The role of copepod grazing in phytoplankton bloom dynamics : a species-based approach

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The role of copepod grazing in phytoplankton bloom dynamics: a species-based approach

by

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ABSTRACT

Seasonal phytoplankton blooms characterize the pelagic communities in temperate oceans and in coastal regions of the Mediterranean Sea. The present study was aimed at understanding the role of trophic interactions in the dynamics of phytoplankton blooms in a long-term time series in the inner Gulf of Naples (LTER-MC) where phytoplankton and mesozooplankton are dominated by diatoms and copepods, respectively. The abundant calanoids *Acartia clausi*, *Centropages typicus*, *Paracalanus parvus*, and *Temora stylifera* peak in different seasons and interact with different co-occurring phytoplankton communities, of which the diatoms *Chaetoceros socialis*, *Leptocylindrus spp.* and *Pseudo-nitzschia* spp. are the most abundant. By following a species-specific approach, the feeding performances and behaviour of the four copepod species on selected bloom-forming diatoms were analyzed by incubation experiments using the food removal method and video recordings of individuals at small scale. Significant differences appeared in the feeding responses of the copepods to the different diatom species, which can be attributed to the species-specific traits of both the predator and prey. Copepods showed behavioral plasticity in presence of different diets, with changes in the duration of feeding bouts and proportion of time allotted to different behaviors, which can in turn affect the feeding rates. Finally, the estimated impact of copepod grazing at st. LTER-MC showed that the copepods can remove a significant portion of the bloom-forming diatoms but have a limited impact on total standing stock of a diversified diatom assemblage. The impact also varies depending on the phases of the blooms with respect to the abundance and composition of the plankton communities. This study discloses the role of key copepod species in the dynamics of phytoplankton blooms for a better understanding of the seasonal and long-term patterns of plankton communities in marine coastal ecosystems.
Dedicated to my grandmother
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CHAPTER 1.

General Introduction
1.1 The role of copepods in pelagic food webs

Copepods are the most abundant and particularly successful metazoans in the world ocean (Kiorboe, 1997). They comprise the bulk of mesozooplankton communities in marine pelagic ecosystems and can contribute a significant proportion to total zooplankton biomass depending on the region and season. In coastal and estuarine regions, they are typically the numerically dominant mesozooplankters and can often account for 80-90% of the total zooplankton abundance (Longhurst, 1985; Raymont, 1983). Feeding by copepods on phytoplankton, i.e., grazing, is one of the most important processes in marine food web dynamics and copepods are regarded as major grazers in all marine ecosystems. Several studies indicate that they are primarily omnivorous, consuming both autotrophic and heterotrophic prey (e.g. Atkinson, 1995; Fessenden and Cowles, 1994; Gifford and Dagg, 1988; Kleppel, 1993; Paffenhofer and Knowles, 1980; Zeldis et al., 2002). As herbivorous feeders, copepods represent an important trophic link between primary producers and higher trophic levels (Cushing, 1989) since they are an important food source for fish and other predators, thus mediating energy and carbon transfer (Runge, 1988) from the euphotic to deeper layers of the oceans (Calbet et al., 2000), and contributing to the functioning of the biological pump (Longhurst and Harrison, 1989). As feeders on heterotrophic prey such as ciliates, copepods participate to the pathway between the microbial loop and the upper trophic levels (Turner, 2004). Copepods can switch from herbivory to carnivory in response to changing food environment (e.g., Gismervik and Andersen, 1997) and are occasionally able to utilize detrital and bacterial sources (e.g., Roman, 1984). Moreover, they can exhibit selective feeding depending on the on the features of the prey: swimming behaviour, speed and jumping, as well as size and palatability. Some copepods can discriminate the prey on the basis of food quality, nutritional value and toxicity (e.g.,
Chapter 1. General introduction

Cowles et al., 1988; Donaghay and Small, 1979; Tiselius, 1989). Copepod grazing therefore may or may not have significant impact on their prey depending on the type of prey and the relative concentrations of predators and prey.

Many studies have reported low copepod grazing rates when feeding primarily upon algae, especially when compared to the high grazing rates of protozoans (Atkinson, 1996; Frost, 1987; Leising et al., 2005; Liu and Dagg, 2003). In coastal bloom waters at temperate and high latitudes worldwide, high microzooplankton grazing rates on diatoms have been measured (e.g., Olson and Strom, 2002; Fonda Umani and Beran, 2003). Direct comparison of microzooplankton grazing with copepod grazing has frequently shown an equivalent to or substantially greater consumption of bloom-forming diatoms by microzooplankton than consumption by copepods (e.g., Landry et al., 2000; Strom et al., 2007; Vargas and González, 2004). Microzooplankton, due to their relatively higher abundance and grazing rates, are now considered the most important grazers of phytoplankton and other microbes within the microbial food web with a more relevant role than copepods (Calbet and Landry, 2004; Liu et al., 2005a).

