinoflagellates). The comparison between the depth ranges is represented by the critical value of the Tukey non-parametric multiple comparison (samples of equal size). Significance levels (p): ns = $p < 0.05$.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 3
Mean ± standard deviation values for abundance (AB; × 10^3 cells mL^{-1}) and biomass (BM; µg C L^{-1}) of nanoplankton assemblages during two seasonal periods (UPW: upwelling; NUPW: non-upwelling) in two depth ranges (0–30, 40–80 m) at the fixed station off Concepción

Depth (m)	Condition	ANF		HNF		NANO	
		AB	BM	AB	BM	AB	BM
0–30	UPW	1.3 ± 3.4	9.6 ± 21.0	0.4 ± 0.4	3.3 ± 4.1	1.9 ± 3.4	39.0 ± 87.5
	NUPW	0.7 ± 0.5	4.8 ± 3.8	0.3 ± 0.2	1.1 ± 0.7	1.1 ± 0.7	10.9 ± 7.6
		1.5 ns	1.0 ns	0.2 ns	1.8 ns	0.1 ns	1.3 ns
40–80	UPW	0.1 ± 0.1	0.3 ± 0.7	0.2 ± 0.2	1.0 ± 1.2	0.2 ± 0.2	2.5 ± 2.6
	NUPW	0.3 ± 0.2	1.1 ± 1.5	0.4 ± 0.4	1.1 ± 0.8	0.7 ± 0.5	4.6 ± 3.5
		4.2***	4.2***	3.0**	1.6 ns	3.9***	2.8**

ANF = autotrophic nanoflagellates, HNF = heterotrophic nanoflagellates, and NANO = total nanoplankton (flagellates, diatoms, dinoflagellates). The comparison between the periods is represented by the critical values of the Dunn non-parametric multiple comparison (samples of different sizes). Significance levels (p): ns = $p < 0.05$;
Q1 * = $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 4
Association between oceanographic variables and the nanoplanktonic structure (abundance data) during the 2-year sampling period at the time series station off Concepción

Variables	Chl-*a* total	Chl-*a* < 20 µm	Stratification	Nitrate	Temperature	Salinity	Oxygen
ANF	0.19***	0.41***	0.22 ns	−0.32***	0.44***	−0.37***	0.41**
HNF	0.29***	0.33***	−0.14 ns	−0.33***	0.23***	−0.18***	0.29***
NANO	0.35***	0.44***	0.06 ns	−0.35***	0.40***	−0.29***	0.39***
Chl-*a* < 20 µm	0.68***	–	–	−0.36***	0.35***	−0.25***	0.38***
Chl-*a* total	–	–	–	−0.25***	0.17***	−0.06 ns	0.21***

Data are for different depths and dates ($n = 195$; except for stratification $n = 22$). The values represent the correlation coefficients from the Kendall Rank Correlation (τ). ANF = autotrophic flagellate, HNF = heterotrophic flagellate, and NANO = total nanoplankton. Significance level (p): ns = $p < 0.05$;
* = $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

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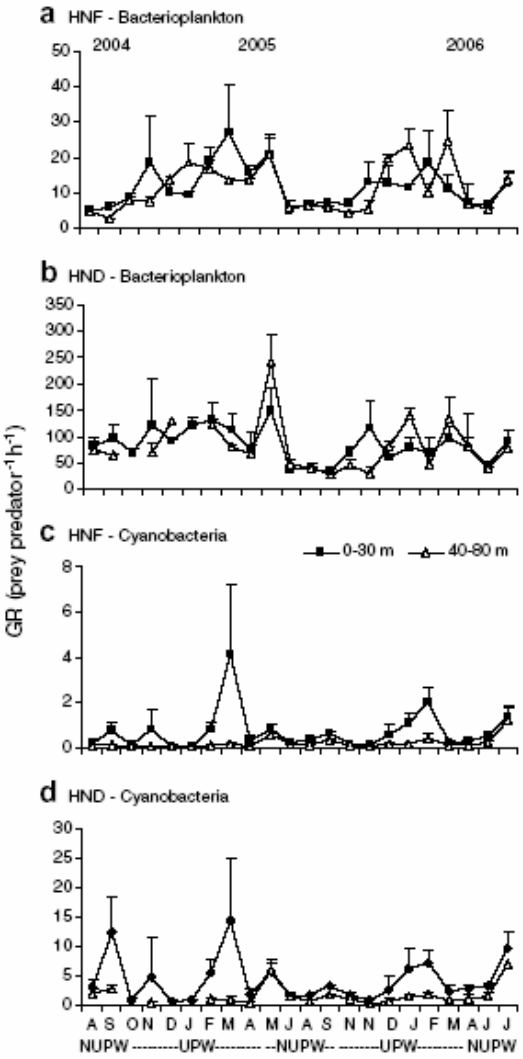

Fig. 7. Mean grazing rates (cells^{-1} predator^{-1} h^{-1}) of (a) HNF and (b) HND feeding on bacterioplankton, and (c) HNF and (d) HND feeding on cyanobacteria at Station 18 during the study period (August 2004–July 2006) and at two depths layers (solid line with squares = 0–30; solid line with diamonds = 40–80 m).

4. Discussion

4.1. Environmental variability and the dominance of nanoplanktonic assemblages in upwelling areas

The main results of our study suggest that there is no clear seasonal variation in the abundance and biomass of nanoplankton assemblages in the upper layer (0–30 m) on the shelf off Concepción, a highly productive area. Both the temporal and spatial resolutions of the sampling may have influenced these results, but we provide arguments that support our results. In this HCS region, the seasonality in the hydrographic conditions was also observed at Station 18 (Sobarzo et al., this issue) but the periods of active upwelling (5–6 d) alternate with periods of relaxation (2–5 d), providing further hydrographic variability (Strub et al., 1998). During the

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upwelling cycle, the contributions of autotrophic nanoplankton to total Chl-*a* can be highly variable (Peterson et al., 1988) and this might explain the high dispersion around the mean values in nanoplankton abundance and biomass observed during the upwelling period in the present study.

The database used here does not include the entire 4-year time series set from Station 18 (August 2002–July 2006; COPAS, unpublished data) since only total nanoflagellate abundances were recorded in the period prior to this study. The nanoflagellates are the numerically dominant component of the 2–20 μm fraction in this region and the dataset confirms the absence of a seasonal pattern of variation, with maximum values appearing during upwelling and non-upwelling periods (Fig. 8). Off Concepción, Cuevas et al. (2004) described temporal (spring *vs.* winter conditions; 2 cruises) and spatial (coastal shelf *vs.* oceanic) variability in the nanoflagellate abundance and also found no differences between upwelling and non-upwelling periods (Table 5). At Station 18, Anabalón et al. (this issue) compared the relative contributions of nano- and microplanktonic assemblages and reported a decrease in the nanoflagellate abundance during the non-upwelling period but their data only covered the first part of the time series (2003–2004; Fig. 8).

A lack of seasonality or variation of the dominant components of the nanoplankton (<10 μm) with a range of mixing/turbulence conditions in the water column has been reported for oceanic (Li, 2002) and coastal upwelling systems (Varela, 1992; Casas et al., 1999; Tilstone et al., 2003; Barlow et al., 2005; Rodríguez et al., 2006). This contrasts with the idea that different regimes of turbulence and/or nutrient availability define the size structure of phytoplankton communities, with dominance of picoplanktonic forms under lower turbulence-nutrient conditions and a shift to larger, micro-phytoplankton cells with increased turbulence and nutrient concentrations (Hutchings et al., 1995; Tilstone et al., 2000; Li, 2002; Irwin et al., 2006). On this basis, we expected the abundance and biomass of the ANF to decrease during the nutrient enrichment caused by upwelling, when chain diatoms become dominant (Anabalón et al., this issue and González et al., this issue). Our results do not confirm this, with most of the maximum values being observed during the upwelling period. Moreover, nitrate concentrations were significantly higher during the upwelling period in both strata, 0–30 m (16 ± 10 μM) and 40–80 m (25 ± 6 μM) (Dunn test after Friedman test, $n = 186$; 0–30 m: $p < 0.01$; 40–80 m: $p < 0.001$), but the values were also relatively high during the non-upwelling period (11 ± 4 at 0–30 m and 18 ± 6 μM at 40–80 m), when river inputs might also act as a source of nitrates.

The lack of correlation between nanoplankton abundance (and biomass) and stratification, and the weak correlation between ANF (and nanoplankton) and nitrate concentration, suggest that nitrate is neither (or rarely) a limiting resource, nor does the degree of stratification influence these assemblages when considered as a size fraction. The co-existence of nano- and microplanktonic autotrophs during the spring–summer months, when the latter are brought to the surface layers by upwelling activity, could be explained by the further nutrient enrichment and improved light conditions for the whole community of phytoplankters. Which, then, are the factors that lead to a reduced level of variation in nanoplankton (mostly nanoflagellates) abundance, with only occasional increases of one to two orders of magnitude? The most likely, based on the information available, is grazing pressure. That this factor might have an impact on the abundance of nanoplankton has been previously suggested as a mechanism for holding their populations at relatively constant levels (Martin et al., 1996; Lewitus et al., 1998; Goericke, 2002). This point is analyzed in detail in Section 4.3.

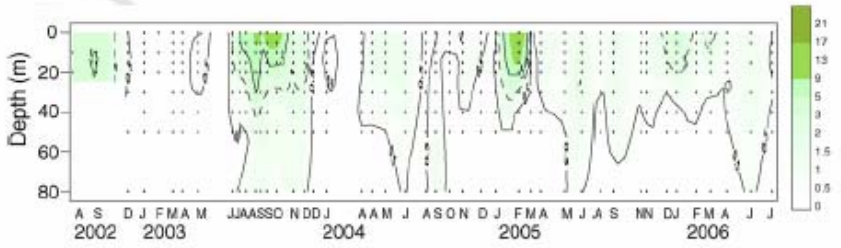

Fig. 8. Temporal distribution of total flagellate abundance ($\times 10^3$ cells mL^{-1}) at Station 18 during the whole sampling period of the COPAS time series (August 2002–July 2006).

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Table 5
Summary of the abundance and biomass data of nanoplanktonic organisms in different upwelling areas

Study area	Abundance	Biomass	Reference
Total flagellates			
Southern Benguela, coastal	$0.0002–8 \times 10^6$ cells L^{-1}	3–173 μg C L^{-1}	Painting et al. (1992)
Concepción Bay	$0.1–3 \times 10^6$ cells L^{-1}		Pacheco and Troncoso (1998)
Off Concepción			Cuevas et al. (2004)
Non-upwelling	$0.06–4 \times 10^6$ cells L^{-1}		
upwelling	$0.07–3 \times 10^6$ cells L^{-1}		
Off Concepción			This study
Non-upwelling	$0.04–3 \times 10^6$ cells L^{-1}	0.3–20 μg C L^{-1}	
upwelling	$0.01–23 \times 10^6$ cells L^{-1}	0.04–139 μg C L^{-1}	
2-year cycle, integrated	$2–315 \times 10^9$ cells m^{-2}	0.01–2 g C m^{-2}	
ANF			
Arabian Sea	$0.02–12 \times 10^6$ cells L^{-1}	0.2–12 μg C L^{-1}	Garrison et al. (1998)
Arabian Sea		0.3–8 μg C L^{-1}	Brown et al. (2002)
Off Concepción	$0–23 \times 10^6$ cells L^{-1}	0–139 μg C L^{-1}	This study
2-year cycle integrated	$0.8–310 \times 10^9$ cells m^{-2}	0.005–1.9 g C m^{-2}	
HNF			
Concepción Bay			McManus and Peterson (1988)
active upwelling	$0.2–2 \times 10^6$ cells L^{-1}		
relaxation	$0.2–4 \times 10^6$ cells L^{-1}		
Arabian Sea	$0.02–2 \times 10^6$ cells L^{-1}	0.2–5 μg C L^{-1}	Garrison et al. (1998)
Off Peru	$0.01–1 \times 10^6$ cells L^{-1}		Sorokin and Kogelschatz (1979)
Off Northern Chile	$0.03–2 \times 10^6$ cells L^{-1}		Cuevas and Morales (2006)
Off Concepción	$0–2 \times 10^6$ cells L^{-1}	0–14 μg C L^{-1}	This study
2-year cycle, integrated	$2–68 \times 10^9$ cells m^{-2}	0.005–0.6 g C m^{-2}	
AND			
Oregon upwelling	$0.006–0.03 \times 10^6$ cells L^{-1}		Hood et al. (1992)
Arabian Sea		0–610 mg C m^{-2}	Garrison et al. (1998)
Off Concepción	$0–0.3 \times 10^6$ cells L^{-1}	0–12 μg C L^{-1}	This study
2-year cycle, integrated	$0.01–3 \times 10^9$ cells m^{-2}	0.3–131 mg C m^{-2}	
HND			
Off Concepción	$0–0.2 \times 10^6$ cells L^{-1}	0–14 μg C L^{-1}	This study
2-year cycle, integrated	$0.03–6 \times 10^9$ cells m^{-2}	2–444 mg C m^{-2}	
Nanodiatoms			
Off Concepción	$0–3.3 \times 10^6$ cellsL^{-1}	0–456 μg C L^{-1}	This study
2-year cycle, integrated	$0–68 \times 10^9$ cells m^{-2}	0.001–10 g C m^{-2}	
Total nanoplankton			
Off Concepción	$0.01–23 \times 10^6$ cells L^{-1}	0.04–482 μg C L^{-1}	This study
2-year cycle, integrated	$3–317 \times 10^9$ cells m^{-2}	0.02–11 g C m^{-2}	

ANF = autotrophic nanoflagellates; HNF = heterotrophic nanoflagellates; AND = autotrophic nanodinoflagellates; HND = heterotrophic nanodinoflagellates; the integrated values refer to the 0–80 m layer in this study.

4.2. Abundance and biomass of nanoplanktonic assemblages in upwelling areas

Except for the maximum value of nanoflagellate abundance registered in February 2005 in this study, the remaining data fall in the same range as other reports on the shelf off Concepción, northern Chile, and southern Peru (Table 5). These values are also similar to those reported for the upwelling area of the Arabian Sea but the biomasses observed are one order of magnitude higher in our study (Table 5). This might be due to differences in size composition but, in general, biomass comparisons are difficult to make since the conversion factors used in the literature differ significantly (Dennett et al., 1999).

Occasionally during this study, the nanodinoflagellates and nanodiatoms contributed significantly to the total integrated nanoplankton abundance (15% and 59%, respectively) and biomass (41% and 91%, respec-

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tively). Few other studies have reported on nanodinoflagellate abundances in pelagic communities (*e.g.*, Stoecker et al., 1994; González et al., 1998; Møller and Nielsen, 2000) and even fewer in upwelling systems (Table 5). In the latter case, this is due to either assigning the dinoflagellates to the microplankton fraction (*e.g.*, Garrison et al., 1998, 2000), not making a distinction between size categories (*e.g.*, Brown et al., 2002), or considering nanoplanktonic components as a whole (*e.g.*, Dennett et al., 1999). In the case of the nanodiatoms, the largest contribution on one occasion (April 2005) was made, but single cells of *T. minuscula* (see also González et al., this issue), counted in the <20 μm range, were also present as clusters in the microplankton size range (data not included in this study; Fig. 3). This species is known to form chains but also appear as solitary cells or embedded in mucilage (Hasle and Syvertsen, 1997). It is difficult to assign this species to the nano- or the microplankton since its contribution to one or the other will change with time, as may happen with other diatom genera which dominate in the upwelling systems (*e.g.*, *Skeletonema*,*Chaetoceros*; Kudela et al., 2005). A closer analysis of the life cycles of diatoms and the factors that modulate their existence as single *versus* aggregated cells will help to understand the link between cell size, trophic transfers, and carbon exportation in upwelling systems.

4.3. The role of grazing by nano-heterotrophs on prokaryote, picoplankton prey assemblages in the upwelling area off Concepción

Nano- and microzooplankton are important grazers of autotrophic and heterotrophic populations in several marine systems (Calbet and Landry, 2004). The HNF have typically been considered as the main consumers of the picoplankton (Weisse, 1993; Christaki et al., 2002; Christaki et al., 2005), whereas dinoflagellates feed on a wide variety of prey including bacterioplankton and cyanobacteria (Verity et al., 1993; Schumann et al., 1994; Jeong et al., 2005), small flagellates, nanodiatoms (Hansen, 1991), and micro-diatoms (Strom and Strom, 1996). HNF grazing rates have been mostly focused on bacterivory with several methods being available, ranging from direct estimates (reviewed in Callieri and Stockner, 2002) to model-derived assessments (*e.g.*, Peters, 1994; Vaqué et al., 1994). In this study, no direct estimates of GR were available but, to obtain them, the Peters (1994) model was used because, compared to others, it includes important sources of variability such as prey and predator volumes. A drawback might be that the model is generic and specific systems such as upwelling areas might (or not) have a different pattern of prey–predator response.

In this study, estimates of grazing rates by HNF feeding on bacterioplankton (range: 3–27 cells $HNF^{-1}\,h^{-1}$) are within the range reported in Cuevas et al. (2004), using the same methodological approach but including coastal and oceanic stations, with no significant differences between the GR under upwelling and non-upwelling conditions. Further data on HNF grazing on bacterioplankton in the HCS system were obtained off northern Chile. Vargas and González (2004), using the same model, obtained GR estimates higher than ours but within the same order of magnitude (range: 12–76 bacterioplankton $HNF^{-1}\,h^{-1}$). Part of this difference may arise from the units they used in the calculations (cells L^{-1}) and those in the original model (cells mL^{-1}), the former resulting in higher values (exercise with our own data, not shown). Considerably lower GR were obtained by Cuevas and Morales (2006) when using the selective inhibitor method (0–0.2 bacterioplankton $HNF^{-1}\,h^{-1}$). Our estimates also fall well within the range of HNF grazing rates on bacterioplankton (~10–80 cells $predator^{-1}\,h^{-1}$) reported for a variety of other marine systems (lake, river, estuary, coastal, oceanic), including different techniques (*e.g.*, selective inhibitor method, dilution technique) or model approaches (*e.g.*, Landry et al., 1984; ?; Weisse, 1990; Weisse and Scheffel-Möser, 1991).

Our study also provides some of the first estimates of GR by HNF on cyanobacteria in the HCS, with HNF having being described as important grazers of cyanobacteria off Concepción in a previous study (Böttjer and Morales, 2005) using the dilution method to estimate specific grazing rates (d^{-1}). Cuevas and Morales (2006), using the food vacuole content method, found similar GR values (0–2.1 cyanobacteria $HNF^{-1}\,h^{-1}$) to those reported here (0.1–4 cyanobacteria $HNF^{-1}\,h^{-1}$). In the literature, the range of values for various other systems is very large (0.0002–23 cyanobacteria $HNF^{-1}\,h^{-1}$) and has involved different techniques (*e.g.*, Christaki et al., 2002, 2005). The present study also provides the first estimates of GR by HND on bacterioplankton and cyanobacteria in HCS, the results for cyanobacteria as prey (0.3–14 cells $dinoflagellates^{-1}\,h^{-1}$) being comparable with the few available estimates (1–64 cells $dinoflagellates^{-1}\,h^{-1}$; Jeong et al., 2005).

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Assessments of the degree of control of picoplanktonic populations by nanoplanktonic grazers have been largely based on the correlations between bacterioplankton and HNF abundances (*e.g.*, Gasol and Vaqué, 1993). High (Berninger et al., 1991; Sanders et al., 1992) and weak (Gasol and Vaqué, 1993) correlations between bacterioplankton and HNF have been reported, whereas in the present study, no significant correlation was found (Kendall Rank correlation, $p < 0.05$, $n = 193$). Instead, the abundances of HNF and HND were significantly correlated ($p < 0.001$) with the abundances of cyanobacteria ($\tau = 0.24$ and 0.29, respectively), as was HND with bacterioplankton abundances ($\tau = 0.14$; $p < 0.01$). This suggests the following alternatives for the case of the HNF: (a) organisms other than HNF (*e.g.*, HND, ciliates) are the main predators of bacterioplankton; (b) there is a top-down control of the abundance of HNF though, in this case, HNF would also not have been correlated with cyanobacteria; or (c) the HNF may be using other sources of carbon than bacterioplankton (apparently cyanobacteria in this case). On the other hand, our results suggest that the grazing impact by small dinoflagellates, usually larger than that of the HNF, might regularly control the prokaryote picoplanktonic populations in the upwelling area off Concepción. In turn, this might explain the lack of large seasonal variations in the abundance of nanoplankton. Altogether, it appears that the microbial pathway in this area sustains the productivity of the system during the year and probably the carbon flows from them to higher trophic levels, the system being mainly under biological controls. The period of upwelling activity seems to provide the setting for the physical disturbances that allow not only micro-diatoms but also nano-diatoms, organized into chains or conglomerates, to become dense blooms, attain higher total primary production, and increase the potential for carbon export to depth.

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4.2.

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SHORT COMMUNICATION

Microzooplankton grazing in a coastal embayment off Concepción, Chile, (~36° S) during non-upwelling conditions

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The impact of grazing by natural assemblages of microzooplankton was estimated in an upwelling area (Concepción, Chile) during the non-upwelling season in 2003 and 2004. Seawater dilution experiments using chlorophyll a (Chl a) as a tracer were used to estimate daily rates of phytoplankton growth and microzooplankton grazing. Initial Chl a concentrations ranged from 0.4 to 1.4 mg Chl a m^{-3} and phytoplankton prey biomass and abundance were numerically dominated by components <20 μm. Phytoplankton growth and microzooplankton grazing rates were 0.19–0.25 day^{-1} and 0.26–0.52 day^{-1}, respectively. These results suggest that microzooplankton exert a significant removal of primary production (>100%) during the non-upwelling period.

Microorganisms of the nano- and micro-plankton community (2–200 μm) comprise autotrophs, heterotrophs, as well as mixotrophs (Sherr and Sherr, 2000). Whereas microzooplankters are effective consumers of prey ranging in size from bacteria to organisms nearly as large as themselves, the diet of nanozooplankton is usually restricted to bacteria sized organisms. Both are important regulators of bacterial and phytoplankton production (Fenchel, 1982; McManus and Fuhrmann, 1988) as well as of the remineralization of organic matter and nutrients in the euphotic zone (Azam *et al.*, 1983; Goldman and Caron, 1985; Goldman *et al.*, 1987; Sherr and Sherr, 2000). Furthermore, they are capable of responding quickly to changes in food supply and, therefore, have potential to maintain a close coupling between production and consumption in the euphotic zone (Verity *et al.*, 1992).

Because a large proportion of the total flux of matter and energy in marine food webs is expected to be channelled through microorganisms (Sherr *et al.*, 1986), several studies have focused on their feeding and metabolic rates. Microzooplankton have been recognized as the main consumers of phytoplankton in both oligotrophic and nutrient-rich regions of the open ocean (Sherr and Sherr, 1992) and their relevance as part of the microbial loop is well accepted for a variety of marine ecosystems (Paranjape, 1990; Neuer and Cowles, 1994; Calbet and Landry, 2004). However, the role of micrograzers in coastal areas, especially in highly productive waters, is still far from being understood. Here we use the term microzooplankton to refer inclusively to both nano- and micro-zooplankton <200 μm and, hence, to the loss process as microzooplankton grazing.

Coastal upwelling areas are highly productive systems which usually feature a classical short food chain (Ryther, 1969). Among these areas, the Humboldt Current System (HCS) is one of the most productive, the area off Concepción (36° S) exhibiting some of the highest primary production rates (~4–20 g C m^{-2} day^{-1}) in the world's oceans (Daneri

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et al., 2000). Here, an important proportion (up to 24%) of the organic matter produced by phytoplankton is channelled through bacteria (Troncoso *et al.*, 2003) making the microbial loop an important pathway for the recycling of organic matter in the water column. Very early studies of the Peruvian upwelling system also found high abundances of microorganisms (Beers *et al.*, 1971; Sorokin, 1978; Sorokin and Kogelschatz, 1979), but beyond that little is known about the role of the microbial loop and its structure and functioning in the HCS upwelling areas. This study focused on the impact of microzooplankton grazing in a coastal embayment located in the upwelling area off Concepción, during the non-upwelling season, when the smaller sized plankton comprises a significant fraction of the planktonic assemblages in the area (COPAS time series, University of Concepción, Chile, unpublished data set).

Microzooplankton grazing estimates were assessed by the seawater dilution method (Landry and Hasset, 1982). Sampling was done at the mouth of Coliumo Bay (36°32′ S, 72°56′ W) on three occasions during austral winter (June 17 and 24, 2003; August 9, 2004). Seawater was collected from a depth of 5 m using 10-L Niskin bottles (General Oceanic Model 1010), equipped with interior rubber-coated springs. The water was sieved immediately to remove mesozooplankton grazers by gently passing it through a 125-µm (experiments in 2003) or a 200-µm mesh (experiment in 2004) into clean, acid-washed, 25-L polyethylene containers, via silicone tubing.

The water was handled as carefully as possible to minimize production of bubbles and physical damage to fragile microplanktonic organisms. Samples were collected for the estimation of *in situ* chlorophyll *a* (Chl *a*) and for pico-, nano- and micro-plankton composition and abundance. Seawater collected for the experiments was transported within 1 h to the laboratory at the Marine Biological Station in Dichato (Chile) and maintained in a cold room at *in situ* water temperature (~12°C).

In the laboratory, the seawater sieved in the field was diluted with filtered seawater (GF/F filters, 47 mm) collected from the same location to obtain approximately the following proportions: 15, 30, 45, 60, 75 and 100% of original undiluted water. The different dilution treatments were distributed into 1.15-L glass bottles (2003) or 1.22-L polycarbonate bottles (2004) with three replicates for each treatment. All bottles were enriched with nitrate (final concentration: 5 µM) and phosphate (final concentration: 1 µM) to minimize nutrient limitation during the incubations (Verity *et al.*, 1996). The incubation bottles were then sealed with parafilm to exclude air bubbles, capped and incubated for 48 h at 12°C, under a 12:12 h light/dark cycle (diffuse and dim light at ~5 µmole photon m^{-2} s^{-1}). Forty-eight hour incubations were done because preliminary experiments conducted for 24 h in 2003 did not provide consistent grazing results. During the incubations, bottles were rotated end over end at 0.5 r.p.m. in order to maintain suspension of both prey and predators.

Chlorophyll *a* samples (100 mL) were collected in triplicate for the *in situ* samples and for each experimental treatment at the beginning (t_0) and the end (t_{48}) of the incubations. These samples were filtered (GF/F filters, 25 mm) and frozen for subsequent analysis. The filters were later extracted in 10 mL 90% acetone for 24 h and analyzed by fluorometry (Holm-Hansen *et al.*, 1965). Microplankton samples from *in situ*, t_0 and t_{48} samples of the undiluted treatments (100%) were concentrated by collection onto a 20-µm mesh and then examined to determine community composition. These samples were preserved in glutaraldehyde (2% final concentration) for later microscopic enumeration by inverted microscopy (Utermöhl, 1958). Following the experiments, a comparison of fixation with glutaraldehyde and acid Lugol's solution (10% vol:vol) was done (COPAS, unpublished data).

Nano- and pico-plankton (<20 µm) in 50 mL water from the *in situ*, t_0 and t_{48} undiluted treatments were also preserved in the dark with glutaraldehyde (2% final concentration). 20 mL subsamples were stained with 1 mL DAPI (0.01% final concentration) and filtered onto black polycarbonate filters (0.8 µm). Randomly selected fields (at least 50–100 cells of cyanobacteria and 25–50 for nanoflagellates) were counted using epifluorescence microscopy (Porter and Feig, 1980).

The grazing rates of the microzooplankton on Chl *a* were calculated by the exponential growth model of Landry and Hasset (Landry and Hasset, 1982). The instantaneous rate coefficients of microzooplankton grazing (g) and phytoplankton growth (k) were estimated from the linear regression of the apparent growth rate ($\mu = (1/t \cdot \ln(C_t/C_0) = k–g)$ plotted against the dilution factor of Chl *a*. The significance of the linear regression was tested using Microsoft Excel version 2000 statistical software. In parallel, dilution plots were tested for nutrient limitation (Gifford, 1988). The estimated g and k values were used to calculate the phytoplankton doubling time, the potential primary production and the percentage of the production removed by the microzooplankton assemblages, following an indirect approach (García-Pámanes and Lara-Lara, 2001; Verity *et al.*, 2002).

In addition, cell counts were used to assess the degree of change in the composition and abundance of prey and predators during the incubations. Autotrophic prey microplankton abundances were converted to carbon biomass using an average cell volume derived from size measurements and the Menden-Deuer and Lessard's

(Menden-Deuer and Lessard, 2000) equations for volume : carbon conversions. Cyanobacteria were converted to carbon using a conversion factor of 400 fg C μm^{-3} (Burkill *et al.*, 1993) and autotrophic nanoflagellates were converted using a factor of 220 fg C μm^{-3} (Børsheim and Bratbak, 1987).

In situ conditions are summarized in Table I. Near-surface seawater temperature was typical of the winter, estuarine period in the area of study (Faúndez-Báez *et al.*, 2001). The *in situ* Chl *a* levels (<125 and <200 μm) were comparatively low (1.5 mg Chl *a* m^{-3}) but within the range expected for this period (Grunewald *et al.*, 2002). All autotrophs (Chl *a* contributors) were considered prey items for the microheterotrophs identified in this table.

Cyanobacteria were numerically dominant (1–2 × 10^4 cells mL^{-1}), their abundances being within the range reported for the winter period in the coastal area off Concepción (COPAS time series, unpublished data). In the nanoplankton size range, the autotrophic flagellates (2–7 × 10^2 cells mL^{-1}) were in the lower part of the range observed off Concepción (COPAS time series, unpublished data). Among the microautotrophs (>20 μm), chain diatoms (mainly *Skeletonema costatum*, *Chaetoceros cinctus*, *Chaetoceros curvisetus* and *Chaetoceros lorenzianus*) were the dominant components (10–60 cells mL^{-1}), in agreement with previous studies in the Coliumo Bay (González, 1982); pennate and centric diatoms were much less abundant (<5 cells mL^{-1}).

Amongst the microheterotrophs, the heterotrophic nanoflagellates were numerically dominant (3–5 × 10^2 cells mL^{-1}), these values being in the lower range (0.6–40 ×10^2 cell mL^{-1}) of those previously reported for the winter period (Cuevas *et al.*, 2004). Other microzooplanktonic components included dinoflagellates (*Protoperidinium* sp., *Gymnodinium* sp.), ciliates (*Tintinnidium* sp., *Strombidium* sp.), copepod nauplii and radiolaria. These numerical abundances are based on samples preserved with glutaraldehyde. However, the comparison of fixatives resulted in an underestimation of diatom and dinoflagellate abundances by a factor of 2 and ciliate abundances by a factor of 3 when using glutaraldehyde. Loss of ciliates may also occur due to the concentration procedure onto a 20-μm mesh (D. Gifford, personal communication). Overall, some of the abundances may have been underestimated but this does not directly affect the estimates of grazing rates.

The sieving of the experimental water (<125 and 200 μm) for the grazing incubations modified the microplankton composition and abundance to different degrees, reflected in their mean values and standard errors, but in general it affected mainly the chain diatoms (data not shown). During the 48-h incubations, the total mean prey (autotrophic) abundance (~2 × 10^4 cells mL^{-1}) and carbon biomass (~16 mg C m^{-3}) at the beginning of each experiment decreased by ~50% in all three experiments.

Variation in the abundances and biomasses of specific groups (Fig. 1), which can be primarily attributed to grazing, mainly affected the pico- and nano-planktonic autotrophic fraction (60–90%). They were numerically dominant (99%) and contributed >95% to total prey carbon-biomass; however, this varied among the 2003 and

Table I: Field conditions in Coliumo Bay from where seawater was collected for the grazing experiments: temperature (°C), chlorophyll a (mg m^{-3}), and mean plankton abundance of preys (cells mL^{-1}) and predators (nanoplankton predators in cells mL^{-1}; microplankton predators in cells L^{-1})

Experiment	1 (June 17, 2003)	2 (June 24, 2003)	3 (August 8, 2004)
Temperature	12	11.5	12
Chl *a*[a]	1.0	0.4	1.4
Chain diatoms	55	14	40
Pennate diatoms	1	1	4
Centric diatoms	2	1	3
Autotrophic nanoflagellates	286	225	724
Cyanobacteria	19845	16478	9727
Dinoflagellates	239	48	1286
Ciliates	192	192	802
Crustacean nauplii	144	96	747
Radiolaria	48	48	0
Heterotrophic nanoflagellates	490	347	290

[a]<125 μm size fraction in the first two experiments and <200 μm in the third experiment.

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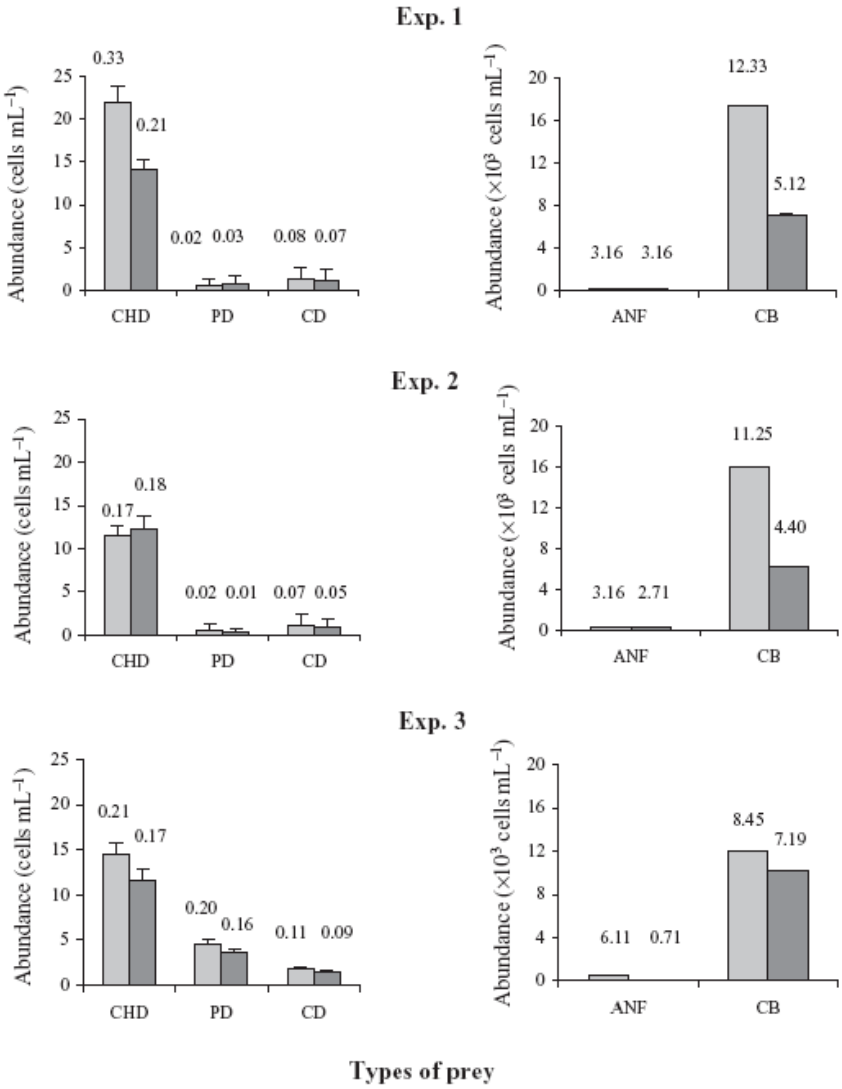

Fig. 1. Prey abundances during the 48 h incubations in the undiluted samples. Grey bars, beginning of the experiment; black bars, end of the experiment and lines from bars, standard error of the mean abundance values. CHD, chain diatoms; PD, pennate diatoms; CD, centric diatoms; ANF, autotrophic nanoflagellates; CB, cyanobacteria. Values on top of each bar indicate the estimated biomass in µg C L^{-1}.

2004 experiments. In 2003, the micro-grazers removed more cyanobacteria than nanoflagellates; in contrast, nanoflagellates were removed at a higher rate than cyanobacteria in 2004. Chain diatoms were grazed moderately (<40%) only during experiments 1 and 3. Other prey abundances, with the exception of the abundances of the pennate and centric diatoms during the third experiment (<20%), did not vary greatly, or at all, during the incubations when considering the associated standard errors. This, however, does not preclude the possibility that they were being consumed, because the experimental design does not control for prey growth.

In general, predator abundances remained relatively constant during the incubations, except in the case of the heterotrophic nanoflagellates which decreased up to 60% in all three experiments (Fig. 2). This suggests that grazing interactions did occur between the predators, affecting mainly the heterotrophic nanoflagellates, and this, in turn, may have influenced the total community grazing upon the autotrophic biomass (Chl *a*).

Microzooplankton dilution experiments, based on Chl *a* changes, displayed the expected pattern of increasing mortality with a decrease in the dilution, resulting in a trend of decrease in the apparent growth rate with

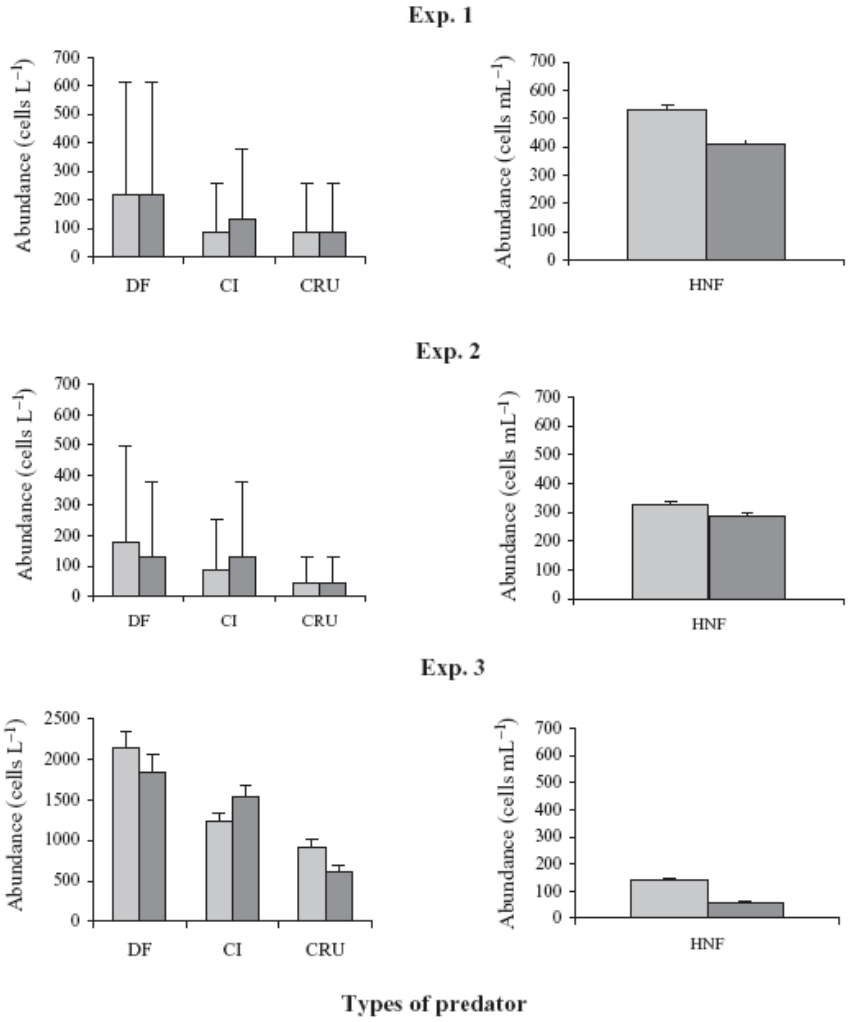

Fig. 2. Predator abundances during the 48-h incubations in the undiluted samples. Grey bars, beginning of the experiment; black bars, end of the experiment and lines from bars, standard error of the mean abundance values. DF, dinoflagellates; CI, ciliates; CRU, crustaceans; HNF, heterotrophic nanoflagellates.

increasing unfiltered (less diluted) water (Fig. 3). Mean estimates for k ranged between 0.19 and 0.25 day^{-1} and for g between 0.26 and 0.52 day^{-1} (Table II). There were, however, some points at the extreme of the curves which could be argued not to fit linearity (Fig. 3), suggesting nutrient limitation and/or feeding thresholds (Gifford, 1988). In this instance, the data from the 100% dilution in experiment 2, as well as the data from the 15% in experiments 1 and 3 were excluded from the analysis. After this, the values of k and g were recalculated (Table II) but the values obtained suggest only a slight change compared with those using the entire data set.