Microzooplankton, due to their broad size range are capable of preying on large phytoplankton, including diatoms and cell chains (e.g., Olson and Strom, 2002; Strom et al., 2007). In particular, dinoflagellates that are able to feed on large chain-forming diatoms through extracellular digestion (Gaines and Taylor, 1984) are the most important microzooplankton consumers of diatoms. Microzooplankton, in turn are important in the diet of copepods and can comprise a high proportion of ingested carbon (e.g., Fessenden and Cowles, 1994; Halvorsen et al., 2001; Kleppel, 1993). Copepod grazing can therefore significantly impact the microzooplankton population and subsequently release the prey of the microzooplankton from grazing pressure (Fessenden and Cowles, 1994; Gifford and Dagg, 1988; Hansen et al., 1993b). Thus, by
regulating the abundance of microzooplankton through their grazing activities, copepods not only affect the standing stock, but also can indirectly alter the size structure of the phytoplankton community and impact diversity of populations (e.g., Broglio et al., 2004; Katechakis et al., 2002; Sipura et al., 2003).

Copepod grazing can not only reduce algal standing crop, but, processes closely linked to grazing, i.e., sloppy feeding, excretion and fecal pellets production can contribute significantly to nutrient regeneration and increasing the pool of dissolved organic matter (DOM), therefore stimulating algal growth (Bergquist and Carpenter, 1986). Sloppy feeding, i.e., the physical breakage of the food item during zooplankton feeding resulting in the release of dissolved nutrients from damaged algae, is considered as the key factor in the production of large amounts of dissolved organic carbon (DOC) during copepod grazing activity (Hasegawa et al., 2001; Moller and Nielsen, 2001; Møller et al., 2003). Fecal pellets produced by zooplankton are ubiquitous component of the oceanic particle flux and an important nutrient source for deep water ecosystems (reviewed by Turner, 2002). Their fast sinking nature can directly contribute to enhance vertical flux of particles (Butler and Dam, 1994; Komar et al., 1981; Turner, 2002; Wexels Risers et al., 2002). While, processes like coprophagy (consumption of fecal pellets), coprorhexy (modification of fecal pellets), leaking and other forms of biological degradation are likely to retain a large fraction of the fecal pellet material in the euphotic zone and contribute significantly to DOC pool in the epipelagic ocean (Frangoulis et al., 2005; Gonzalez and Smetacek, 1994; Poulsen and Kiørboe, 2005; Urban-Rich, 1999). Copepods through grazing can thus provide an alternative source to the dissolved organic matter pool and fuel the bacterial community through production of DOC (Moller, 2005; Titelman et al., 2008; Vargas et al., 2007). An important percentage (~25 to
50%) of the carbon grazed by copepods may go directly to the microbial food web through the production of organic matter (Moller, 2005). Grazing can thus influence the biological pump by modifying the quality and quantity of the particle flux and potentially structure plankton communities (Kiorboe, 1997).

1.2 Importance of small copepods in pelagic environment

Small copepods (~ 1 mm) comprise a substantial proportion of the copepod assemblage, which generally dominates the mesozooplankton not only in terms of abundance, but also in terms of biomass in different marine environments (e.g., Hopcroft et al., 2001; Turner, 2004; Vidjak et al., 2006). However, for a long time, the use of coarser mesh resulted in the under sampling of this important fraction and failed to provide a realistic representation of this size fraction of copepod community (Turner, 2004). As a result, small copepods remained under-studied and their trophic, ecological and biogeochemical significance has been less understood as compared to their larger counterpart (as reviewed by Turner, 2004). In both open ocean and coastal ecosystems, small copepods can be more abundant than their larger counterparts, and their population biomass has been proposed to equal that of large calanoids (e.g., Dubischar et al., 2002; Sun et al., 2011).

Small-size classes are known to feed as omnivores on diverse food sources including autotrophic and heterotrophic protists, and marine snow (Turner, 2004 and reference therein). Many studies have now highlighted the important role of small copepod species as important trophic link between pelagic primary production and higher trophic levels (Calbet et al., 2001; Hirst and Lampitt, 1998) as well as in the classical and microbial food webs (e.g., Böttjer et al., 2010; Calbet et al., 2000; Schnetzer and Caron, 2005). Several species of small copepods often depend on constant food availability and are therefore more sensitive to variations in the food
environment. As a result, these species are also quick to respond to higher food availability as during phytoplankton blooms (e.g., McManus and Foster, 1998; Paffenhöfer and Stearns, 1988)

The biomass of particulate matter in the average pelagic environment can be considered to be approximately constant in equal, logarithmic size classes (Sheldon et al., 1972). Consequently, the biomass of food available to the different size classes of copepods, i.e. both small and large copepods, is the same even though they feed on differently sized particles (Berggreen et al., 1988; Kiorboe and Sabatini, 1995). Considering that copepods feed on prey about a factor of 18 smaller than their linear body dimension (Hansen et al., 1993a), small copepods are closer to the base of the microbial food web than the larger copepods (Calbet et al., 2000). This also implies that the small-sized prey fraction not available to the larger copepods due to the physical constraint on their feeding can be utilized by the small copepods. In addition to this, small copepods have much higher mass specific rates of feeding and metabolism than the larger species (Marshall and Orr, 1966). Consequently, when the small copepods dominate numerically, they can be major grazers and can have a strong top down effect on smaller food items and phytoplankton available to other feeders (Kiorboe and Nielsen, 1994; Morales et al., 1991).