Estimates of less than one doubling per day (Table III) for the autotrophs and a potential primary production in the order of 6–17 mg C m^{-3} day^{-1} were obtained when assuming a C : Chl *a* conversion factor of 60, according to estimates in the same area and season (Grunewald *et al.*, 2002). The potential primary production values are in the lower range of the winter primary production values (3–129 mg C m^{-3} day^{-1}) reported in the coastal area off Concepción (Cuevas *et al.*, 2004) and may have been light limited by our experimental set up. However, there are no reports of light regime for this area, but Montecino *et al.* (Montecino *et al.*, 2004) suggest that the higher turbulence in the mixed layer during winter may prevent specific populations from remaining under appropriate light conditions.

In all experiments, the grazing impact represented a significant (>100%) fraction of the potential primary

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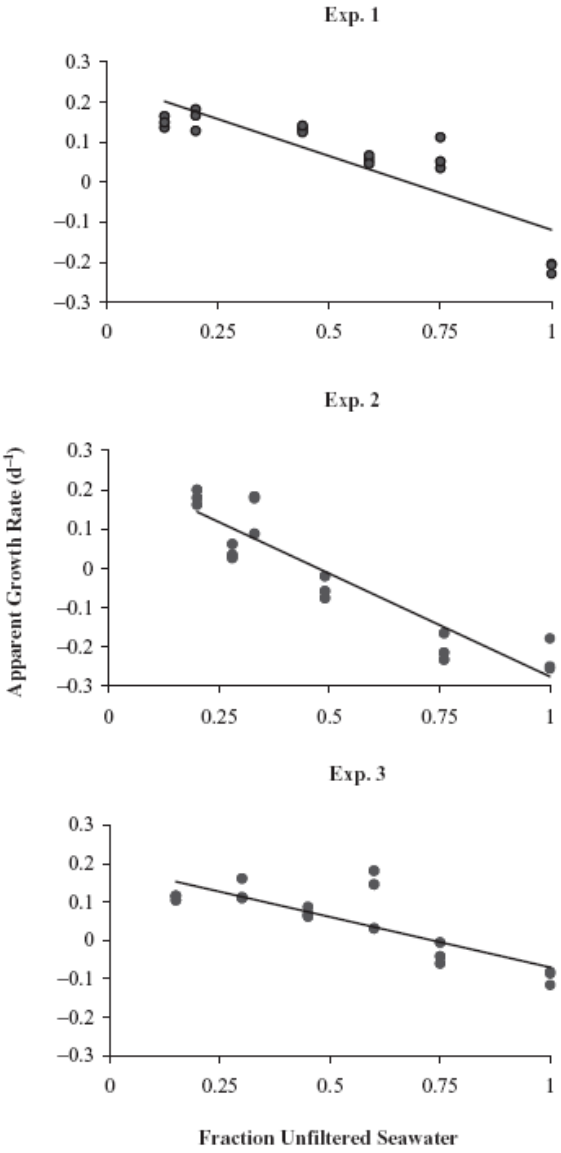

Fig. 3. Plot of the apparent growth rate of autotrophic prey (represented as chlorophyll *a*) versus the dilution factor of the first (June 17, 2003), second (June 24, 2003) and third experiment (August 9, 2004). Lines fitted by least-square regression; number of data points in each experiment = 18.

production (Table III). Overall, these Chl *a* based estimates of grazing, as well as the general observation of the removal of autotrophic plankton during the experiments, suggests that microzooplankton grazing has a significant impact on total primary production in Coliumo Bay during the non-upwelling winter period. In contrast, for shelf waters off Concepcion, and using an indirect approach for estimating grazing, Cuevas *et al.* (Cuevas *et al.*, 2004) suggest that most of the primary production is first channelled through bacteria and, subsequently, through heterotrophic nanoflagellates.

The results of this study represent the first direct attempt to evaluate the trophic interactions mediated by micrograzers in the upwelling system off central Chile. There are few other studies on microzooplankton grazing in upwelling regions with which the data in this study may be compared. In the Oregon upwelling system, where the phytoplankton population consisted mainly of chain-forming or solitary diatoms, the grazing impact on primary production was 44% (mean value) during the upwelling period and 82% (single value) during the non-upwelling period (Neuer and Cowles, 1994). In the Gulf of California, approximately 100% of the primary production was removed by the microzooplankton assemblages, independent of the season (García-Pámanes and Lara-Lara, 2001). In the upwelling system off the northwest coast of Galicia, grazing impact ranged between 60 and 160% during an upwelling/relaxation event (Fileman and Burkill, 2001). In the Arabian Sea, which exhibits both upwelling and oligotrophic conditions, different rates of primary production removal by microheterotrophs have been estimated. Burkill *et al.* (Burkill *et al.*, 1993) reported that 76–111% (mean: 104%) of the production was grazed when the phytoplankton was dominated by cyanobacteria during the non-upwelling period. In contrast, Edwards *et al.* (Edwards *et al.*, 1999) estimated lower rates (4–29%) during the upwelling period when the phytoplankton was dominated by diatoms and cyanobacteria, and moderate rates (24–60%) during the oligotrophic period when cyanobacteria and autotrophic flagellates were dominant. It appears, therefore, that in the typical high productivity, coastal upwelling areas, a significant proportion of primary production might be removed by microzooplankton communities.

Microzooplankton grazing activity is assumed to depend strongly on the composition and size structure of the food supply (Heinbokel, 1978; Paranjape, 1990); it is also known that microzooplankton graze on food particles nearly as large as themselves (Capriulo *et al.*, 1988; Strom, and Strom, 1996). Furthermore, selective feeding behaviour of grazers has been reported in the literature (Burkill *et al.*, 1987). Overall, these facts suggest that micrograzers are potentially able to control autotrophic and heterotrophic biomasses and may regulate species composition in planktonic assemblages. In this study, the microzooplankton assemblages appeared to have fed primarily on the smaller food (<20 μm) during all experiments, as represented by the greatest decline of cyanobacterial and nanoflagellate abundances and, to a lesser degree, on larger autotrophs. The higher consumption of autotrophic nanoflagellates during the third experiment as compared with the first two

Table II: Estimated g (instantaneous grazing rate) and k (instantaneous growth rate) values by linear regression, considering all of the data, nutrient limitation and/or feeding thresholds

Date	Calculation method	k (d^{-1})	g (d^{-1})	r^2
17 June, 2003	Whole data set	0.25 ± 0.03	0.37 ± 0.05	0.75
	Feeding threshold	0.30 ± 0.04	0.43 ± 0.06	0.69
24 June, 2003	Whole data set	0.25 ± 0.03	0.52 ± 0.05	0.86
	Nutrient limitation	0.30 ± 0.04	0.67 ± 0.08	0.85
9 August, 2004	Whole data set	0.19 ± 0.03	0.26 ± 0.05	0.67
	Feeding threshold	0.24 ± 0.04	0.33 ± 0.07	0.65

All values are significant at $p < 0.001$.

Table III: Grazing impact of microheterotrophs on the potential primary production in Coliumo Bay during non-upwelling conditions off Concepción

Experiment	Phytoplankton doublings (d^{-1})	Potential primary production (mg C m^{-3} d^{-1})	Production grazed (% d^{-1})
1	0.39	7	140
2	0.37	6	185
3	0.28	17	132

experiments may be explained by the higher abundance of dinoflagellates (mainly *Gymnodinium* sp. and *Protoperidinium* sp.) in the last experiment, with *Gymnodinium* being a predator of nanoflagellates (Hansen, 1991).

Cyanobacteria are generally considered to be a poor food source in comparison to eukaryotic phytoplankton due to their potential toxicity (O'Neil, 1999) though they represent an important proportion of the total primary production in many different marine ecosystems (Burkill *et al.*, 1993 and references therein). A possible explanation for the higher grazing impact on cyanobacteria might be related to their numerical dominance, so that the grazer encounter rate was higher for them compared with the larger particles. Nevertheless, autotrophic flagellates were also numerically abundant but their standing stocks were not affected to a large extent; suggesting that the micrograzers might have been selecting the cyanobacteria as food (Fig. 1). In agreement with this, Burkill *et al.* (Burkill *et al.*, 1993) found that nanozooplankton (mainly flagellates) consumed 31–71% of the cyanobacteria standing stock in the northwest Indian Ocean. On the other hand, the consumption of diatoms (mainly chain diatoms) that occurred during two of the experiments (1 and 3) suggests that at least part of the grazers, probably copepod nauplii and/or the dinoflagellates (Jacobsen, 1999), were also selecting for larger food particles.

A decrease in the abundance of heterotrophic nanoflagellates was observed during all experiments in the undiluted treatment (Fig. 2), suggesting that they were grazed by the rest of the predators feeding on nanoplankton size fractions and not distinguishing between autotrophic and heterotrophic prey. This implies that the grazing pressure on the autotrophic biomass might have been higher if the grazing rates were underestimated. Dolan *et al.* (Dolan *et al.*, 2000) have already pointed out that only a few studies consider the response of the grazers during the incubations. Changes in aloricate ciliate concentrations were observed by Gifford (Gifford, 1988), the biggest loss occurring in the undiluted (100% seawater) treatments. Based on this, it can be assumed that the decrease of heterotrophic nanoflagellates during our study would have diminished with increasing dilution due to decreased predator–prey interactions.

In conclusion, the impact of microzooplankton grazing in the coastal embayment in the upwelling area off Concepción during the non-upwelling winter season can be high and produces a significant removal of primary production, primarily from the <20 μm size fraction. Whether this grazing pressure is maintained over the upwelling season remains to be assessed though the system becomes dominated by larger autotrophic size fractions, mainly chain diatoms (COPAS time series, unpublished data).

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4.3.

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Are small cyclopoid copepod nauplii (*Oithona* spp.) important grazers in the highly productive, upwelling system off central Chile?

Limnology and Oceanography

Are small cyclopoid copepod nauplii (*Oithona* spp.) important grazers in the highly productive upwelling system off central Chile?

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Running Head: Grazing by nauplii of cyclopoid copepods

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ABSTRACT

Copepod grazing impact on planktonic communities has been commonly underestimated due to the lack of information on nauplii feeding behaviour and ingestion rates. The trophic role of nauplii of the cyclopoid copepod *Oithona* spp., a numerically dominant component of the metazoan microzooplankton in the coastal upwelling area off Concepción (central Chile, ~36ºS), was investigated during the highly productive, upwelling season. Diet composition, ingestion rates, and food-type preferences were assessed through grazing experiments with: a) different size fractions of natural planktonic assemblages (<3, <20, <100 and <125 μm), and b) cultures of the nanoflagellate *Isochrysis galbana*. Under natural concentrations of nano- and microplankton, the nauplii ingested nanoflagellates, small-sized dinoflagellates, and diatoms in solitary form (range: 5 - 73 x 10^3 cells nauplii^{-1} d^{-1}). Under a mixture of pico- and nanoplankton, the nauplii ingested mainly nanoflagellates (9 - 17 x 10^3 cells nauplii^{-1} d^{-1}) but picoplankton was also ingested when it was the solely food available (5 - 18 x 10^6 cells nauplii^{-1} d^{-1}). Ingestion rates on *I. galban*a (28 - 31 x 10^3 cells nauplii^{-1} d^{-1}) were in the range of those estimated for natural nanoflagellates. Carbon uptake by the *Oithona* nauplii was mainly derive d from the nanoflagellates (mean of 350 ng C nauplii^{-1} d^{-1}). At highest abundance levels of the nauplii in the system under study, their daily grazing impacts on the prey standing stocks range from <21% for picoplankton, 2 - 68% for nanoflagellates (mean = 34%), <24% for dinoflagellates, and <13% for diatoms. These results suggest that *Oithona* spp. nauplii exert a significant control on the abundances of the nanoplankton assemblages and, thereby, represent an important trophic intermediate between the classical and microbial food webs in this coastal upwelling system.

KEY WORDS: *Oithona* nauplii, cyclopoid copepods, microzooplankton grazing, coastal upwelling

INTRODUCTION

Small cyclopoid copepods of the genera *Oithona* (<1 mm in length) are ubiquitous and one of the most abundant copepods in the world's ocean (Gallienne and Robins 2001; Turner 2004). Compared to the large calanoids, the knowledge of the biology and ecology of the small cyplopoids, and on their trophodynamic and biogeochemical roles in pelagic systems, is minimal (Paffenhöfer 1993 and references therein). In the case of *Oithona* species, trophic studies have been concentrated mostly on the feeding rates of adults and most of this information has been derived from laboratory studies based on limited diet offers of cultured organisms (e.g. Lampitt and Gamble 1982; Nakamura and Turner 1987; Lonsdale et al. 2000; Castellani et al. 2005). In general, copepod nauplii are expected to exhibit a different feeding behaviour than copepodites and adults (Paffenhöfer and Lewis 1989) but, due to the lack of information on nauplii feeding, the assessments of copepod grazing impacts on planktonic communities has been commonly underestimated (Fessenden and Cowles 1994).

The optimal food cell size for copepods is related to their body size, with small copepods ingesting nano- and picoplankton more efficiently than large copepods (Berggreen et al. 1988; Webber and Roff 1995). The classical view of copepods feeding mainly on diatoms has been questioned as being too simple to describe their trophic interactions (e.g. Paffenhöfer et al. 2005) and today, herbivorous protists are considered to constitute their main food (see review of Stoecker and Capuzzo 1990). Moreover, preferences for motile to non-motile prey types have been observed for *Oithona* spp. (Turner and Graneli 1992, Svensen and Kiørboe 2000; Paffenhöfer and Mazzocchi 2002). The few reports available on the feeding of copepod nauplii indicate that they feed on a variety of prey types, including bacterioplankton (Roff et al. 1995), small sized phytoplankton (e.g. Berggreen et al. 1988) as well as protists (e.g. Merell and Stoecker 1998; Lonsdale et al. 2000; Turner et al. 2001), and detritus (Green et al. 1992). In turn, they are prey items for larval fish (e.g. Conway et al. 1998), heterotrophic protists (Jeong 1994) and mesozooplankton (Bonnet et al. 2004) and, on this basis, they are considered as an important link between the microbial and classical food chains.

In the productive upwelling system off Concepción, central Chile, *Oithona* spp. (mostly *O. similis* and *O. nana*) is a common component of the coastal zooplankton (Escribano et al. in press) and it appears to reproduce throughout the year (Torres 2006). The latter implies that the nauplii experience adequate food quantity and/or quality for their development even when seasonal differences in primary production and chlorophyll-a concentration occur (Montecino et al. 2004; González et al. in press; Morales et al. in press). However, the feeding behaviour

and grazing rates of these oithonids in this upwellling system remains unexplored. Moreover, there is only one previous study which describes the grazing rates of adult *Oithona* species inhabiting the Humboldt Current System (northern upwelling region off Chile; Vargas and Gonzalez 2004a).

Most of the naupliar stages of *Oithona* species in the Concepción upwelling area are smaller than 200 µm (NI to NVI in *O. nana* and NI to NV in *O. similis*) and, therefore, constitute part of the microzooplankton size fraction. In this area, micro-grazers (20 - 200 µm) can exert a high grazing impact (>100%) on the potential primary production during the non-upwelling condition, when the pico- and nano-size fractions are the most abundant components of the planktonic assemblages (Böttjer and Morales 2005). Since the *Oithona* nauplii appear to be a regular component in the area (Torres 2006), and given that the pico- and nanoplankton assemblages display low seasonal abundance variation in this strongly seasonal environment (Böttjer and Morales in press), we propose that the *Oithona* spp. nauplii exert a high and permanent grazing pressure upon the abundance/biomass of microbial assemblages and, thereby, probably act as an important link in channelling organic carbon to higher trophic levels. To test this, nauplii grazing experiments were performed during the upwelling period, using both natural assemblages and cultured cells as food.

MATERIAL AND METHODS

Field collection of copepods and acquisition of naupliar stages

Plankton samples were taken at the mouth of Coliumo Bay (36°32` S, 72°56`W), central Chile, during the upwelling season (austral spring-summer 2004, 2005, 2006, and 2007). Copepods were collected by slow horizontal hauls from 0 to 10 m depth using a WP-2 plankton net (mesh size 200 µm) fitted with a non-filtering cod-end. These samples were immediately diluted with surface seawater, placed in thermo-boxes and transported within 1 h after collection to the laboratory of the Marine Biological Station in Dichato. In the same location, water for the subsequent incubation of the adult females of *Oithona* spp. was sampled (Niskin bottles; General Oceanic Model 1010, equipped with interior rubber-coated springs) and the surface temperature was measured. Once in the laboratory, the samples were maintained in a cold room at the appropriate *in situ* water temperature (Table 1) for a couple of hours until the completion of sample processing.

For the acquisition of nauplii, undamaged adult females of *Oithona* spp. and especially those carrying ovigerous sacs (Fig. 1a and b), were sorted out from the field samples using a stereomicroscope (Zeiss Stemi 2000-C). The selected females (~100 - 150 individuals) plus natural food (natural seawater screened through 100 μm) were haltered in 1 L glass beakers (~30 females per beaker) until they produced nauplii. These incubations, as well as those of the grazing experiments, were maintained at in situ temperature (11.3 - 12.2°C) and irradiance of 110 μmole photon m^{-2} s^{-1} on a 12:12 light:dark cycle. The water + food contained in each beaker were completely changed daily and eggs, freshly hatched nauplii, and dead females were removed. The naupliar stages of *Oithona* spp. were identified according to their length and characterizations provided by Murphy (1923), Gibbons and Oglivie (1933), and Haq (1965). The eggs and nauplii obtained were then incubated in separate containers (eggs were kept in filtered seawater (<0.2 μm) and the nauplii in water + the food type offered in the subsequent experiment) until a sufficient number (21 - 60) of NIV and NV stages of *O. nana* (size range: 120 - 145 μm; Fig. 1c and 1d) and/or NIII and NIV of *O. similis* (size range: 140 - 165 μm) was achieved to start the grazing experiments.