Some authors have also shown correlation between the dominance of small copepods and low vertical flux of fecal pellets in the water column suggesting that small copepods degrade fecal pellets at higher rates than large copepod species (e.g. Svensen and Nejstgaard, 2003; Viitasalo et al., 1999). This may be due to the fact that small copepods produce small pellets, which have lower sinking speed and are degraded faster than pellets produced by larger copepods (Poulsen and Kiorboe, 2005). In food environments dominated by larger diatoms, where grazing of the
smaller copepods may not have a direct substantial effect, the nutrients recycled by the small copepods can be utilized by the larger diatoms. Thus, the activities of small copepods may indirectly support the build-up of the large phytoplankton blooms dominated by large diatoms (Dubischar et al., 2002).

1.3 Phytoplankton blooms

Blooms are events of rapid production and accumulation of phytoplankton biomass that are usually responses to changing physical forcing (Legendre, 1990). The photoautotrophic biomass during blooms may increase by up to 3 orders of magnitude (Irigoien et al., 2005). Blooms arise when the growth rates of one or more species of the phytoplankton assemblage exceed their mortality rates. This can happen when there is an imbalance or a lag between the production of microalgae and their removal or advection/sedimentation out of the production zone (Legendre, 1990). Several factors, referred to as the bottom-up control mechanisms like light, temperature, depth and rates of vertical mixing and nutrients availability are critical to bloom formation (Mann and Lazier, 2006; Tian et al., 2009; Townsend et al., 1994). The major top-down control resulting in mortality and breakdown of the bloom can be grazing (e.g., Banse, 1994; Behrenfeld, 2010; Irigoien et al., 2005). Several other loss factors like nutrient depletion (Leblanc et al., 2009), sedimentation (Brussaard et al., 1995; Keller and Riebesell, 1989), viral lysis (Brussaard, 2004 and references therewithin) are also considered to be effective in the removal of the phytoplankton biomass accumulated during blooms.

Spring blooms have been of interest for many decades and a good theoretical understanding has been achieved (Behrenfeld et al., 2013; Evans, 1988; Huppert et al., 2002; Sverdrup, 1953). In most temperate and subpolar oceanic regions, phytoplankton populations undergo strong seasonal cycles, with prominent blooms
occurring particularly in the winter-spring season but also to a lesser extent in the autumn. Spring phytoplankton blooms in temperate waters are generally dominated by comparatively few species of unrelated genera of diatoms (Assmy and Smetacek, 2012). According to the classical “critical-depth theory” of Sverdrup (1953), the depth of the upper mixed layer must be less than the critical depth for a spring bloom to initiate. According to this theory, the spring bloom initiates when the upper mixed layer depth of the water column is shallower than the depth above which the depth-integrated photosynthesis exceeds the depth-integrated phytoplankton losses (e.g., Nelson and Smith, 1991; Siegel et al., 2002; Smetacek and Passow, 1990). The nutrient enrichment of the water column by conventional mixing in the winter months followed by increasing solar radiation are favourable factors contributing to the development of spring blooms. Following the depletion of silicate and then other nutrients, diatoms are replaced by small nanoplankton (prymnesiophytes, prasinophytes and cyanobacteria). Toward late spring, the water column continues to stratify and hinders nutrient replenishment, and as a result coccolithophores, which have lower nutrient requirements, can become prominent (Leblanc et al., 2009; Lochte et al., 1993). Increasing zooplankton abundance and decreasing nutrient concentrations within the upper mixed layer have been suggested to be important in controlling the bloom after its initiation (Fasham et al., 1993).

Autumn blooms are typical of temperate regions, and their occurrence has been widely documented in the literature (e.g., Estrada, 1984; Siokou-Frangou et al., 2010; Smetacek, 1985; Zingone et al., 1995). As compared to the spring blooms, autumn blooms are less well studied. The classical hypothesis states that phytoplankton blooms in autumn are caused by increased vertical mixing and a breakdown of the stratification causing an influx of nutrients into the upper layers of the ocean (Findlay et al., 2006),
while light levels remain high enough so as not to limit photosynthesis. In addition, increased vertical mixing is considered to dilute the grazer population which can further promote phytoplankton growth. This combination of factors allows phytoplankton populations to increase their growth rate and initiate a bloom (Findlay et al., 2006). Few studies have also postulated mechanisms like coastal upwelling (Estrada, 1984), enrichment from sediments (Smetacek, 1985), land-driven nutrient enrichment (Zingone et al., 1995) all of which cause episodic enrichment of the photic layer that gives rise to an autumn bloom. Reduced grazing pressure has also been considered to explain the development of a phytoplankton maximum (Smayda, 1957). The fate of the phytoplankton bloom thus depends on the intensity of various complex physical, biological and biogeochemical processes in concert with the composition and structure of the pelagic communities.

During algal blooms, higher rates of phytoplankton production and change in biomass are typically coupled, with increased production leading to increased standing stock. The winter-spring blooms, which dominate the seasonal cycle of productivity in many temperate estuarine and coastal areas, can contribute up to 80% of the total annual primary production over a relatively short (3-4 weeks) time (e.g., Durbin et al., 1975; Lignell et al., 1993). The organic matter synthesized by phytoplankton, when fixed into particulate material (POM), can be released subsequently to the dissolved phase (as DOM) by a variety of processes such as cell leakage, grazing and viral lysis (Gobler and Sanudo-Wilhelmy, 2003). The release of DOM by marine phytoplankton has been shown to significantly affect nutrient cycling (Kirchman et al., 1991), trace metal availability (Hutchins et al., 1999), bacterial growth (Baines and Pace, 1991; Cole et al., 1982), and microbial food webs (Kirchman et al., 1991).
A spring bloom may be important in providing a brief but plentiful supply of food against a background of generally low chlorophyll $a$ levels and small food cells for herbivorous or omnivorous zooplankton (Smetacek et al., 1990). Higher food availability may also reduce the inter-specific competition for food and leads to higher diversity of grazers. The quality of food, i.e. both its biochemical composition and diversity is one of the essential factors affecting growth, reproduction and mortality of individuals, and therefore, plays an important role influencing the population dynamics of marine copepods (e.g. Koski et al., 2008; Mazzocchi et al., 2006; Nejstgaard et al., 2001; Vargas et al., 2006). Under favorable food conditions, copepods grow fast and build up a large population biomass, which in turn can be exploited by higher trophic levels such as fish larvae (Runge, 1988; Vargas and González, 2004). The productive areas of the coastal regions offer to copepods a complex mix of potential food particles, including not only phytoplankton, but also heterotrophic and mixotrophic protists (e.g. Gifford and Dagg, 1988; Halvorsen et al., 2001; Verity and Paffenhofer, 1996; Yang et al., 2010). Under such conditions, both, food quality and concentration can drastically influence their gross growth efficiency, with important implications for trophic transfer in coastal food webs.

1.4 Grazer responses to phytoplankton blooms

Several authors have reported enhanced copepod feeding, growth and fecundity in parallel with chlorophyll $a$ concentration peaks and dominance of large cells. This occurs in highly productive environments during upwelling, spring blooms and storm events (Bautista et al., 1994; Gómez-Gutiérrez and Peterson, 1999; Nielsen and Kiørboe, 1991; Peterson and Kimmerrer, 1994). Increase in quality or quantity of food is also known to affect the developmental rates and moulting (Yamaguchi et al., 2010) or lipid build up (Atkinson et al., 1996; Hagen and Schnack-Schiel, 1996) and can induce
behavioral changes (e.g., in diel vertical migration patterns) (Tsuda et al., 2006) in copepods.

Zooplankton grazing is one of the most important processes responsible for loss terms of phytoplankton (Calbet et al., 2003). It can exert a significant impact on phytoplankton increase such as during diatom blooms (Bathmann et al., 1990; Campbell et al., 2005; Huskin et al., 2001). While physical and biological factors such as advection, mixing, sinking and disease can all contribute to the loss of phytoplankton populations, fast-growing grazers are most likely to keep pace with increased phytoplankton growth (Juhl and Murrell, 2005). Several modelling studies have demonstrated the potential of grazer populations to maintain low phytoplankton standing stock and the role of grazing in phytoplankton bloom dynamics (Behrenfeld, 2010; Findlay et al., 2006; Griffin et al., 2001; Morozov, 2010). An early study focused on the role of grazers in bloom dynamics suggested that the spring diatom bloom in Narragansett Bay established because of the low abundance of grazers and that the winter-spring bloom ended when rising spring temperatures become favourable to zooplankton reproduction (Martin, 1965). Studies in different marine environments have identified both microzooplankton (e.g., Calbet and Landry, 2004; Fonda Umani and Beran, 2003; Levinsen and Nielsen, 2002; Strom et al., 2007) and mesozooplankton (Halvorsen et al., 2001; Leising et al., 2005; Nielsen, 1991; Takahashi et al., 2008) as important grazers of bloom worldwide. Copepods are thought to be the major grazers of phytoplankton, particularly when abundant and when the phytoplankton assemblage consists of larger cells (Bautista et al., 1992).

An algal bloom typically undergoes several distinct phases in its temporal course including initiation, growth, maintenance and senescence (Assmy and Smetacek, 2012). Copepod grazing can have different impact on the different phases of the bloom. It has
been suggested that the point at which grazing exerts the most control over the progression of a phytoplankton bloom is in its initial stage, when phytoplankton cell numbers are low (Uye, 1986). Buskey et al. (1997) suggested that a disruption of grazers may aid in the initiation of algal blooms. An ecosystem model demonstrated that copepod grazing can control the timing of the end of the spring diatom bloom in the Northwestern Pacific Ocean (Fujii et al., 2002). A nutrient-phytoplankton-zooplankton (NPZ) model parameterized for the Oyashio regions (Pacific Ocean) predicted that the grazing pressure was an important factor controlling the magnitude and the duration of the spring bloom. (Saito et al., 2002). Takahashi et al. (2008) further confirmed these implications with grazing rates estimated with gut fluorescence for the copepod community and also demonstrated that copepod grazing potentially terminated the phytoplankton bloom. The importance of grazing in controlling artificially induced phytoplankton blooms like in mesoscale iron-enrichment experiments have also been reported in the literature. For example, Tsuda et al. (2007) emphasized that the copepod community grazed large portion of the phytoplankton bloom during the Subarctic Pacific Iron Experiment for Ecosystem Dynamics Study II (SEEDS II), because the seasonal increase of the copepod biomass was synchronized with that of primary production. Bottle experiments carried out in the high nutrient low chlorophyll (HNLC) regions also demonstrated the role of copepod grazers both in preventing the accumulation of large phytoplankton cells by direct feeding and enhancing the increase of small phytoplankton cells by consumption of their microzooplankton predators (Dagg et al., 2009, 2006; Liu et al., 2005b).