Collection and preparation of food

Natural plankton assemblages were offered in 14 and cultured microalgae in 3 experiments (Table 1). The natural food was collected on the same day before starting the grazing experiments, from the same site where the adult copepods had been collected before. For this purpose, the water was sampled at 5 m using 10 L Niskin bottles. Immediately after collection, this water was brought to the Marine Biological Station in Dichato and maintained in the same cold room as the copepods. Immediately, the collected water was screened through an appropriate mesh size, according to the selected food type treatment (<125, <100, <20 or <3 μm; Table 1), and directly transferred to clean, acid-washed, polyethylene containers (10 L) using silicone tubing. In the experiments with cultured food, the nanoflagellate *Isochrysis galbana* (~5 μm in length) was used in the exponential phase of growth. In each case, 3 mL of the microalgae were added to each of the experimental beakers (500 mL) containing filtered seawater <0.2 μm.

Grazing experiments

The grazing incubations were carried out following a standard protocol (Gifford 1993), including sampling of the food at the beginning (t_1) and end (t_2) of the incubation period. A total of 9 bottles were used in each experiment: 6 as controls (3 for t_1, 3 for t_2), containing

only the prey, and 3 as grazing treatments, including the prey and a known density of nauplii (Table 1). All the incubation bottles (500 mL) were filled to the top and sealed with parafilm© to avoid the production of air bubbles which might damage the fragile organisms. These bottles were placed on a rotation wheel (~0.5 r.p.m.) to keep the food and nauplii in suspension, and incubated for ~24 h under the light and temperature conditions described before. The initial food samples in the controls were collected after 1 h incubation and included the analyses of micro-, nano-, and/or picoplankton cells, as well as total Chl-*a*. For microplankton, 100 mL samples were preserved with Lugol (5% final concentration) and stored in the cold and dark. Samples for nano- and picoplankton (20 and 10 mL, respectively) were preserved with Glutaraldehyde (2% final concentration) and stored as the microplankton samples. For Chl-*a* analysis, triplicate 100 mL samples were filtered onto fibreglass GF/F filters and frozen; these filters were extracted in 10 mL 90% Acetone for ~24 h and the fluorescence was measured using a Turner Designs TD-700 Fluorometer (Holm-Hansen et al. 1965). At the end of the incubations, samples from the control and grazing bottles were collected and treated as described above.

Food cell counts and carbon content

Micro- and nanoplanktonic cells in samples from experiments 1 to 6 (Table 1) were analysed with an inverted microscope (Nikon® TE2000S) equipped with a digital camera (Nikon® Coolpix 4500). For this purpose, 50 mL of the preserved samples were concentrated in settling chambers for at least 24 h (Utermöhl 1958). Cells were enumerated at 400x or 1000x magnification and categorized into main groups (e.g. nanoflagellates, diatoms, dinoflagellates and ciliates); in the case of chain-forming diatoms, the counts refer to the number of cells contained in each chain. The nanoflagellates and dinoflagellates were also distinguished according to size categories (nanoflagellates: 2 - 5, 5 - 10, and 10 - 15 µm; dinoflagellates: <10, 11 - 19, 20 - 39, 40 - 59, and 60 - 99 µm). At least 150 cells in each taxon were counted. In these experiments (1 to 6), the picoplankton size fraction was included in the water but not in the analyses as it was initially assumed that this fraction was not part of the food size range available for the nauplii; this assumption however was tested in subsequent experiments.

For the analyses of pico- and nanoplankton samples in experiments 7 to 14 (Table 1), subsamples of 3 mL (picoplankton) and 20 mL (nanoplankton) were stained with DAPI (4',6-diamidino-2-phenylindole), at a final concentration of 0.01% (Porter and Feigh 1980), and filtered onto black polycarbonate membrane filters (0.2 µm for picoplankton and 0.8 µm for

nanoplankton), supported underneath by 0.45 µm membrane filters. The filters with samples were then stored at –20°C in the dark until analysis. These samples were examined at 1000x magnification with the same microscope described above, equipped with an epifluorescence unit and using UV, blue or multiple excitations (NIKON Filter Blocks DAPI UV-2E/C, NB-2A and DAPI/FITC/TRITC). Picoplanktonic cells were distinguished as bacterioplankton (heterotrophic) and cyanobacteria (autotrophic) and nanoplanktonic cells as autotrophic/mixotrophic or heterotrophic nanoflagellates according to the type of fluorescence emitted. During the enumeration of the food items, photos of representative groups were taken to measure cells size dimensions using the software Image Pro Plus® (Version 4.5).

Micro-, nano-, and picoplanktonic carbon contents were derived from the measured cell dimensions, assigning an appropriate geometric formula, and calculating cell volumes (Chrzanowski and Simek 1990; Sun and Lui 2003). Cell volumes were converted to carbon biomass using a factor of 220 fg C μm^{-3} for flagellates (Børsheim and Bratbak 1987) and 82 fg C $cell^{-1}$ for cyanobacteria (mainly *Synechoccocus*) assuming it represents a coastal population (Worden et al. 2004). Bacterial cellular carbon (BOC) was estimated from: BOC (fg) = 90.06 x BVOL $(\mu m^3)^{0.59}$, where BVOL = bacterial volume (Simon and Azam 1989). The following volume to carbon relationships were applied for the remaining prey types (Menden-Deuer and Lessard 2000): diatoms, $\log_{10}$ pg C $cell^{-1}$ = -0.541 + 0.811 x $\log_{10}$ volume (μm^3); dinoflagellates, $\log_{10}$ pg C $cell^{-1}$ = -0.353 + 0.864 x $\log_{10}$ volume (μm^3); and ciliates, $\log_{10}$ pg C $cell^{-1}$ = -0.639 + 0.984 x $\log_{10}$ volume (μm^3).

Estimation of nauplii feeding rates and selective feeding

Clearance (filtration) and ingestion rates were assessed from the changes in Chl-*a* (as total) and cell abundances (per prey type) between the beginning and the end of the incubations, using the equations provided by Frost (1972). The instantaneous growth coefficient of the prey (k) is obtained from the changes in prey concentration (C) in the control treatments at time t_1 (C_1) and t_2 (C_2) of the incubation:

$$C_2 = C_1 \cdot e^{k(t_2 - t_1)}$$

The instantaneous grazing coefficient (g) is calculated from:

$$C_2^* = C_1 \cdot e^{(k-g)(t_2 - t_1)}$$

where C_2^* is the prey concentration at t_2 in the treatment containing the consumers. A mean food concentration $\left(\langle C \rangle\right)$, expressed in terms of Chl-*a* concentration, cell numbers or biomass, is calculated from:

$$\langle C \rangle = C_1(e^{(k-g)(t_2-t_1)} - 1)/(t_2 - t_1)(k - g)$$

The clearance rate (F = volume cleared copepod^{-1} time^{-1}) is obtained from the volume of the incubation bottle (V, in mL) and the copepod density in each bottle (N):

$$F = V \cdot g / N$$

The ingestion rate (I = food concentration or biomass copepod^{-1} time^{-1}) is calculated from:

$$I = F \cdot \langle C \rangle$$

Ingestion rates were further considered in the analysis only when the difference in prey concentration between the control and grazing treatments at the end of the incubation proved to be significantly higher in the control (Student's *t*-test; Sokal and Rohlf 1981). Since the natural prey assemblages in the incubation bottles usually contain multiple trophic levels (Tang et al. 2001), a verification of the potential interactions was done by calculating the potential grazing rate of the "additional grazers" (e.g. nanoflagellates, dinoflagellates, ciliates). For this purpose, a generic model for planktonic protistan grazing (Peters 1994) was used:

$$ln\ GR = -2.701 - 0.344\ ln(V_{PY}) + 0.477\ ln(V_{PD}) + 0.489\ ln(C_{PY}) - 0.270\ ln(C_{PD}) + 0.033T$$

where T = temperature (ºC), V = cell volumes (μm^3), and C = abundances (cells mL^{-1}) of both the prey (PY) and predators (PD).

Selective feeding by the nauplii in the grazing experiments was assessed by using the Vanderploeg and Scavias's electivity index (E*) (Vanderploeg and Scavia 1979a, b):

$$E_i^* = \frac{W_i - (1/n)}{W_i + (1/n)}$$

with n as the total number of prey kinds in a given experiment and the selectivity coefficient W_i is defined by:

$$W_i = \frac{F_i}{\sum F_i}$$

where F_i is the clearance rate of the ith food type and $\sum F_i$ is the sum of clearance rates on all food types. The electivity index E* ranges between -1 and +1; negative values correspond to avoidance; zero values represent neutrality, and positive values selectivity. The use of this index has been recommended especially in the cases where the different food types are not equally abundant (Lechowicz 1982).

RESULTS

Food conditions during the grazing experiments

Food composition and concentration (as total Chl-*a*, abundance, and/or biomass) at the beginning of the grazing experiments are summarized in Table 1; detailed prey composition is provided in Tables 2 and 3. The initial Chl-*a* concentrations in the experiments with natural assemblages in the <100 or <125 μm fractions (experiments 1 to 6) were distributed over a wide range (0.3 - 7 μg L^{-1}), as were those in the experiments with cultured microalgae (1 - 12 μg L^{-1}). The initial prey abundances (excluding the picoplankton) in experiments 1 to 6 (Fig. 2a) were generally dominated by nanoflagellates (31 - 82%) and/or by diatoms (7 - 60%). The nanoflagellates (range: 7 - 39 x 10^5 cells L^{-1}) were mainly distributed in the 2-5 μm size range and the diatoms (range: 0.7 - 35 x 10^5 cells L^{-1}) were frequently dominated by the chain forming *Skeletonema* spp. (Table 2). Ciliates (mostly in the microplankton size range), dinoflagellates (in the nano- and microplankton size range), and other diatoms in solitary or chain forms, were less abundant in these experiments (<2 x 10^5 cells L^{-1}; Table 2) and contributed <8 % of the total abundance (Fig. 2a). In terms of initial carbon biomass (Fig. 2b; Table 2), chain forming diatoms (range: 6 - 386 μg C L^{-1}) were most frequently the largest contributors to the total (11 - 70%); occasionally, the nanoflagellates (range: 14 - 47 μg C L^{-1}) and dinoflagellates (range: 11 - 30 μg C L^{-1}) constituted an important component (up to 41%).

During the grazing experiments with only the picoplankton (<3 μm) and the pico- to nanoplankton (<20 μm) fractions as food for the nauplii (experiments 7 to 14; Table 3), the cyanobacteria (range: 2 - 31 x 10^5 cells L^{-1}; 0.02 - 0.3 μg C L^{-1}) and the nanoflagellates, mostly in the lowest size fraction (range: 2 - 6 x 10^5 cells L^{-1}; 0.7 - 2.6 μg C L^{-1}), were a minor component. Instead, the bacterioplankton represented >90% of the total initial abundance and biomass (range: 591 - 2962 x 10^6 cells L^{-1} and 14 - 72 μg C L^{-1}, respectively). In the experiments with *I. galbana* as food, the initial abundances and biomasses (Table 1) were one to two orders of magnitude higher than those of nanoflagellates in the natural assemblages (Table 2 and 3).

The potential interference of grazing by multi-trophic levels during the different experiments was tested (interactions: diatoms-dinoflagellates-nauplii, nanoflagellates-dinoflagellates-nauplii, cyanobacteria-nanoflagellates-nauplii and bacterioplankton-nanoflagellates-nauplii) and was found to be minimal (<1% of the total grazing by the "additional grazer"). Therefore, no correction was applied to account for the effect of other

grazers (mainly nanoflagellates and dinoflagellates) in the estimates of *Oithona* spp. nauplii ingestion.

Nauplii feeding on nano-to micro-planktonic size fractions

Among the different prey types in the nano- and microplanktonic size fractions (experiments 1 to 6; Table 2), the *Oithona* spp. nauplii consumed some of them (Table 4). There was no evidence that the nauplii fed on ciliates, chain diatoms, or large dinoflagellates (>40 μm). The nauplii ingested chain-forming centric diatoms only when they were present as solitary cells (*Skeletonema* spp. and *Thalassiosira* spp.; 12 and 17 μm cell length, respectively), except for C*haetoceros* spp. (14 μm length). Also, solitary cells of *Navicula* spp. (24 μm length) were present in all the experiments (1-6) but were fed upon by the nauplii only in two of them; other pennate genera (e.g. *Asterionellopsis*, *Cylindrotheca*, *Pseudo-nitzschia;* length range: 27-50 μm) were not consumed. Feeding on dinoflagellates was concentrated on the small-sized cells (<10 - 39 μm) and occurred in 5 of these experiments. Significant feeding on nanoflagellate cells was detected in all 6 experiments, more often on the smallest size fraction (2 - 5 μm).

In terms of the ingestion rates by the *Oithona* spp. nauplii (Table 5), those on solitary centric diatoms were much higher (range: 229 - 3633 cells nauplii^{-1} d^{-1}; 49 - 506 ng C nauplii^{-1} d^{-1}) than on solitary pennate diatoms (range: 74 - 143 cells nauplii^{-1} d^{-1} ; 4 - 7 ng C nauplii^{-1} d^{-1}). Ingestion rates on dinoflagellates were in the same order of magnitude as those estimated for the solitary diatoms (range: 20 - 1524 cells nauplii^{-1} d^{-1}; 1 - 162 ng C nauplii^{-1} d^{-1}). Ingestion rates on nanoflagellates (range: 5000 -51000 cells nauplii^{-1} d^{-1}; 28 - 1076 ng C nauplii^{-1} d^{-1}) represented between 27 and 90% of the total carbon consumed by the nauplii in the different experiments (Table 5). Also, the ingestion of Chl-*a* was detected to be significant, except for experiment 5 although carbon ingestion was significant in the latter (Tables 4 and 5).

Nauplii feeding on pico- to nanoplanktonic size fractions

The *Oithona* spp. nauplii displayed significant feeding on nanoplankton cells (nanoflagellates size = 2 - 5 μm) but less frequently on picoplankton cells (bacterioplankton and cyanobacteria cellvolumes = 0.15 and 1.5 μm^3, respectively) when both, pico- and nanoplanktonic preys were available (experiments 7 to 10). Consumption of autotrophic/mixotrophic (AMNF) and heterotrophic nanoflagellates (HNF) was detected during these 4 experiments and it was, in most cases, the main carbon source for the nauplii

(Table 6). Ingestion rates on HNF were in general similar (range: 5 - 9 x 10^3 cells nauplii^{-1} d^{-1}; 15 - 27 ng C nauplii^{-1} d^{-1}) to those on AMNF (range: 2 - 8 x 10^3 cells nauplii^{-1} d^{-1}; 10 - 42 ng C nauplii^{-1} d^{-1}) but these rates were in the lower range of those estimated under a more diverse food size spectrum (experiments 1 to 6; Table 5). Ingestion of cyanobacteria and bacterioplankton was observed only once in these experiments but it was more common when they were the only food size available (experiments 11 to 14; Table 6). Ingestion rate ranged on cyanobacteria between 9 and 20 x 10^3 cells nauplii^{-1} d^{-1} (1 - 1.7 ng C nauplii^{-1} d^{-1}) and on bacterioplankton between 4.8 and 18 x 10^6 cells nauplii^{-1} d^{-1} (116 - 444 ng C nauplii^{-1} d^{-1}).

Nauplii feeding on cultured *Isochrysis galbana*

In the three grazing experiments with *I. galbana* offered as a mono-food type to *Oithona* spp. nauplii (experiments 15 to 17), the abundance and biomass were determined only twice (Table 1). The statistical analysis of the differences between the final abundances in the control and grazing bottles indicated that the nauplii fed on this microalgae; the same was true in terms of Chl-*a* concentration (Table 7). Ingestion rates on *I. galbana* (Table 7) were similar in terms of cells and Chl-*a* even though one experiment had almost twice the food concentration of the other (Table 1). Cell ingestion rates on this nanoflagellate were in the range (experiments 1 to 6) or one order of magnitude higher (experiments 7 to 10) compared with those estimated from incubations with natural nanoflagellates (Tables 5 and 6).

Response of Oithona *spp. nauplii to food concentration and food type*

The functional relationships between cell ingestion rates by the *Oithona* spp. nauplii and food concentration (in terms of abundance), for each prey type, presented no saturation response albeit the wide range of food concentrations in the experiments. Instead, ingestion was linear over this wide range (Fig. 3a-d). However, the relationship between Chl-*a* concentration and Chl-*a* ingestion (Fig. 3e) was not significant ($p > 0.05$). In terms of food selection (Fig. 4), the values of the electivity index (E_i*) for the different food types are mostly in the -0.25 to +0.25 range and suggest non-selective feeding by the nauplii. Only on three occasions out of 21 the E_i* values were strongly negative (> -0.5), suggesting avoidance of diatoms (experiment 1), nanoflagellates (experiment 4) and of the picoplankton (experiment 8).

DISCUSSION

Food spectrum and ingestion rates by *Oithona* spp. nauplii

The ingestion rate data of *Oithona* spp. nauplii derived from this study probably represent the first one to include a variety of natural prey assemblages, in terms of food size (0.2 – 125 μm), food type (bacterioplankton, cyanobacteria, nanoflagellates, dinoflagellates, ciliates and diatoms), and prey motility. Most previous studies on *Oithona* feeding refer to adults, and their diet appears to be wide, including diatoms, nanoflagellates, dinoflagellates, ciliates and copepod nauplii (e.g. Marshall and Orr 1966; Lampitt and Gamble 1982; Nakamura and Turner 1997; Vargas and Gonzalez 2004a; Castellani et al. 2005), as well as detritus (González and Smetacek 1994). The fewer studies on the feeding of *Oithona* nauplii indicate that flagellates, dinoflagellates and ciliates are suitable prey (Eaton 1971 *fide* Nielsen and Sabatini 1996; Drits and Semenova 1984 *fide* Nielsen and Sabatini 1996; Uchima and Hirano 1986; Lonsdale et al. 2000); detritus has not been found to be important (Uchima and Hirano 1986).

Our results on the feeding of *Oithona* spp. nauplii in the upwelling area off Concepción suggest that their diet is largely based on the picoplankton and nanoplankton size ranges (0.2-20 μm), including nanoflagellates (mean ingestion rate= 350 ng C nauplii^{-1} d^{-1}) , small dinoflagellates (98 ng C nauplii^{-1} d^{-1}), single diatom cells (106 ng C nauplii^{-1} d^{-1}), as well as bacterioplankton (121 ng C nauplii^{-1} d^{-1}) and cyanobacteria (0.4 ng C nauplii^{-1} d^{-1}). Chain diatoms, a dominant component of the plankton biomass during the upwelling period in this system (Vargas et al. 2007; Gonzalez et al. in press), were not at all consumed by the nauplii. This item might be either too big or too heavily armoured for the nauplii to be handled efficiently. In the upwelling region off northern Chile, small copepods (*Acartia tonsa, Oithona similis* and *Paracalanus parvus*) also have been shown to feed on solitary cells but not on chain diatoms (Vargas and Gonzalez 2004a). Ciliates were not included in the diet of the *Oithona* nauplii in this study, although other prey of similar size (20 - 39 μm) and abundance (~0.9 x10^3 cells L^{-1}) were ingested.