The response of the grazers to the bloom is subject to many factors which can vary over temporal and spatial scales. The characteristics of food environment, i.e., phytoplankton composition, can be crucial in determining the extent of grazing on the
spring bloom (e.g., Bautista et al., 1992; Sautour et al., 1996). Some studies concluded that the size of the copepod population at the time of the bloom is crucial in determining the level of grazing. Slow rate of population increase of small neritic copepod species and the time delay between the spring increase in phytoplankton and peak in copepod abundance has been suggested to be the main reason why only a small proportion of the bloom in the North Sea is likely to be grazed (Nielsen and Richardson, 1989; Roff et al., 1988). In contrast, Gowen (1999) concluded that the timing of maximum abundance of small copepod species in the western Irish Sea in relation to the spring bloom was the most important factor in determining the role of these copepod species during the bloom.

Although the literature on copepod grazing is vast, there is no consensus about grazer impact on primary production and the result of studies carried out in different marine environments globally are contradictory. While some studies concluded that copepods grazed daily most of the primary production during upwelling and bloom events (Huskin et al., 2001; Pakhomov and Perissinotto, 1997), others showed that losses due to copepod grazing are of minor importance during blooms following upwelling phenomena (Barquero et al., 1998; Bode et al., 2003; Halvorsen et al., 2001; Head et al., 1999; Morales et al., 1991). As per the study conducted to investigate the feeding habits and grazing rates of the ontogenetically migrating copepods (go through different developmental stages and mature as they migrate) in the Oyashio region of the northwestern Pacific Ocean, the copepod community did not graze down the spring phytoplankton bloom, even though it had a significant impact on microbial food web (Kobari et al., 2010). These controversial results highlight the complexity of trophic interactions and call for more studies on the key grazers and phytoplankton community at regional scale.
1.5 Aims of the thesis

The objective of the present study was to i) investigate the role of copepod grazing in the phytoplankton bloom dynamics in a typical Mediterranean coastal environment and ii) evaluate whether and how copepod grazing can impact phytoplankton blooms.

In the Gulf of Naples (Tyrrhenian Sea, Western Mediterranean), the phyto- and mesozooplankton communities have been studied extensively thanks to the regular sampling program *MareChiara* (http://szn.macisteweb.com), which started in 1984 at a fixed coastal station that is part of the international network of the Long Term Ecological Research sites (st. LTER-MC). As a result there is a fairly good understanding of their population structure, diversity, distribution and seasonal dynamics (e.g., Casotti *et al.*, 2000; Mazzocchi and Ribera d’Alcalà, 1995; Mazzocchi *et al.*, 2011, 2012; Modigh, 2001; Ribera d’Alcalà *et al.*, 2004; Zingone *et al.*, 2010).

In the very diversified copepod assemblage (Mazzocchi *et al.*, 2012), four key calanoid species together comprise nearly 46% (1984-2006) of total group abundance and peak in succession from the spring to the autumn, i.e., *Acartia clausi* and *Centropages typicus* (June-July), *Paracalanus parvus* (July-August), and *Temora stylifera* (September-October). Due to their ample annual occurrence and notable numerical dominance in respective seasons, these four populations interact with different co-occurring phytoplankton communities. These interactions can be important but variable during the different phases of the phytoplankton time course, including the development and demise of the blooms. However, to date, the effect of copepod grazers on the blooms in the Gulf of Naples is unknown.

A species-based approach has been chosen with the focus on the four above mentioned calanoid species and the bloom-forming diatoms. These species, due to their
prevalence, can be considered as key species for predator-prey interactions at the study site. Outcomes of such interactions are directly related to the characteristic species-specific traits of both the predators and prey, namely, the morphological properties of the prey or feeding habits, modes and motion behaviour of the predators. Analysis of bulk communities cannot unveil the species-specific interactions and can mask subtle but significant processes that occur at the individual level. Identification of key organisms and their function is therefore crucial to eventually understand the processes that are likely to govern the functioning of the ecosystems.

The diatoms species chosen for this study are *Chaetoceros socialis*, *Leptocylindrus aporus*, *Leptocylindrus danicus* and *Pseudo-nitzschia calliantha*. These species vary in size and morphology (shape and type of occurrence, i.e., single celled or colonial) and occur as the most abundant species throughout the year in the Gulf of Naples. These species are the dominant ones during the bloom periods as well as most common during the non-bloom periods (Ribera d’Alcalà *et al.* 2004; Sarno and Zingone, unpublished).

Towards the primary objective of the thesis, grazing experiments were carried out to estimate copepod grazing rates at bloom and non-bloom concentrations in presence of monospecific and mixed diets with the four selected bloom-forming diatom species. The hypothesis driving this part of the work was that the target copepod species show species-specific differences in the feeding rates and selectivity when feeding under two very different food concentrations (Chapter 3).

Copepods are known to exhibit differences in their feeding modes depending upon the prey and food environment. Given the morphological difference in the diatoms species, it is possible that the feeding behaviour of copepods changes when feeding on
different diatoms. In order to test this hypothesis, the small-scale feeding behaviour of individual copepods was recorded with a high-speed camera and analysed (Chapter 4).

The role of each of the target copepod species on the bloom dynamics in terms of grazing impact at st. LTER-MC was estimated based on the rates from the grazing experiments and the phytoplankton and zooplankton abundances recorded in the period 1984-2009 (Chapter 5).

Eventually, the copepod species-specific feeding rates, selectivity, feeding behaviour and grazing impact in coastal phytoplankton dynamics has been commented in an integrated discussion (Chapter 6).
CHAPTER 2

Study site and target species
2.1 The *MareChiara* plankton time series at st. LTER-MC

The Long Term Ecological Research station *MareChiara* (st. LTER-MC) is a fixed site located two nautical miles from the shore (40°48.5′N, 14°15′E) in the Gulf of Naples (GoN), on the south-west coast of Italy (Ribera d’Alcalà *et al.*, 2004). The GoN is a wide and deep embayment (170 m average depth, 900 km² area) with the inner shelf area strongly coupled with the offshore waters of the Southern Tyrrhenian Sea (Western Mediterranean) (Carrada *et al.*, 1980). As a result, it has hydrological features typical of both oligotrophic systems (in its offshore area) and eutrophic coastal zones (Ribera d’Alcalà *et al.*, 2004). The inner part of the gulf is characterized by a high temporal and spatial variability for physical, chemical and biological parameters (Ribera d’Alcalà *et al.*, 1989; Zingone *et al.*, 1990).

![Figure 2.1 Map of the Gulf of Naples (Western Mediterranean Sea) with the sampling site (st. MC) of the LTER-MC time series.](image)

*Figure 2.1* Map of the Gulf of Naples (Western Mediterranean Sea) with the sampling site (st. MC) of the LTER-MC time series.
Sampling has been carried out at the study site fortnightly from 1984 to 1991 and weekly from 1995 to date to analyze various physical, chemical and biological parameters. The mean seasonal cycle of temperature in surface layer of the water column (0-10 m) shows a minimum and maximum monthly averages in March (14 ± 1°C) and August (26 ± 1.5°C), respectively. The annual cycle of temperature drives the time course of water column stratification at the seasonal scale. Stratification due to the seasonal cycle of cooling and warming can be represented as the depth of the mixed layer starting in April and completely disrupted from December onwards. The GoN also experiences a lateral advection of fresher water from the coast frequently resulting in a decrease in surface salinity leading to a sharp halocline. As a consequence of this terrestrial runoff, a pronounced pycnocline is frequently recorded also in winter in an otherwise homogeneous water column. In stratified water column, low salinity waters float at the surface thereby enhancing the water column stability and reducing the mixed layer depth (Ribera d’Alcalá et al., 2004).

In case of the plankton, the analysis of the data from this long-term time series has not only provided information on the taxonomy, distribution and abundance of species but has also depicted the general patterns in the seasonal evolution of both phytoplankton (Ribera d’Alcalá et al., 2004; Zingone et al., 1995, 2010) and zooplankton (Mazzocchi and Ribera d’Alcalá, 1995; Mazzocchi et al., 2011, 2012). Details on the sampling and analytical methods have been published elsewhere (e.g., Mazzocchi et al., 2011; Ribera d’Alcalá et al., 2004).

In brief, the chl $a$ concentration was determined from water samples collected with Niskin bottles at selected depths and analyzed with a spectrophotometer. Phytoplankton samples were taken at the surface (0.5 m) with a Niskin bottle and were fixed with neutralized formaldehyde. Cell identification and counts were performed.
using an inverted microscope after sedimentation of variable volumes of seawater (1-100 ml), depending on cell concentration. Phytoplankton biomass was indirectly derived from cell abundance through the measurement of cell biovolume and carbon content. Biovolumes were measured approximating the cell shape of each species to geometric solids. Carbon content was estimated from mean cell biovolume using the formulas by Menden-Deuer and Lessard (2000). Mesozooplankton samples were collected using a 200 μm mesh Nansen net with vertical hauls taken from 50 m depth to the surface. Samples were immediately fixed after collection and preserved in 4% formaldehyde-seawater solution and were later analyzed at a dissecting microscope for species identification and counts (Mazzocchi et al., 2011).