The ingestion of natural bacterioplankton and cyanobacteria by *Oithona* nauplii in the present study, mostly when no larger prey (nanoflagellates) was available, apparently is the first evidence of the inclusion of this type of item in the diet of planktonic copepods. This observation is strengthened by previous studies using fluorescent labeled bacteria (FLB). Roff et al. (1995) reported the presence of FLB in the gut of many copepod nauplii, among them NII-NIII stages of *Oithona* spp. (but did not provide rate estimates), and discarded incidental

feeding since some nauplii of larger copepods (e.g. *Centropages velificatus* and *Euchaeta marina*) constantly failed to ingest the FLB. Turner and Tester (1992) reported mean ingestion rates of 5.7 x 10^6 FLB nauplii^{-1} d^{-1} for NI-NIII stages of *Acartia tonsa* (mean body length = 75 - 132 μm). This estimate is about half of the mean value estimated in this study (10 x 10^6 picoplankton cells nauplii^{-1} d^{-1}) for NIII-NV stages of *Oithona* spp. (mean body length = 120 - 165 μm). However, the average cell volume of the FLB was 0.7 μm^3 in the first case, compared to the smaller volume (0.15 μm^3) of the natural bacterioplankton in the present study.

The present study suggests that most prey items of *Oithona* spp. nauplii are motile. Preference for motile prey types has been documented before for *Oithona* spp. (Nielsen and Sabatini 1996; Svensen and Kiørboe 2000; Paffenhöfer and Mazzocchi 2002). Moreover, Uchima and Hirano (1986) concluded that the developmental stages of *O. davisae* only grew and survived on motile food particles. *Oithona* is not known to generate a feeding current; they have a limited swimming performance and act as an ambush predator using hydromechanical detection of prey (Svensen and Kiørboe 2000; Saiz et al. 2003). Turbulence influences this detection and, consequently, their feeding rates; at lowest turbulence intensities (10^{-4} cm^2 s^{-3}), feeding is enhanced (Saiz et al. 2003; Maar et al. 2006). In part then, the experimental set up in this study, with low turbulence levels, may explain the relatively high ingestion rates.

In terms of food selection, the general lack of strong selectivity in the *Oithona* nauplii (Fig. 4) suggests that they probably act as opportunistic feeders, a strategy that might favor their persistence in this upwelling system. Only occasionally the electivity index (E_i*) indicated avoidance of certain food types. In experiment 1, this was the case for diatoms; most probably, single cells of *Skeletonema* sp. were solely ingested because they occurred at high abundance compared to other experiments (Table 2). In experiment 4, avoidance occurred for nanoflagellates; here, the abundance of them was lowest, as was the ingestion rate, compared to the rest of the experiments (Table 2). In this case, total ingestion was complemented with the diatoms *Thalassiosira* and *Navicula* spp., as single cells (Table 5).

Commonly, cyclopoids have been assumed to have lower ingestion rates compared to similarly sized calanoids, based on arguments of lower metabolic requirements in cyclopoids (Paffenhöfer 1993; Saiz and Calbet 2007). A few other studies indicate that this group is comparable with the calanoids in terms of ingestion, growth and development (Kiørboe and Sabatini 1994; Sabatini and Kiørboe 1994; Calbet et al. 2000). In the present study, total carbon ingestion rates (31 - 2184 ng C nauplii^{-1} d^{-1}) by the *Oithona* nauplii are: i) in the range

or higher than those reported for some calanoid nauplii and adult cyclopoids, and ii) in the lower range of those of copepodite and adult stages of some small-sized calanoids (Table 8). On the other hand, the comparatively lower carbon ingestion rates of adult females of *O. nana* (Lampitt and Gamble 1982) and adults + CV copepodites of *O. similis* (Nakamura and Turner 1997; Castellani et al. 2005), can be explained by food limitation during the incubations (density of 15 to 50 copepods in 100 or 200 mL bottles incubated for 24 h). In fact, ingestion rates have been shown to be higher for *O. similis* adults in incubations at a density of 10 copepods in 1 L^{-1} for ~24 h (Vargas and Gonzalez 2004a).

Daily carbon rations were assessed by using an estimate of the carbon content of *Oithona* nauplii (0.26 µg C $nauplii^{-1}$; Swadling et al. 1997) combined with the carbon ingestion rates obtained in the experiments (Tables 5 and 6). The values range from 40 to 840% of the body carbon (291 ± 295%) when the nauplii fed on nanoflagellates, dinoflagellates and diatoms (experiments 1 to 6), and from 12 to 172% (63 ± 57%) under a nanoflagellate and/or picoplankton diet (experiments 7 to 14). There is a trend of increase in the mean daily ration with food concentration (73% at <50 µg C L^{-1}, n = 6; 156% at 50-100 µg C L^{-1}, n = 4; 449% at 100-500 µg C L^{-1}, n = 3). This trend was recently described by Saiz and Calbet (2007) in a review of the patterns of ingestion rates in small calanoid copepods, together with high daily rations (up to 300%). Similarly, high values (up to 308%) have been documented before for calanoid copepod nauplii of *Acartia grani* (Ingerslev Henriksen 2005). In the case of Oithonids, data are available for *O. similis* females (Sabatini and Kiørboe, 1994; Castellani et al., 2005) and naupliar stages of *O. davisae* (Ingerslev Henriksen 2005), all documenting lower daily rations compared to calanoids. Sabatini and Kiørboe (1994) and presented values between 10 and 22%, Castellani et al. (2005) of ~3 to 32%, and Ingerlev Henriksen of 121%. To sustain some modest growth and basic metabolic needs, Paffenhöfer (1998) suggested that at least 30% of the body carbon needed to be ingested by *Oithona* spp. nauplii (during a North Atlantic spring bloom); this requirement was accomplished during nearly all the experiments in this study (Tables 5 and 6) and suggests that growth is not food limited.

Grazing impact by *Oithona* spp. nauplii in the upwelling area off Concepción

In the highly productive, coastal upwelling area off Concepción, the microbial food web is a fundamental and almost permanent feature of the trophic pathways in the water column, and micro-heterotrophs have been shown to be an important component in channeling primary and/or bacterial production (Cuevas et al. 2004; Böttjer and Morales 2005; Vargas et al. 2007). Among the micro-heterotrophs, metazoans <200 µm have not been explicitly included

as grazers in these studies, nor have they been in many other studies of planktonic food webs in coastal environments (Turner 1991). Ingestion rates of *Oithona* nauplii obtained in this study are compared with that of other micro-heterotrophs which potentially compete with them for the same prey types in the coastal system (Table 9). In terms of carbon, the ingestion of picoplankton by the nauplii can be two to five orders of magnitude higher than that shown by nanoflagellates; ingestion of picoplankton by ciliates can be similar to that of the nauplii but the maximum ingestion of the latter can be three orders of magnitude higher. Also, carbon ingestion of diatoms + nanoflagellates by the nauplii can be one to four orders of magnitude higher than those displayed by ciliates and heterotrophic dinoflagellates. In terms of cell ingestion, the estimates are also orders of magnitude higher for the nauplii. This is not surprising considering the size difference between the *Oithona* nauplii and that of most protistan grazers.

Given the lower abundances of copepod nauplii compared to that of heterotrophic protists in most of the coastal marine systems, their carbon consumption rates might be similar, or lower, than those of the protistan grazers (e.g. Verity et al. 1993). To compare this for the present data, consumption rates by the *Oithona* nauplii were calculated using the daily carbon ingestion rates on different prey types (Tables 5 and 6) and a maximum naupliar (*Oithona nana*) abundance in the area of study (15 nauplii L^{-1}; Torres, 2006). The results are represented in Figure 5, a scheme of the trophic interactions of the microbial food web in the Concepción upwelling area; the consumption rates of the *Oithona* nauplii appear to be mostly similar to those of the protistan micro-grazers. This data also suggest that the *Oithona* spp. nauplii can exert from a small to a large grazing impact on the standing stock of nanoflagellate assemblages (range: 2 - 68%; mean = 34%) whereas the impact is lower on picoplankton (<21%) and dinoflagellates (<24%), and minimal on diatoms (<13%). These grazing impact estimates are substantially higher than those previously reported for different stages in Oithonids (mostly <5% of prey standing stocks; Lonsdale et al. 2000; Zeldis et al. 2002; Antienza et al. 2006).

In order to estimate the grazing impact of the *Oithona* nauplii upon primary production (PP) in the upwelling area off Concepción, the following estimates were considered: a) consumption rates were derived using the maximum nauplii abundance of 15 ind^{-1} L^{-1} and integrating this value over the top 35 m (= 525000 ind^{-1} m^{-2}), and b) total PP values of 5061 (spring 2004) and 5393 mg C m^{-2} d^{-1} (summer 2005) reported by Vargas et al. (2007) for the same area of study. The grazing impact of the nauplii on the relatively high PP values during the upwelling period was fairly low (4 - 5%). In comparison with the impact of the metazoans

in the mesozooplankton size range, in the same area and seasonal period as in this study, these estimates are higher than those reported by Vargas et al. (2007; <2%) but lower than those in Grünewald et al. (2002; 17%). In general, the grazing impact of mostly small-sized copepods on PP in several ecosystems varies over a wide range (4 - 82%; mean = 35%, n= 8 for nets <100 μm; Gallienne and Robins 2001). Estimates of the grazing impact by metazoan in the microzooplankton size range (mainly small-sized calanoid and cyclopoid copepod nauplii) are less frequent in the literature; White and Roman (1992) reported it to be between 8 and 28% in Chesapeake Bay; Paffenhöfer (1998) provides estimates for early copepodites of small copepods of between 15 and 21%.

In terms of the total grazing impact of heterotrophic protists (including nano- to microzooplankton assemblages) on total PP in the same area of study, Vargas et al. (2007) reported values between 13% (spring) and 18% (summer) during the upwelling period but they only considered protistan grazers. To obtain the total grazing impact of whole micro-grazer assemblages on total PP, values obtained for the *Oithona* nauplii in the present study were added to those in Vargas et al. (2007). The estimates increase the impact to 18 - 22%, values that are relatively high considering the high levels of PP in the system; they are lower than those reported for whole nano- to microzooplankton assemblages during the winter, non-upwelling period (132 - 180 %; Böttjer and Morales 2005), when PP is lower (Montecino et al. 2004; Vargas et al. 2007) and the *Oithona* nauplii are scarce in abundance (Torres 2006).

This study indicates that the *Oithona* nauplii in the upwelling area off Concepción predate mostly on the nanoflagellate size fraction and, therefore, the grazing impact upon PP should be higher when considering only this size fraction. Since there are no size-fractionated PP values available for the system under study, an estimate was obtained based on the data reported by Iriarte and González (2004) for the upwelling system of northern Chile; a contribution of ~20% by the nano-photoautotrophs to total PP in the study region was assumed to be representative of the system. With this, the grazing impact by the *Oithona* nauplii alone increases to 21 - 24% of the PP within their food size range. Also, their grazing impact on PP <20 μm and/or on the standing stocks of micro-organisms might increase considering that the maximum abundance of the *Oithona* nauplii in this study (15 nauplii L^{-1}) is relatively low compared to the values reported for other systems (up to 100 nauplii L^{-1}), for example in Turner (2004), Hansen et al. (2004), and Ward and Hirst 2007). Overall, the *Oithona* spp. nauplii in this upwelling system are important in controlling the abundance, and probably the production of nano-assemblages. At the same time, the data suggest that the nauplii play a minor role in controlling the abundance of the dominant component of the

system, the chain diatoms. On the other hand, the diversity of food types ingested by *Oithona* spp. nauplii undoubtedly contributes to their presence throughout the year in this upwelling system and their significant grazing impact on nanoplankton assemblages certainly allows them to link the microbial and classical food web in this system.

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Table 1. Experimental set up during the *Oithona* spp. nauplii feeding experiments. Initial prey concentration in terms of chlorophyll-a (µg Chl-*a* L^{-1}), abundance (x 10^6 cells L^{-1}), and biomass (µg C L^{-1}) are included. T = *in situ* temperature; --- = not determined; presence of *Oithona nana* ([a]) and/or *O. similis* ([b]).

Exp.	Date	T	Food offered	Nauplii density	Initial food concentration		
	(D/M/Y)	(°C)	(µm)	(N°/bottle)	Chl-*a*	Abundance	Biomass
1	22/11/2004	11.5	natural < 125	20 [a]	6.7	3.4*	143*
2	20/01/2005	11.5	natural < 125	12 [a]	3.1	2.3*	69*
3	02/02/2005	11.5	natural < 100	9 [a]	3.2	1.7*	70*
4	30/03/2005	12.0	natural < 100	9 [a]	0.3	0.8*	40*
5	26/09/2005	12.0	natural < 100	7 [a]	5.9	5.3*	587*
6	27/10/2005	11.5	natural < 100	13 [a,b]	6.4	6.5*	306*
7	04/10/2006	11.3	natural < 20	11 [a]	---	1346	34
8	05/10/2006	11.3	natural < 20	12 [a]	---	1330	34
9	28/02/2007	12.2	natural < 20	13 [a,b]	0.8	2089	54
10	01/03/2007	12.0	natural < 20	10 [a,b]	0.9	2964	75
11	04/10/2006	11.3	natural < 3	11 [a]	---	591	14
12	05/10/2006	11.3	natural < 3	12 [a]	---	736	18
13	28/02/2007	12.2	natural < 3	11 [a, b]	0.02	1138	28
14	01/03/2007	12.0	natural < 3	11 [a,b]	0.02	1289	31
15	23/11/2004	11.5	*Isochrysis galbana*	15 [a]	6.4	6.0	79
16	18/01/2005	11.5	*I.galbana*	13 [a]	1.1	---	---
17	19/01/2005	11.5	*I.galbana*	10 [a]	11.7	10.5	167

* = Picoplankton size-fraction not included

Table 2. Composition and mean ± SD (n = 3) abundance (cells mL^{-1}) and biomass (µg C L^{-1}, in parenthesis) of the different food types at the beginning of the grazing experiments with *Oithona* spp. nauplii incubated with natural nano- to microplankton assemblages from the upwelling area off Concepción (experiments 1 to 6). Diatom are identified to genera, ciliates were mostly represented by oligotrichous and dinoflagellates by gymnodinoids.

	1	**2**	**3**	**4**	**5**	**6**
Nanoflagellates						
2-5 µm	908 ± 117 (5.1 ± 0.7)	1525 ± 204 (8.6 ± 1.2)	809 ± 57 (4.6 ± 0.3)	423 ± 31 (2.4 ± 0.2)	1010 ± 100 (5.7 ± 0.6)	3519 ± 303 (20 ± 1.7)
5-10 µm	213 ± 14 (11 ± 0.7)	281 ± 50 (14 ± 2.6)	183 ± 14 (9.4 ± 0.7)	154 ± 43 (7.8 ± 2.2)	461 ± 55 (24 ± 2.8)	413 ± 112 (21 ± 5.7)
10-15 µm	29 ± 8.2 (2.8 ± 0.8)	2.1 ± 0.4 (0.2 ± 0.04)	3.5 ± 0.8 (0.3 ± 0.1)	111 ± 20 (11 ± 2.0)	146 ± 19 (14 ± 1.9)	58 ± 7.1 (5.7 ± 0.7)
Dinoflagellates						
< 10 µm	0.9 ± 0.2 (0.03 ± 0.01)	0	1.2 ± 0.2 (0.04 ± 0.01)	0	5.5 ± 0.7 (0.2 ± 0.02)	56 ± 16 (2.0 ± 0.6)
11-19 µm	2.7 ± 1.0 (0.2 ± 0.1)	3.5 ± 0.6 (0.3 ± 0.04)	7.1 ± 0.4 (0.6 ± 0.1)	53 ± 2.9 (3.8 ± 0.2)	27 ± 1.3 (2.5 ± 0.2)	95 ± 5.2 (9.9 ± 0.4)
20-39 µm	8.7 ± 0.7 (3.6 ± 0.3)	7.7 ± 1.6 (3.7 ± 0.8)	11 ± 1.3 (5.9 ± 0.5)	13 ± 0.4 (7.2 ± 0.5)	14 ± 1.0 (6.4 ± 0.5)	8.7 ± 2.0 (5.9 ± 1.2)
40-59 µm	4.2 ± 0.3 (6.8 ± 0.2)	3.4 ± 0.6 (13 ± 2.3)	1.3 ± 0.5 (2.9 ± 1.7)	2.6 ± 1.1 (5.6 ± 3.1)	5.4 ± 1.6 (15 ± 2.0)	4.4 ± 3.7 (7.2 ± 5.2)
60-99 µm	0	0.5 ± 0.5 (1.6 ± 1.1)	0.3 ± 0.1 (2.4 ± 0.8)	0	0.6 ± 1.0 (5.7 ± 9.9)	0
Ciliates	0.9 ± 0.3 (1.5 ± 0.4)	0.7 ± 0.2 (1.6 ± 0.7)	0.8 ± 0.1 (2.0 ± 0.4)	1.3 ± 0.1 (5.5 ± 0.6)	0.1 ± 0.03 (0.4 ± 0.2)	1.3 ± 0.1 (3.2 ± 0.5)
Pennate diatoms						
Asterionellopsis (s)	0	0	22 ± 4.4 (1.4 ± 0.3)	5.3 ± 3.7 (0.3 ± 0.2)	0	0
Asterionellopsis (ch)	9.4 ± 2.3 (0.6 ± 0.1)	0	0	0	31 ± 12 (2.1 ± 0.7)	36 ± 12 (2.2 ± 0.7)
Cylindrotheca (s)	6.3 ± 1.4 (0.5 ± 0.1)	1.2 ± 0.4 (0.1 ± 0.03)	1.1 ± 0.5 (0.1 ± 0.04)	2.9 ± 1.7 (0.2 ± 0.1)	53 ± 11 (4.0 ± 0.8)	213 ± 7.2 (16 ± 0.5)
Navicula (s)	9.4 ± 2.4 (0.5 ± 0.1)	2.2 ± 0.9 (0.1 ± 0.04)	0.5 ± 0.4 (0.03 ± 0.03)	3.5 ± 0.9 (0.2 ± 0.04)	27 ± 7.2 (1.3 ± 0.4)	31 ± 5.4 (1.5 ± 0.3)
Pseudonitzschia (s)	0	17 ± 2.8 (1.0 ± 0.2)	0	0.7 ± 1.2 (0.04 ± 0.1)	0	0
Pseudonitzschia (ch)	0	24 ± 4.8 (1.4 0.3)	0.9 ± 0.8 (0.05 ± 0.05)	2.6 ± 0.2 (0.1 ± 0.1)	0	17 ± 7.2 (1.0 ± 0.4)
Centric diatoms						
Chaetoceros (s)	0	0	0.4 ± 0.6 (0.1 ± 0.1)	4.8 ± 0.5 (0.6 ± 0.1)	113 ± 19 (14 ± 2.4)	20 ± 19 (2.6 ± 2.4)
Chaetoceros (ch)	0	0	69 ± 4.6 (8.7 ± 0.6)	25 ± 4.8 (3.2 ± 0.6)	1365 ± 177 (173 ±22)	133 ± 80 (17 ± 10)
Coscinodiscus (s)	7.0 ± 2.4 (3.3 ± 1.1)	0.8 ± 0.7 (0.4 ± 0.4)	2.1 ± 0.3 (1.0 ± 0.1)	2.2 ± 0.7 (1.0 ± 0.3)	139 ± 9.8 (65 ± 4.6)	17 ± 2.7 (8.0 ± 1.3)
Eucampia (ch)	0	0	0	1.0 ± 1.7 (0.6 ± 0.1)	0	0
Leptocylindrus (ch)	0	0	0	0	0	25 ± 22 (3.7 ± 3.3)
Skeletonema (s)	154 ± 48 (7.7 ± 2.4)	6.2 ± 2.2 (0.3 ± 0.1)	0	0	0	0
Skeletonema (ch)	2015 ± 312 (100 ± 16)	465 ± 82 (23 ± 4.1)	610 ± 98 (30 ± 4.8)	27 ± 3.7 (1.4 ± 0.2)	114 ± 19 (5.7 ± 0.9)	839 ± 80 (42 ± 4.0)
Thalassiosira (s)	0	0	0	3.5 ± 1.2 (0.5 ± 0.2)	294 ± 31 (41 ± 4.3)	246 ± 109 (34 ± 15)
Thalassiosira (ch)	0	0.9 ± 1.6 (0.1 ± 0.2)	0.8 ± 0.8 (0.1 ± 0.1)	1.7 ± 1.8 (0.2 ± 0.2)	1490 ± 77 (207 ± 11)	742 ± 341 (103 ± 47)

Table 3. Composition and mean ± SD (n = 3) abundance (cells mL^{-1}) and biomass (µg C L^{-1}, in parenthesis) of the different food types at the beginning of the grazing experiments with *Oithona* spp. nauplii incubated with natural picoplankton and/or nanoplankton assemblages from the upwelling area off Concepción (experiments 7 to 14). BPL= bacterioplankton (abundance values x 10^3; mean volume = 0.15 μm^3); CYB= cyanobacteria (1.5 μm^3); AMNF= autotrophic/mixotrophic nanoflagellates (24.5 μm^3); HNF= heterotrophic nanoflagellates (15.1 μm^3); --- = not included.

Exp.	BPL	CYB	AMNF	HNF
7	1346 ± 25 (33 ± 0.6)	377 ± 27 (0.03 ± 0.00)	61 ± 20 (0.3 ± 0.1)	110 ± 8 (0.4 ± 0.0)
8	1330 ± 98 (32 ± 2.4)	456 ± 33 (0.04 ± 0.00)	105 ± 23 (0.6 ± 0.1)	162 ± 42 (0.5 ± 0.1)
9	2086 ± 86 (51 ± 2.1)	3142 ± 486 (0.3 ± 0.04)	225 ± 18 (1.2 ± 0.1)	398 ± 56 (1.3 ± 0.2)
10	2962 ± 199 (72 ± 4.8)	1562 ± 322 (0.1 ± 0.03)	246 ± 33 (1.3 ± 0.2)	391 ± 27 (1.3 ± 0.1)
11	591 ± 30 (14 ± 2.0)	263 ± 23 (0.02 ± 0.00)	---	---
12	736 ± 107 (18 ± 2.6)	193 ± 33 (0.02 ± 0.00)	---	---
13	1138 ± 38 (28 ± 0.9)	658 ± 219 (0.05 ± 0.02)	---	---
14	1289 ± 20 (31 ± 0.5)	731 ± 127 (0.06 ± 0.01)	---	---

Table 4. Statistical analysis (Student´s t-test) of the differences between the final abundance or Chl-*a* concentration in the control and the grazing bottles in the experiments with natural nano- to microplankton assemblages (experiments 1 to 6). Significance levels: *** = p <0.001, ** = p <0.01, * = p <0.05, ns = not significant, --- = not present.

	1	**2**	**3**	**4**	**5**	**6**
Nanoflagellates						
2-5 µm	***	**	*	*	*	**
5-10 µm	*	ns	**	ns	ns	***
10-15 µm	ns	ns	ns	ns	**	*
Dinoflagellates						
Gymnodinoids < 10 µm	*	---	*	---	ns	**
Gymnodinoids 10-19 µm	**	ns	*	ns	*	**
Gymnodinoids 20-39 µm	ns	*	*	ns	*	*
Gymnodinoids 40-59 µm	ns	ns	ns	ns	ns	ns
Gymnodinoids 60-99 µm	ns	ns	ns	---	ns	---
Oligotrichous ciliates	ns	ns	ns	ns	ns	ns
Pennate diatoms						
Asterionellopsis spp. (s)	---	---	ns	ns	---	---
Asterionellopsis spp. (ch)	ns	---	---	---	ns	ns
Cylindrotheca spp. (s)	ns	ns	ns	ns	ns	ns
Navicula spp. (s)	ns	*	ns	*	ns	ns
Pseudonitzschia spp. (s)	---	ns	---	---	---	---
Pseudonitzschia spp. (ch)	---	ns	---	ns	---	ns
Centric diatoms						
Chaetoceros spp. (s)	---	---	---	ns	ns	ns
Chaetoceros spp. (ch)	---	---	ns	ns	ns	ns
Coscinodiscus spp. (s)	ns	ns	ns	---	ns	ns
Leptocylindrus spp. (ch)	---	---	---	---	---	ns
Skeletonema spp. (s)	*	ns	---	ns	---	---
Skeletonema spp. (ch)	ns	ns	ns	ns	ns	ns
Thalassiosira spp. (s)	---	---	---	***	ns	**
Thalassiosira spp. (ch)	---	---	---	ns	ns	ns
Chl-a	***	***	***	***	ns	**

Table 5. Mean rates of ingestion (± SD; n=3) of cells (first row; nanoflagellates in x 10^3 cells nauplii^{-1} d^{-1}, dinoflagellates and diatoms in cells nauplii^{-1} d^{-1}) and carbon (second row; in ng C nauplii^{-1} d^{-1}) by *Oithona* spp. nauplii incubated with nano- to microplankton assemblages (experiments 1 to 6). Total ingestion in terms of Chl-a (ng Chl-*a* nauplii^{-1} d^{-1}), cells (x10^3 cells nauplii^{-1}d^{-1}), and carbon (ng C nauplii^{-1} d^{-1}).

	Nanoflagellates			**Dinoflagellates**			**Diatoms** (solitary forms)			**Total**		
Exp.	2-5 µm	5-10 µm	10-15 µm	<10 µm	10-19 µm	20-39 µm	*Skeletonema*	*Thalassiosira*	*Navicula*	Chl-*a*	Cells	Carbon
1	14 ± 1	4 ± 1	---	20 ± 4	36 ± 2	28 ± 4	984 ± 282	---	---	54	19	372
	78 ± 6	207 ± 50	---	1 ± 0.1	4 ± 0.3	33 ± 5	49 ± 14	---	---			
2	30 ± 4	---	---	---	---	167 ± 86	---	---	74 ± 27	18	30	224
	167 ± 23	---	---	---	---	54 ± 28	---	---	4 ± 1			
3	16 ± 6	8 ± 2	---	58 ± 25	251 ± 26	137 ± 61	---	---	---	21	24	705
	93 ± 31	425 ± 93	---	2 ± 0.9	24 ± 3	162 ± 73	---	---	---			
4	5 ± 0.4	---	---	---	---	---	---	229 ± 19	143 ± 16	4	5	104
	28 ± 2	---	---	---	---	---	---	68 ± 5	7 ± 1			
5	33 ± 6	---	7 ± 1	---	509 ± 139	156 ± 40	---	---	---	---	41	950
	184 ± 35	---	666 ± 105	---	50 ± 14	50 ± 13	---	---	---			
6	51 ± 3	21 ± 0.2	1 ± 0.6	844 ± 116	1524 ± 181	77 ± 44	---	3633 ± 282	---	19	79	2184
	290 ± 19	1076 ± 96	106 ± 59	29 ± 4	153 ± 23	25 ± 14	---	506 ± 39	---			

Table 6. Statistical analysis (Student´s t-test) of the differences between the final abundance in the control and grazing bottles and estimated mean ingestion rates (± SD; n = 3) of *Oithona* spp. nauplii in terms of cells (x 10^3 cells nauplii^{-1} d^{-1}) and carbon (ng C nauplii^{-1} d^{-1}) during incubations with natural pico- to nanoplankton assemblages (experiments 7 to 14). Significance levels: *** = p <0.001, ** = p <0.01, * = p <0.05, ns = non significant. BPL = bacterioplankton; CYB = cyanobacteria; AMNF = mixotrophic/autotrophic nanoflagellates; HNF = heterotrophic nanoflagellates; nd = no data.

Experiment		**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
BPL	Difference in abundance	ns	*	ns	ns	*	*	*[1]	***
	Cell ingestion	---	9734 ± 3362	---	---	7079 ± 3078	4768 ± 1989	---	18238 ± 723
	C ingestion	---	237 ± 82	---	---	173 ± 75	116 ± 48	---	444 ± 18
CYB	Difference in abundance	***	ns	ns	ns	*	ns	ns	*
	Cell ingestion	6.2 ± 0.5	---	---	---	8.8 ± 2.9	---	---	20.5 ± 4.9
	C ingestion	0.51± 0.04	---	---	---	0.7 ± 0.2	---	---	1.7 ± 0.4
AMNF	Difference in abundance	**	**	**	*				
	Cell ingestion	2.1 ± 0.5	3.4 ± 0.6	8.3 ± 1.3	5.4 ± 0.7	nd	nd	nd	nd
	C ingestion	10.7 ± 2.6	16.8 ± 3.2	41.6 ± 6.4	27.1 ± 3.4				
HNF	Difference in abundance	***	**	*	*				
	Cell ingestion	6.7 ± 0.4	5.1 ± 0.9	9.1 ± 2.2	5.3 ± 3.7	nd	nd	nd	nd
	C ingestion	20.2 ± 1.1	15.3 ± 2.6	27.2 ± 6.5	15.8 ± 11.0				
Total C ingestion		**31**	**269**	**69**	**43**	**173**	**116**	---	**446**

[1] = Difference in prey concentration between the control and grazing treatments at the end of the incubation significantly higher in the grazing treatment (negative grazing).

Table 7. Statistical analysis (Student´s t-test) of the difference between the final abundances and Chl-*a* concentrations of *Isochrysis galbana* in the control and grazing bottles and the estimated ingestion rates (± SD; n = 3) of *Oithona* spp. nauplii (experiments 15 to 17). Significance level: ***= $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, nd= no data.

Experiment		**15**	**16**	**17**
I.galbana	Difference in abundance	**	nd	*
	Cell ingestion ($x10^3$ cells $nauplii^{-1}d^{-1}$)	28 ± 3.5	nd	31 ± 9.3
	Carbon ingestion (ng C $nauplii^{-1}d^{-1}$)	365 ± 46	nd	495 ± 149
I.galbana	Difference in Chl-*a*	***	***	***
	Chl-*a* ingestion (ng Chl-a $nauplii^{-1}d^{-1}$)	18 ± 3.7	6 ± 0.9	20 ± 5.0

Table 8. Daily carbon ingestion rates (IR = µg C individual^{-1} d^{-1}) of small cyclopoid and calanoid metazoans in coastal areas. PIP = picoplankton, NF = nanoflagellates, COC = coccolithophorids; DF = dinoflagellates, CI = ciliates, DT = diatoms, CN = copepod nauplii, IG = *Isochrysis galbana*, and PHY = phytoplankton <200 µm. T = temperature (°C), FC = food concentration (* = x 10^6 cells L^{-1}; ** = µg C L^{-1}); --- = no data.

Copepods	Food spectra	FC *	FC**	IR	T	Reference
cyclopoids						
Oithona nana [a]	NF, DT, DF, CN	---	1-350	0.1 – 0.3	10	Lampitt & Gamble, 1982
O. similis [a,b]	DF,CI,CN	0.001-3.2	---	0.15	19-21.2	Nakamura & Turner, 1997
O. similis [a]	PIP, NF, DF, CI, DT	---	~400-600	1.7 – 3.2	12-16.5	Vargas & Gonzalez, 2004a
O. similis [a]	NF, COC, DF, CI, DT	---	3-169	0.001 – 0.1	---	Castellani et al., 2005
Oithona spp. [d]	PIP, NF,DF, CI, DT, IG	0.8-2964	14-587	0.03 – 2.2	11.3-12.2	This study
calanoids						
Calanus helgolandicus [d]	DF, DT	---	32-108	0.2 – 0.8	15	Paffenhöfer, 1971
C. helgolandicus [d]	NF,DF,DT, COC	---	364-768	0.3-1.9	15	Rey et al., 2001
Calanus spp. [d]	NF, DF, CI, DT	0.07-0.16	12-30	0.02 - 0.07	5	Turner et al., 2001
Acartia tonsa [a]	PIP, NF, DF, CI, DT	--	40-1800	4.3 - 5.8	~11-13	Vargas et al., 2007
A. tonsa [b]	NF, DF, CI, DT	---	~50-700	0-9.6	20.2-31.2	Kleppel & Hazzard, 2000
Paracalanus parvus [a]	PIP, NF, DF, CI, DT	---	40-1800	4.4 - 6.0	~11-13	Vargas et al., 2007
Paracalanus sp. [a]	DF, CI	0.04-0.13	7-25	0.5 - 1.4	23.7-25.2	Suzuki et al., 1997
A. tonsa + *P. parvus* [c]	PIP, NF, DF, CI, DT	---	40-1800	2.7 - 4.6	~11-13	Vargas et al., 2007
calanoids + cyclopoids						
< 200 µm [d]	PHY	---	80-160	0.1-0.3	---	Verity et al., 1993

[a] = adults, [b] =adult females, [c] = copepodites, [d] = nauplii

Table 9. Daily ingestion rates (IR) by different types of micro-grazers that potentially compete for the same prey types as the *Oithona* spp. nauplii in the area of study. Rates have been directly (grazing experiments) or indirectly obtained (generic model of Peters (1994) for protistan grazing). PIP = picoplankton; FLB = fluorescent labelled bacteria; BAC = heterotrophic bacteria; SYN = *Synechococcus*; PRO = *Prochlorococcus*; CRYP = cryptophytes; NF = nanoflagellates; COC = coccolithophorids; DF = dinoflagellates; CI = ciliates; DT = diatoms; --- = no data); IRa= (x 10^3 cells ind^{-1} d^{-1}); IRb= (ng C ind^{-1} d^{-1}).

Micro-grazer type	Food spectra	IRa	IRb	Method	Reference
Copepod nauplii					
Oithona spp.	NF, DT	5-77	104-1980	Direct	This study
Oithona spp.	PIP	6-18238	0.5-444	Direct	This study
Ciliates					
Tintinnids	NF, DF, DT	0.1-2.2	---	Direct	Capriulo & Carpenter, 1980
Strombidium sulcatum	SYN, PRO	22	---	Direct	Christaki et al., 1999
Mixed ciliates	NF	0.02-0.5	---	Indirect	Vargas & González, 2004b
Mixed ciliates	PIP	0.17-1.8	---	Direct	Ichinotsuka et al., 2006
Oligotrichous	PIP	15-31	0.4-0.7	Indirect	This study
Oligotrichous	NF, DT	0.07 -0.25	1-10	Indirect	This study
Dinoflagellates					
Oxyrrhis marina	NF, COC	0-0.1	---	Direct	Hansen et al., 1996
Gyrodinium galatheanum	CRYP	0-0.0002	---	Direct	Li et al., 2001
Mixed dinoflagellates	DT	0.01-0.012	---	Indirect	Vargas & González, 2004b
Gonyaulax polygramma	NF	0.001*	0.2*	Direct	Jeong et al., 2005
Mostly Gymnodinoids	NF, DT	0.01-0.06	0.1-1.1	Indirect	This study
Heterotrophic nanoflagellates					
Mixed	FLB	0.1-0.7	---	Direct	Sherr et al., 1988
Mixed	SYN,PRO	0.13	---	Direct	Christaki et al., 2005
Mixed	BAC	0.4-1.8	---	Indirect	Vargas & Gonzalez, 2004b
Mixed	PIP	0.1-1.3	0.04-0.2	Indirect	Böttjer & Morales, in press
Mixed	PIP	0.2-0.2	0.005-0.006	Indirect	This study

***** = *estimated maximum value*

Figure legends

Fig. 1. Photographs of two adult *Oithona* spp. females carrying ovigerous sacs (a = *O. similis* and b = *O. nana*), and two naupliar stages of *O. nana* (c = NV and d = NIV).

Fig. 2. Relative contribution (%) of the main taxonomic groups in terms of a) total abundance (cells mL^{-1}) and b) biomass (µg C L^{-1}) of the natural nano- to microplanktonic assemblages at the beginning of the grazing experiments with *Oithona* spp. nauplii (experiments 1 to 6). CI= ciliates, DF= dinoflagellates, SD= solitary diatoms, CHD= chain-forming diatoms, NF= nanoflagellates.

Fig. 3. Ingestion rates (x 10^3 cells $nauplii^{-1}$ d^{-1}) of *Oithona* spp. nauplii versus food concentration (x 10^3 cells mL^{-1}): a) picoplankton, b) nanoflagellates, c) dinoflagellates, and d) diatoms; e) Chl-*a* ingestion (µg Chl-*a* $nauplii^{-1}$ d^{-1}) versus Chl-*a* concentration (µg Chl-*a* L^{-1}). R^2 = regression coefficient; p = significance level and n = number of cases. Note the log-log scale for picoplankton. Regression analysis: $y = a \cdot x + b$.

Fig. 4. Electivity index for the different food types ingested by *Oithona* spp. nauplii during the experiments with natural assemblages of nano- and microplankton (experiments 1 to 6), pico- and nanoplankton (experiments 7 and 8) or solely picoplankton (experiments 11 and 14). NF = nanoflagellates, DF = dinoflagellates, DT = diatoms, PIPL = picoplankton, NANOPL = nanoplankton, BPL = bacterioplankton and CYB = cyanobacteria.

Fig. 5. Conceptual scheme of the trophic interactions linking *Oithona* spp. nauplii in the coastal upwelling area off Concepcion during the upwelling (spring/summer) period. Mean standing stocks (µg C L^{-1}) are shown below the different prey/predator type (boxes). The numbers on the arrows represent the mean consumption rates (µg C L^{-1} d^{-1}) and the percentages in parentheses indicate the grazing impact on the total standing stock (biomass) of each prey category. The thickness of the arrows indicates the strength of the interaction in terms of carbon uptake by the predator. Other trophic interactions are also shown: ciliates-prokaryotes, ciliates - nanoflagellates, ciliates - diatoms, dinoflagellates - prokaryotes, dinoflagellates - nanoflagellates, dinoflagellates - diatoms and nanoflagellates - prokaryotes. A model approach was used to predict these mean protistan grazing rates (Peters, 1994) and

were derived by using data from the present study, except for nanoflagellates feeding on prokaryotes (data from Böttjer and Morales in press).