In the following, the synthetic average patterns of chl $a$ and phyto-and zooplankton seasonal variability are shown for the period 1984-2009 as elaborated for this thesis with the aim of presenting the main features of plankton communities at the study site. These seasonal patterns are integrated with other qualitative and quantitative information acquired during previous studies focused on different periods of the LTER-MC time series

### 2.1.1 Chlorophyll $a$

The average annual cycle of surface (0-10 m) and integrated (0-60 m) chlorophyll $a$ (chl $a$) in the period 1984-2009 showed a slight increase in the surface concentration from January onward, followed by an annual major peak during March-May, decreasing through June-August and a new increase in September-October, followed by a subsequent decrease in November-December (Fig. 2.2). The biomass observed in May-June (late spring) and September (summer), when the water column is highly stratified, was confined to surface layers. In October (autumn), the increase in the integrated chl $a$ is due to the deepening of the mixed layer from disruption of thermal stratification. As a
result of the different vertical distribution of the biomass, the annual pattern of integrated chl $a$ values differed from that observed at the surface, with less remarkable seasonal variability (Fig. 2.2).

![Figure 2.2](image)

**Figure 2.2** Seasonal cycles of surface (0-10 m) and integrated (0-60 m) chlorophyll $a$ (monthly averages) at st. LTER-MC during the years 1984-2009.

### 2.1.2 Phytoplankton

The total phytoplankton abundance started increasing from February-March and reached the highest peak in May (Fig. 2.3A). A secondary peak occurred in August after which the abundance decreased till the minimum value at the end of the year. The total phytoplankton biomass also followed a pattern similar to the abundance, reaching the peak in May. Following the peak, the values started decreasing gradually through autumn and were lowest during the winter (Fig. 2.3B). Diatoms contributed to the bulk of phytoplankton biomass throughout the year (Fig. 2.3), except during June (late spring), when dinoflagellates and phytoflagellates (not shown in the figure) were equally important. Occurrence of winter blooms, mainly caused by small flagellates and small non-colonial diatoms have also been reported (Zingone et al., 2010).
Figure 2.3 Annual cycle (monthly average) of total phytoplankton and total diatoms in terms of A) abundance and B) carbon and C) % contribution of target diatom species to total
Diatoms are represented by a high number of species (~150) at st. LTER-MC (Sarno and Zingone, personal communication), a few of them constituting the bulk of the group, and show a regular succession pattern (Fig. 2.3C). Species such as *Thalassiosira* spp., *Thalassionema* spp., *Chaetoceros* spp., *Leptocylindrus* spp., *Skeletonema* spp., *Pseudo-nitzschia* spp. occur throughout the year, although in variable abundance (based on present analysis of the dataset provided by Sarno and Zingone).

The late winter-early spring bloom communities in the years 1984-2009 were dominated by large colonial diatoms including several *Chaetoceros* spp., *Pseudo-nitzschia delicatissima* and *Thalassionema bacillaria*, and phytoflagellates. From the onset of the stratification throughout the summer, the colonial diatom species were substituted by small-sized ones, often in a non-colonial stage (*Skeletonema pseudocostatum*, *C. tenuissimus*, *C. socialis*). Intense phytoflagellate blooms, and an increase in dinoflagellate abundance were also recorded in this period of the year. The coccolithophore *Emiliania huxleyi* occasionally contributed to summer blooms. In autumn, several spring and summer diatoms showed a second peak (Ribera d’Alcalà *et al.*, 2004).

Based on the annual cycle of chl *a* (Fig. 2.2), phytoplankton abundance (Fig. 2.3A) and biomass (Fig. 2.3B), three main periods of phytoplankton development corresponding different stages of to phytoplankton blooms could be identified during an average annual cycle:

1) Spring (March - June)
2) Summer (July- September)
3) Autumn (October-November)
During these periods, the phytoplankton composition and abundance changes extensively over a temporal scale and there can be periods of low and high cell concentrations resembling a pre-bloom or bloom condition respectively (Table 2.1).

Table 2.1 Phytoplankton carbon concentration corresponding to the average of highest and lowest total phytoplankton carbon values for different periods of phytoplankton peak at st. LTER-MC. These highest and the lowest values were considered as “bloom” and “non-bloom” concentrations respectively and included all values more than and less than the threshold value of 400 µg C (spring), 250 µg C (summer) and 80 µg C (autumn). Values are reported as avg.± stdev.

<table>
<thead>
<tr>
<th>Season</th>
<th>Months</th>
<th>Bloom carbon conc. (µg C L⁻¹)</th>
<th>Non-bloom carbon conc. (µg C L⁻¹)</th>
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<tr>
<td>Spring</td>
<td>March-June</td>
<td>812.8±244.9</td>
<td>127.1±62.8</td>
</tr>
<tr>
<td>Summer</td>
<td>July-September</td>
<td>462.6±135.7</td>
<td>47.8±27.2</td>
</tr>
<tr>
<td>Autumn</td>
<td>October-December</td>
<td>159.3±179.1</td>
<td>14.3±12.6</td>
</tr>
</tbody>
</table>

2.1.3 Mesozooplankton

The mesozooplankton community at st. LTER-MC showed the highest values of abundance during mid-spring (April-May) and summer (July-September), while the lowest values were in winter (December and January) (Fig. 2.4 A). Copepods were the most important group during the whole year, except summer (Fig. 2.4 A). Their annual peak occurred in spring and was mainly due to the high abundance of *Acartia clausi* and *Centropages typicus* (Fig. 2.4 B). The summer peak observed for the total mesozooplankton was equally due to copepods and cladocerans (Ribera d' Alcalà et al., 2004).