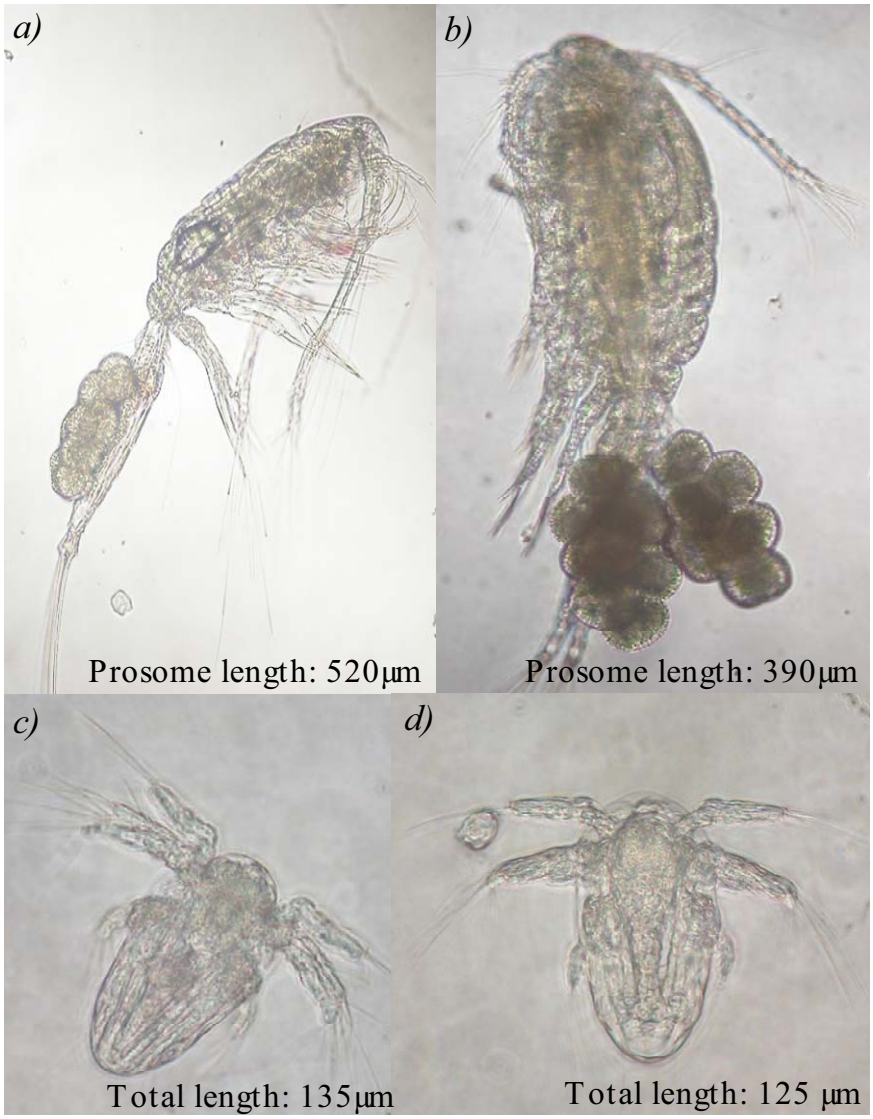

a)
Prosome length: 520µm
b)
Prosome length: 390µm
c)
Total length: 135µm
d)
Total length: 125 µm

Figure 1

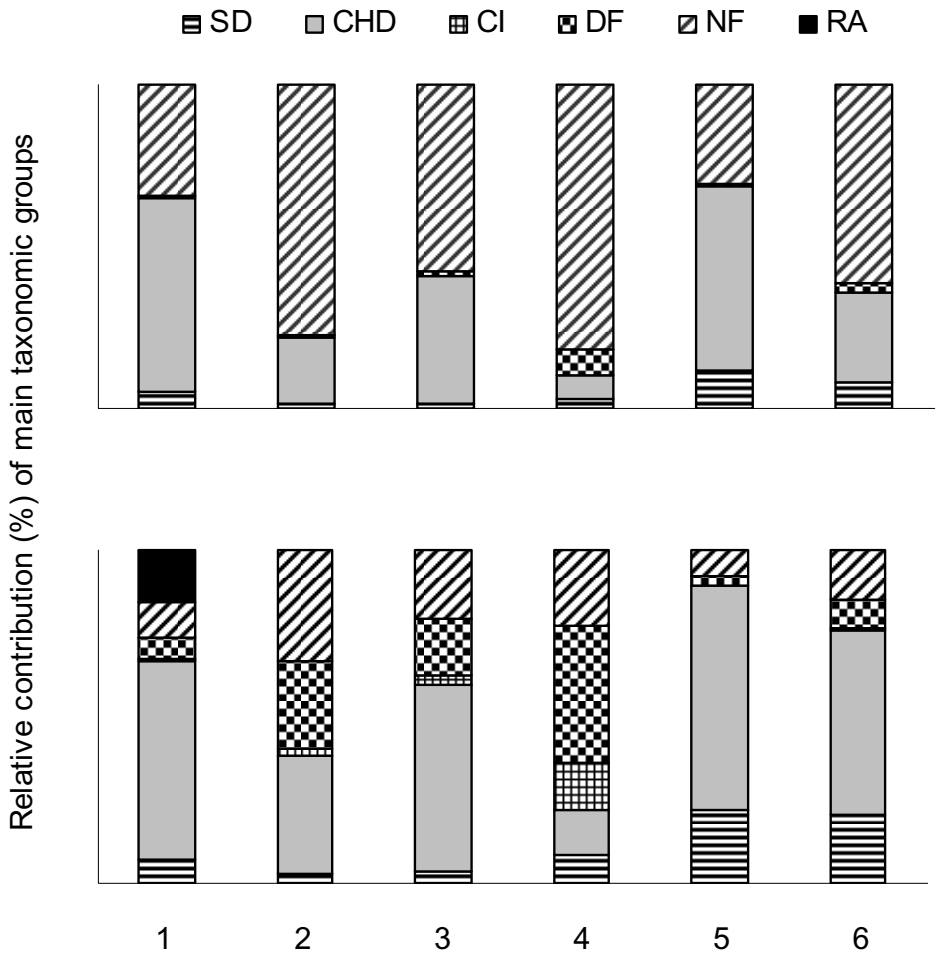

SD
CHD
CI
DF
NF
RA
Relative contribution (%) of main taxonomic groups
1
2
3
4
5
6

Figure 2

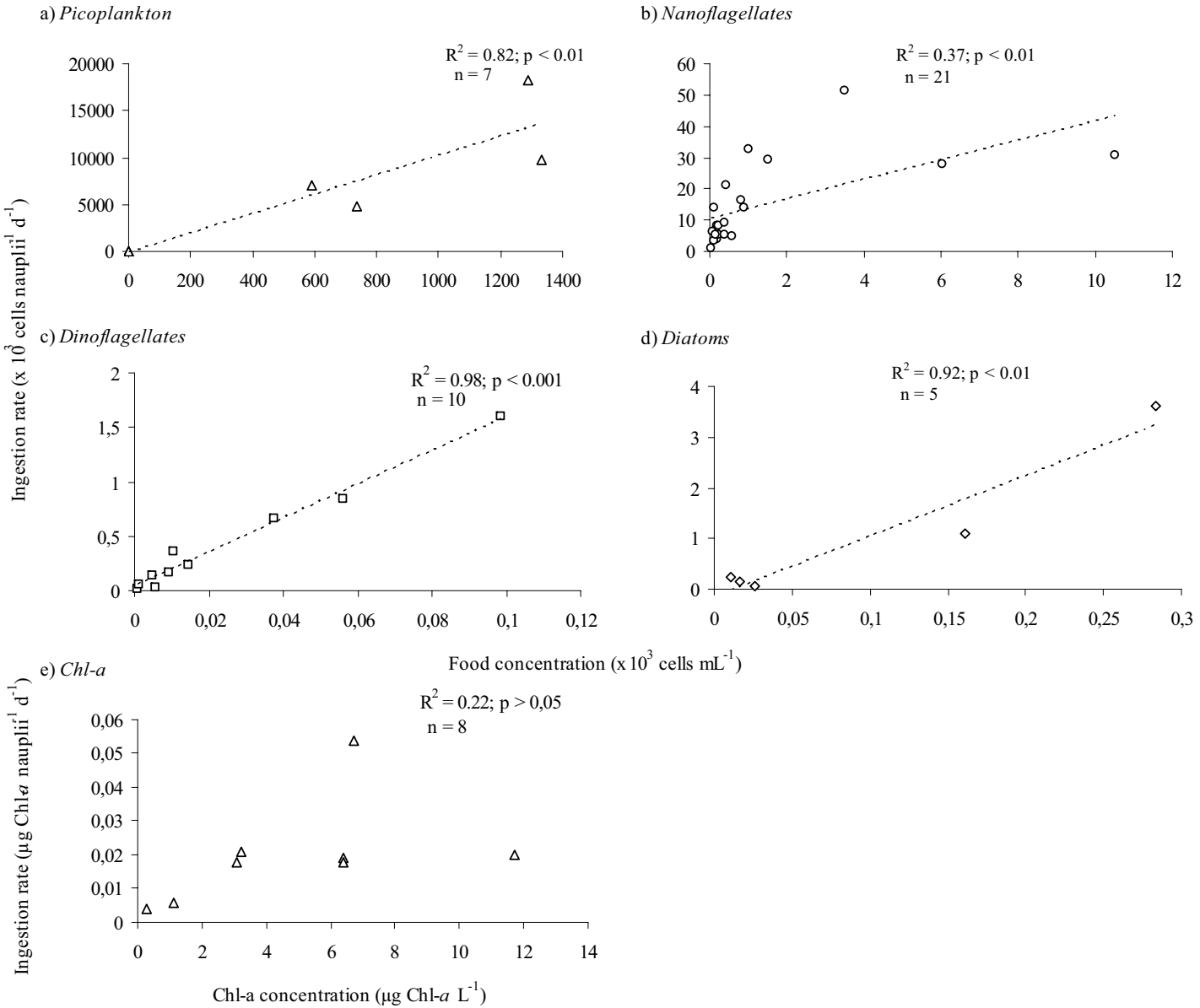

a) Picoplankton
R2 = 0.82; p < 0.01
n = 7
b) Nanoflagellates
R2 = 0.37; p < 0.01
n = 21
c) Dinoflagellates
R2 = 0.98; p < 0.001
n = 10
d) Diatoms
R2 = 0.92; p < 0.01
n = 5
e) Chl-a
R2 = 0.22; p > 0,05
n = 8
Ingestion rate (x 10^3 cells nauplii^-1 d^-1)
Food concentration (x 10^3 cells mL^-1)
Ingestion rate (µg Chl-a nauplii^-1 d^-1)
Chl-a concentration (µg Chl-a L^-1)

Figure 3

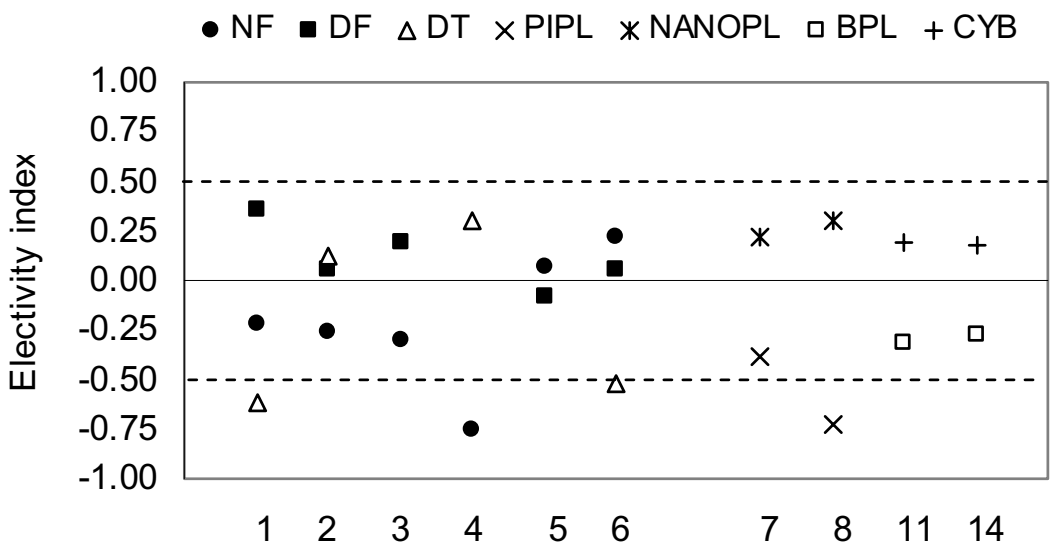

NF
DF
DT
PIPL
NANOPL
BPL
CYB
Electivity index
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
1
2
3
4
5
6
7
8
11
14

Figure 4

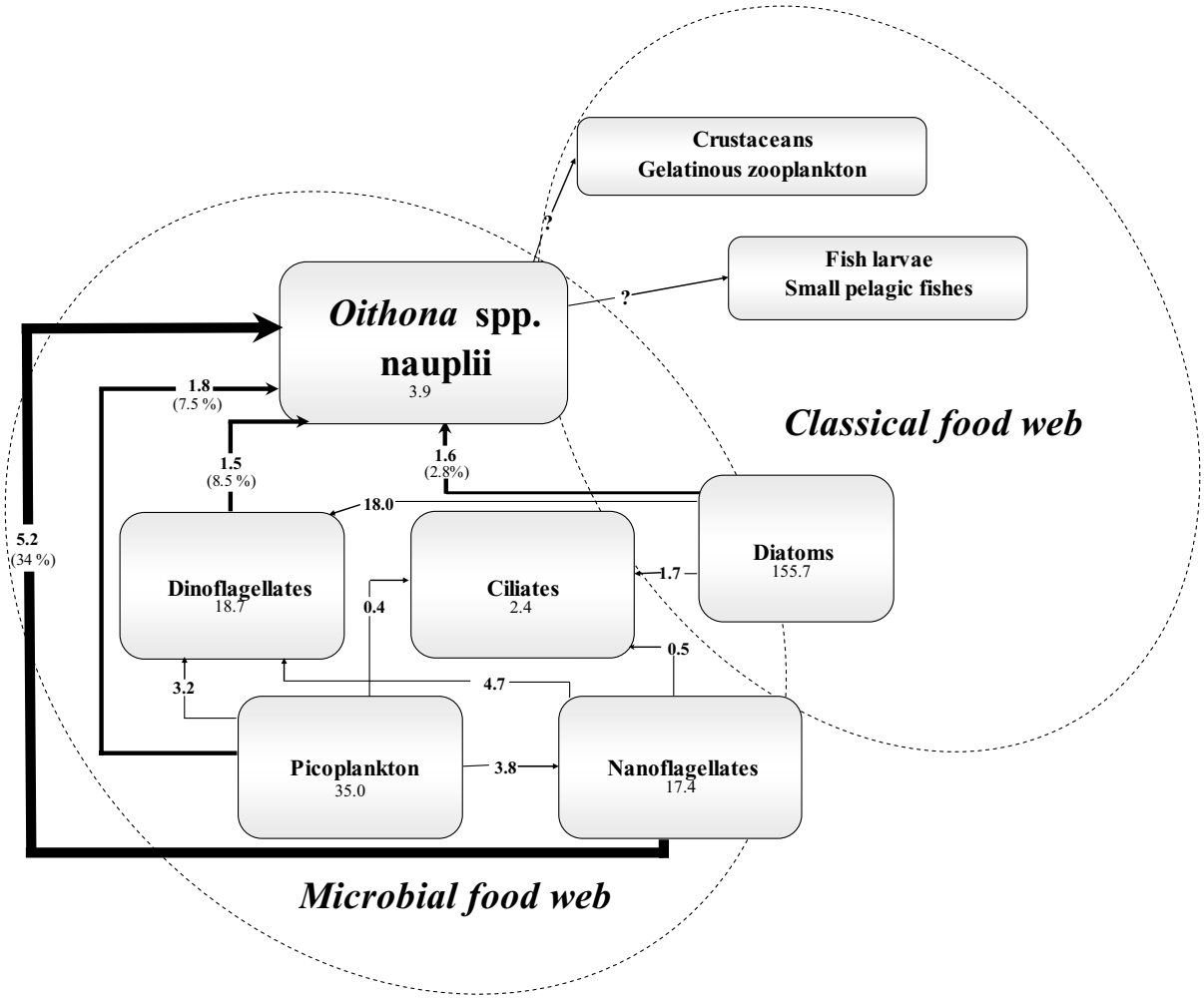

Crustaceans
Gelatinous zooplankton
?
?
Fish larvae
Small pelagic fishes
Oithona spp.
nauplii
3.9
Classical food web
1.8
(7.5 %)
1.5
(8.5 %)
1.6
(2.8%)
18.0
5.2
(34 %)
Dinoflagellates
18.7
Ciliates
2.4
Diatoms
155.7
1.7
0.4
0.5
3.2
4.7
Picoplankton
35.0
3.8
Nanoflagellates
17.4
Microbial food web

Figure 5

5. Discussion

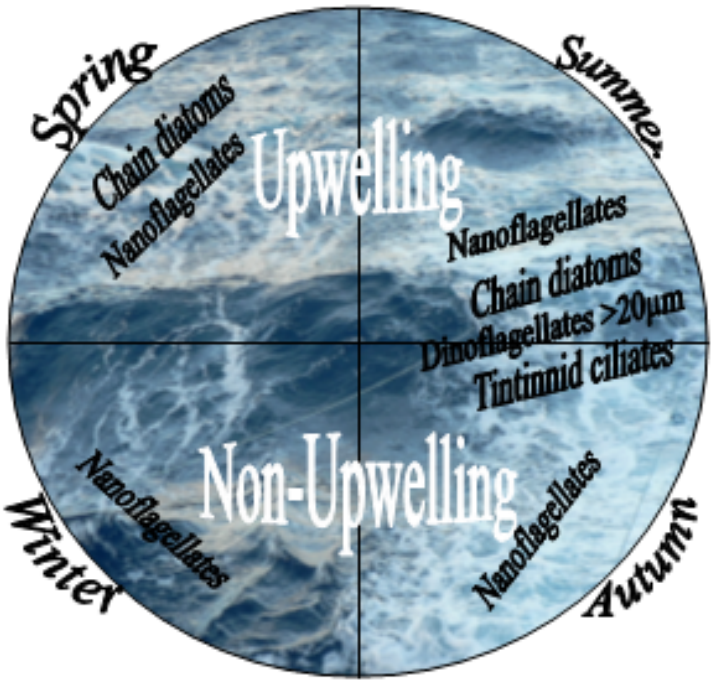
The present thesis focuses on the role and relevance of micro-organisms (<200 μm) and their impact on the ocean's carbon flow in a highly productive coastal upwelling system off central Chile. A comprehensive 2-year data set on the structure of nanoplanktonic components and the impact of seasonal hydrographic variability on the abundance and biomass of the nanoplanktonic assemblages is discussed in section 5.1. The experimental results on rates and grazing impacts of protists and metazoan micro-heterotrophs, and their role in controlling primary production and/or different prey abundances, is discussed in section 5.2.

5.1. The impact of environmental variability on nano- and microplankton assemblages in the coastal upwelling area off Concepción

In the HCS off Chile, the central-southern zone (35 - 38ºS) has been identified to present the most intense and persistent coastal upwelling activity, with ESSW being the main source of upwelled water (Strub *et al.*, 1998). Recently, Sobarzo *et al.* (*in press*) described the seasonal changes in the hydrography of this region and denoted two periods with different processes influencing the water column structure: 1) October to March (austral spring/summer), when upwelling and increased solar radiation play a larger role, and 2) May to July (austral autumn/winter), predominated by river influx and precipitation. These environmental changes are expected to result in temporal changes in the functional groups and/or species composition of planktonic assemblages. The changes in phytoplankton biomass and composition in upwelling systems has usually been related to water-column stratification, nutrient availability, and the intensity and persistence of upwelling conditions (Hutchings *et al.*, 1995). A typical annual succession of micoplankton species in upwelling areas is thought to be characterized by diatom spring and dinoflagellate autumn blooms (associated with highest Chl-*a* and nutrient concentrations), the winter period being dominated by small flagellate species (Blasco *et al.*, 1980; Kudela *et al.*, 2005). Recent findings from time series studies off Concepción partially back up and another part contradict this typical view of the annual succession in upwelling areas (Figure 5).

It has been shown that rapid growth of large-sized phytoplankton (mostly chain-forming diatoms of *Chaetoceros* spp., *Skeletonema* spp. and *Thalassiosira* spp.) follows the upwelling of nutrient-rich ESSW (Anabalón *et al.*, *in press*; González *et al.*, *in press*) and, certainly, they are the dominant autotrophic component in the system during the spring/summer period. González *et al.* (*in press*) described maximum abundances of dinoflagellates and tintinnid

ciliates in the microplankton fraction to occur at the same time, or just slightly after, the chain-diatom blooms. In considering both the nano- and micro-plankton fractions, Anabalón *et al.* (*in press*) found maximal abundance and biomass of the dominant genera and



morphotypes to co-occur during the upwelling period.

Figure 5. Principal nano- and micro-planktonic components found during the annual cycle at the shelf off Concepción, central Chile (~36°S). Modified from Kudela *et al*. (2005).

As part of this thesis, it was found that the integrated <20 µm Chl-*a* size fraction (11 - 86 mg m^{-2}), corresponding to the nano- and picoplanktonic autotrophs, is highly variable throughout the annual cycle and contributes >60% of the total Chl-*a* whenever the latter are low (<60 mg m^{-2}). The microplankton (>20 µm) is the dominant fraction of the Chl-*a* concentrations mostly during the upwelling period; nevertheless, the maximum values in the <20 µm fraction (> 60 mg m^{-2}) were observed during the same period of time (Böttjer & Morales, *in press*). Furthermore, a comparison of the abundance and biomass of autotrophic nanoflagellates between the upwelling and non-upwelling periods revealed no clear seasonal pattern, in agreement with previous studies in other coastal upwelling systems (Probyn, 1992; Varela, 1992; Casas *et al.*, 1999; Tilstone *et al.*, 2003; Barlow *et al.*, 2005; Rodríguez *et al.*, 2006). Anabalón *et al.* (*in press*) and Gonzalez *et al.* (*in press*) noted an absence of large nutrient differences between upwelling and non-upwelling conditions on the shelf off Concepción, with NO_3 and Si $(OH)_4$ concentrations not accounting for changes in the dominance of the

nano- and micro-plankton fractions in the study area. In contrast, Böttjer & Morales (*in press*) found NO_3 concentration to be significantly higher during the upwelling compared to the non-upwelling period, although they are still relatively high during the non-upwelling period, probably because river inputs. It was also found that the autotrophic nanoflagellates were weakly correlated with NO_3 concentration and, furthermore, there was a lack of correlation with water column stratification..

These findings contrast with the idea that different regimes of turbulence and/or nutrient availability define the size structure of phytoplankton communities, with dominance of pico- and nano-planktonic forms under lower turbulence-nutrient conditions and a shift to larger, micro-phytoplankton cells with increased turbulence and nutrient concentrations (Hutchings *et al.*, 1995; Tilstone *et al.*, 2000; Irwin *et al.*, 2006), but point to the importance of the nanoplanktonic fraction as a year-round component in the coastal upwelling region off Concepción, contributing to sustain the system's productivity. At the same time, the lack of seasonality of this size fraction implies that their potential grazers (*e.g.* metazoan microzooplankton) experience adequate food quantity and quality during the whole annual cycle. Which, then, are the factors that structure the autotrophic nanoflagellates off Concepción? The most likely seems to be the exposure to a constant grazing pressure by the microzooplankton holding their populations at relatively stable level throughout the year, with only occasional increases of one or two orders of magnitude.

To conclude, the system under study may principally act as source and high exporter of organic carbon fixed by large-sized phytoplankton cells during the upwelling period but it also sustains maxima in small autotrophic cell abundances and biomasses under contrasting hydrodygraphic conditions.

5.2. The impact of micro-heterotrophic grazing and the carbon flow in the coastal upwelling area off Concepción

Heterotrophic protists have been shown to be important in controlling bacterioplankton abundance and biomass through a range of different ecosystems (review in Sanders *et al*., 1992) although a partial control by viruses has also been reported (Fuhrman, 1999 & 2000). Heterotrophic nanoflagellates (HNF) have typically been considered as the main consumers of the picoplankton (Weisse, 1993; Christaki *et al.,* 2002 & 2005) and less attention has been paid to other protists, such as the heterotrophic nanodinoflagellates (HND). Estimates of GR by nanoheterotrophs (HNF and HND) feeding on autotrophic and heterotrophic prokaryotes in the upwelling area off Concepción are well in the range of GR values reported for a variety of

other marine systems (lake, river, estuary, coastal, and oceanic), including different direct techniques (*e.g*. selective inhibitor method, dilution technique) or model approaches (Landry *et al.*, 1984; Sherr *et al.*, 1986; Weisse, 1990; Weisse & Scheffel-Möser, 1991; Christaki *et al.*, 2002 & 2005; Jeong *et al.*, 2005; Cuevas & Morales, 2006). Results of this thesis indicate that the grazing impact by nanoheterotrophs (HNF and HND) on prokaryotic standing stocks (mean: 59%) does not differ between upwelling and non-upwelling periods, suggesting their high relevance in controlling prokaryotic picoplankton populations in the upwelling area off Concepción (Böttjer & Morales, *in press*). HNF and HND are an important food source for larger protists (Edwards *et al.*, 1999), as well as for metazoans (Turner & Granéli, 1992). Results of this thesis also indicate that in the upwelling system off Concepción, metazoan microplankton (cyclopoid copepod nauplii of *Oithona* spp.) exert a significant grazing impact on the nanoplankton size fraction (principally on the nanoflagellate standing stocks: 34%), implying their ability to control these assemblages (Böttjer *et al*., submitted). They also revealed their minor role in controlling the abundance of the dominant autotrophic component, chain-forming diatoms, during the upwelling period.

As a summary of these results, the overall utilization of autotrophic production (pico to microplankton) in the upwelling system off Concepción is represented in Figure 7. This scheme highlights the fate of PP due to the grazing impact of different grazers during contrasting hydrographic conditions and stresses the importance of the microbial food web in the system.

Microzooplankton are important consumers of PP (132 - 185 %) during winter time when the phytoplankton community is numerically dominated by mostly small-sized autotrophic forms (cyanobacteria and flagellates) (Böttjer & Morales, 2005). Vargas *et al.* (2007) found a comparatively lower grazing impact on primary production (39 - 84%) during the same seasonal period and area of study. They, however, considered only protists grazers and excluded small metazoan microzooplankton in their estimates. This component has been shown here (Böttjer *et al*., submitted) to be an important predator upon the nanoplankton during the spring/summer period, including autotrophic nanoflagellates and nanodiatoms; they are also expected to be important grazers during the autumn/winter period since they are present all year-round, as well as their main food. Excluding metazoan microzooplankton underestimates the total micro-heterotrophic grazing impact by two to three times.

The scenario of microzooplankton herbivory in the coastal system off Concepción, Chile changes during the austral/spring summer, upwelling period, when the system becomes dominated by large autotrophic forms (chain-forming diatoms), generating dense blooms and

high primary production values (Montecino *et al*., 2006; Vargas *et al.*, 2007 González *et al.*, *in press*). Attempts by Böttjer & Morales (unpublished data) to estimate microzooplankton grazing by carrying out dilution experiments during the upwelling period, when Chl-*a* concentrations exceeded ~5 mg Chl-*a* m^{-3} (range: 5.6 – 23.3 mg Chl-*a* m^{-3}), displayed negative microzooplankton grazing, that is, higher net growth in less diluted treatments.

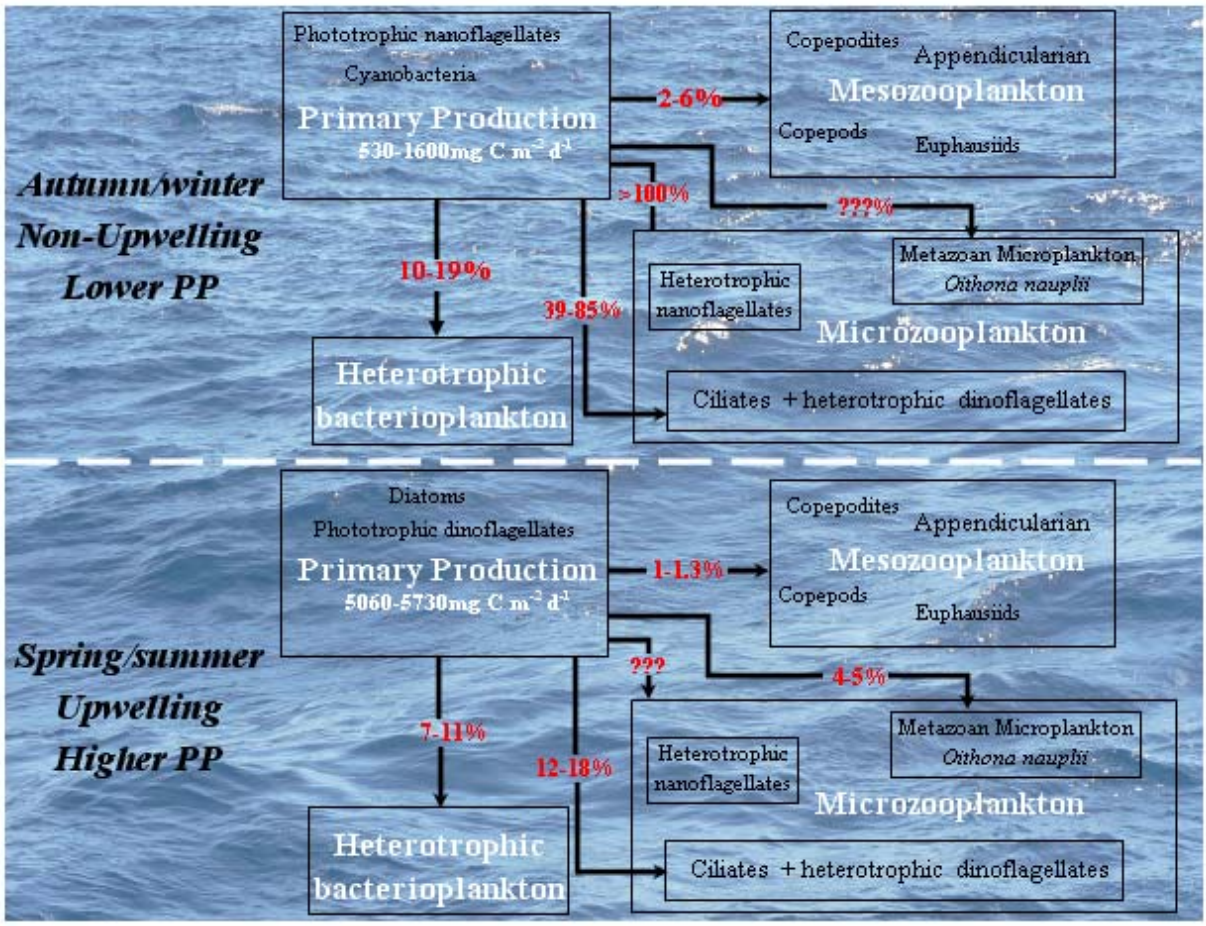


Figure 7. Utilization of primary production by different planktonic size fractions during two contrasting seasons in the coastal upwelling system off Concepción, central Chile. Data are from the studies of Cuevas *et al.* (2004), Böttjer & Morales (2005), Vargas *et al.* (2007) and Böttjer *et al.* (submitted).

Certainly, the dilution method is not without problems, in particular when feeding becomes saturated at very high food levels (Landry & Hasset, 1982; Gifford, 1988; Gallegos, 1989; Evans & Paranjape, 1992; Dolan *et al*., 2000). That could possibly explain the "failure" of the dilution method during this thesis experiments, although the same approach has been successfully applied in other productive systems with high Chl-*a* concentrations (*e.g.* 4 - 55 mg Chl-*a* m^{-3}; Neuer & Cowles, 1994; Strom & Strom, 1996). The recent publication of Vargas *et al.* (2007) described a moderate impact of protists (11 - 18%) on a relatively high primary production (5061 - 5725 mg C m^{-2} d^{-1}) during the spring/summer period off Concepción by applying the size-fractionation method (Capriulo & Carpenter, 1980). They

concluded that the microzooplankton community is not able to keep up with the phytoplankton biomass growth when diatoms are forming into dense blooms, even though larger-sized micro-heterotrophs, known to be able to feed on diatoms, are concurrently present. Therefore, what might be the explanation for the phytoplankton to be able to escape the control of micro- and meso-zooplankton grazing during the upwelling period in this system? Irigoien *et al.* (2005) have recently posed the hypothesis that blooming species (diatoms and dinoflagellates), through a combination of predation avoidance mechanisms, elude predation by zooplankton, opening so called "loopholes". Bloom forming diatoms have evolved morphological (*e.g.* increasing cell size by forming chains, spines, frustules) or chemical (aldehydes) defense strategies to deter planktonic protist grazers (Smetacek, 2001; Strom, 2002) as well as metazoans (reviewed in Pohnert, 2005). The phenomenon of chemical defense has received little attention (Wolfe, 2000 *fide* Strom, 2002), and demonstrating that size is truly an effective strategy against predation needs some further experimentation. Top-down grazing upon heterotrophic protists by mesozooplankton, in particular copepods, may further elucidate the lack of control of phytoplankton blooms by microzooplankton herbivory (trophic cascading). Mesozooplankton abundance and biomass has been shown to increase during the spring/summer upwelling period in the system under study (Escribano *et al.*, *in press*) but Vargas *et al.* (2007) imply that they only incorporate a very small part of the PP (~1%).

Altogether, the findings of this dissertation, in agreement with the studies of Troncoso *et a*l. (2003), Cuevas *et al*. (2004), and Vargas *et al*. (2007), suggest that the microbial food web is a fundamental and permanent component in the upwelling system off Concepción. However, the microbial food web has usually been considered to be an inefficient carbon pathway and a *sink* for biogenic carbon in terms of recycling within the euphotic zone rather than transfer onto higher trophic levels (*e.g*. Legendre & Le Fèvre, 1995). I propose that this hypothesis needs a careful revision since the upwelling system off Concepcion is highly productive during the whole year due to the persistence of the microbial food web. The microbial food web does not strictly include various grazing steps to incorporate the photosynthetically fixed carbon into higher trophic levels. Small sized autotrophs can be channelled to higher trophic levels as effective as their larger counterparts (*e.g*. cyanobacteria → ciliate → fish or autotrophic nanoflagellate → metazoan microzooplankton → fish, compared to diatom → herbivorous zooplankton → fish). Therefore, the microbial food web might transfer carbon as efficient as the herbivorous food web and, thereby, be able to sustain a high, year-round productivity of the coastal upwelling system off Concepción.

The main goal of this thesis was to elucidate the relevance of small micro-organisms and the carbon flow in a highly productive, coastal upwelling system; a "little-known, yet fascinating" part of marine microbial ecology and by doing so, the following has been concluded:

1. Minor impact of the strongly seasonal hydrographic variability on the abundance and biomass of nanoplanktonic assemblages

2. Grazing by nano-heterotrophs controls prokaryotic picoplankton populations

3. Total microzooplankton (including micro- and nano-heterotrophs) exert an important impact on the potential primary production (>100%) in the system during the non-upwelling, autumn/winter period.

4. Metazoan microzooplankton (*Oithona* spp. nauplii) control the nanoplankton assemblage and, thereby, represent an important trophic intermediate between the classical and microbial food webs in this coastal upwelling system.

5. The microbial food web is a fundamental and permanent feature of the trophic pathways in the water column in the system and probably it is also efficient in channelling primary and/or secondary production to higher trophic levels

6. Perspectives

This thesis revealed several new aspects in the food web dynamics of the coastal upwelling area off central Chile, and further knowledge on the carbon flow in this system was gained. Yet, a lot of new questions emerged, certainly a sign of the vitality of marine microbial ecology. One ends up with more questions than answers, the unknowns still outnumber the established facts, and with this, leaving much room for further research. This chapter will briefly present the most burning issues.

Certainly, the most 'urgent' topic is the pelagic food web structure and the trophic transfer between the herbivorous and microbial food web. The upwelling area off Concepción is highly productive; most of the photosynthetically fixed carbon is channelled through the microbial food web, which actually has been described as inefficient in trophic transfer. Further explorations and possibly a revision of the microbial food web model and the related carbon transfer in highly productive regions are strongly needed in order to understand the year-round productivity in these systems.

Studies of pure cultures of micro-organisms definitely provide fundamental information of important species and to culture a species under carefully controlled conditions allows the examination of its biology in the absence of potentially confounding interactions with other living organisms. Therefore, the understanding of the ecological role of micro-organisms in nature might improve with more species brought into culture. Predominant protists found in the coastal waters off Concepción could be isolated from natural assemblages (Caron, 1993; Gifford, 1993; Lessard, 1993) to examine their nutritional mode, feeding behaviour, feeding rates as well as preferences and/or growth rates of specific species. Nevertheless, it should be kept in mind that studies of feeding and growth rates under natural conditions are further required and the application of newer technologies such as flow cytometry should facilitate such studies (Sherr & Sherr, 2002).

Dinoflagellates are known for their complex feeding behaviours and apparatus that enable them to ingest prey as large as themselves, as well as chains and colonies (Strom & Strom, 1996; Jacobson, 1999), and also choreotrich ciliates have been reported to feed on prey organisms that measure nearly half of their oral diameter (Jonsson, 1986). Nevertheless, dense phytoplankton blooms occur during the upwelling period in the system under study and emphasis needs to be placed on the understanding of the processes that regulate the uncoupling of phytoplankton growth and microzooplankton grazing. Why are large-celled, herbivorous protists not capable of controlling the enormous biomass build-up of bloom-forming diatoms by using their various different feeding modes? Do defense strategies (*e.g.*

chemical, morphological) enable the phytoplankton to escape microzooplankton grazing? These are only some questions that should be addressed in future projects on phytoplankton-microzooplankton interactions under upwelling conditions.

Microbial composition and diversity are influenced by environmental (*e.g.* turbulence, organic substrates and nutrients) and biological factors (competition and predation), and in this context, viral infection has been recently assumed as one of the key factors in regulating the structure and composition of prokaryotic communities in aquatic ecosystems (*e.g.* Weinbauer & Rassoulzadegan, 2004; Winter *et al.*, 2005; Bouvier & del Giorgio, 2007). Viruses infecting eukaryotic, marine phytoplankton (diatoms, chrysophytes, pyrmnesiophytes, haptophytes, rhaphidophytes and cryptomonads) are found in the euphotic zone and are abundant, so that they might control algal blooms (Fuhrmann, 1999). So far, little attention has been paid to the role and relevance of viruses and virus-induced mortality of bacteria and specific phytoplankton in the system under study, but certainly need to be included in further microbial food web studies.

The application of molecular biology tools remains another challenge. Molecular biology has swept through the field of aquatic microbial ecology and the phylogenetic diversity and gene function of bacteria and protists have revealed spectacular discoveries (*e.g.* Giovannoni *et al.*, 1999). Protists biodiversity through DNA analysis would be of great interest for a future development and molecular tools could be included in upcoming grazing experiments to study the effect of predation on prey community structure: Are some prey species selected? Does selection of certain prey species favour the dominance and persistence of other species? This new line of research is only the beginning since these novel approaches undoubtedly continue to be a major theme in the field of marine microbial ecology.

7. Literature cited

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Anlage zur Dissertation

Eidesstattliche Erklärung
gem. § 6 (5) Nr. 1-3 PromO

Hiermit erkläre ich, dass ich

1. Die Arbeit ohne unerlaubte fremde Hilfe angefertigt habe,

2. Keine anderen, als die von mir angegebenen Quellen und Hilfsmittel benutzt habe und

3. Die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Concepción, Chile 15.10.2007

Daniela Böttjer