The four copepods, *Acartia clausi*, *Centropages typicus*, *Paracalanus parvus* and *Temora stylifera* are considered key species of the zooplankton assemblages at st. LTER-MC (Mazzocchi et al., 2012). On a mean annual basis, these species (adults and juveniles) comprised 47.41% (±6.03%) of total copepod abundance in the period 1984-
2009 (Mazzocchi and Di Capua, unpublished). These four calanoids showed a clear succession in their seasonal cycle, with *A. clausi* and *C. typicus* reaching the peak abundance in spring, followed by *P. parvus* in summer and *T. stylifera* in autumn (Fig. 2.4 B).

The highest abundance of total population was for *A. clausi*, which showed two peaks during early spring (April) and late-spring (June) (Fig. 2.4 B). The abundance of *C. typicus* total population started to increase from April onwards, reaching its peak in June (Fig. 2.4 B). The total population of *P. parvus* and *T. stylifera* reached their peak abundance in August and October respectively (Fig. 2.4 B). The contribution of females to the total population of all the copepod species was always less than 32% throughout the year, except *P. parvus* whose females accounted for more than 70% of the population (Fig 2.4 C).
Chapter 2. Study site and target species

Figure 2.4 Annual cycle (average monthly abundance) of A) total mesozooplankton and total copepods, B) total population (f+m+j) of the four target copepod species and C) % contribution of adult females of target copepod species to total copepod population for the period 1984-2009 at st. LTER-MC. Bars indicate standard error.
2.2 Ecological and biological traits of the target species

2.2.1 Diatoms

2.2.1.1 Chaetoceros socialis (Lauder)

*Chaetoceros socialis* is one of the dominant diatoms species in the GoN, and constitutes the bulk of total diatom abundance on a mean annual basis. *C. socialis* is common during the spring bloom as colonies as well as during summer and autumn when it occurs in a non-colonial stage (Ribera d'Alcalà et al., 2004). Single cells are cylindrical with an oval or circular base and they appear square with rounded corners, when seen in girdle view. They have an apical axis of 10-18 µm and a single central chloroplast. Several cells form curved chains, which in turn group together to form spherical colonies. Each cell has 4 setae emerging from inside the valve margins, one longer than the other three. The longer setae unite with its homologue in the adjacent cell giving the colony a fan shape. The extremities of the longer setae weave together and converge at the centre, to form more or less spherical colonies (website of Mediterranean plankton, via www.szn.it).

*Figure 2.5* Chaetoceros socialis colony. Scale bar, 10 µm. (Picture courtesy of D. Sarno).
2.2.1.2 *Leptocylindrus aporus* (French III and Hargraves 1986)

The species of the genus *Leptocylindrus* are considered widespread, mostly coastal, with numerous records of their occurrence and often being a major contributor of the diatom blooms. Ribera d’Alcalà *et al.* (2004) reported specimens assignable to *L. danicus* throughout the year, with two distinct bloom phases, a conspicuous one in summer and a modest one in autumn. A recent study of this genus in the GoN has identified five species with distinct or partially overlapping periods of occurrence over the year (Nanjappa *et al.*, 2013). *L. aporus* is apparently the species responsible for the remarkable summer blooms in the GoN, and it is also found in autumn (Nanjappa *et al.*, 2013).

Single cells are cylindrical in shape and measure 4–7.5 µm in diameter, solitary or forming short filamentous chains. Solitary cells of this species are common in both cultures and summer field samples. Chains can contain up to 24 cells (Nanjappa *et al.*, 2013).

![Leptocylindrus aporus colony](image)

**Figure 2.6** *Leptocylindrus aporus* colony. Scale bar, 10 µm. (Picture courtesy of D. Nanjappa)
2.2.1.3 *Leptocylindrus danicus* (Cleve)

The recent study by Nanjappa *et al.*, (2013) has shown that the different species belonging to the genus *Leptocylindrus* at the study site are present with different cell concentrations and have different seasonal patterns. Of all the congeneric *L. danicus* has a wider distribution in time, spanning from mid-summer through late autumn.

![Figure 2.7](image.png)

**Figure 2.7** *Leptocylindrus danicus* colonies with multiple cells. Scale bar, 10 µm and 50 µm (Picture courtesy of D. Nanjappa)

Single cells are cylindrical and measure 3–13 µm in diameter and 22–75 µm in length. It is a colonial diatom, forming filamentous chains with multiple cells joined by their valve. Field samples of the species are often composed of more than one hundred cells (Nanjappa *et al.*, 2013).

2.2.1.4 *Pseudo-nitzschia calliantha* (Hasle)

*Pseudo-nitzschia calliantha* belongs to the *Pseudo-nitzschia pseudodelicatissima* complex, which includes several species which are morphologically similar, but show a high degree of genetic diversity (Amato *et al.*, 2007; Lundholm *et al.*, 2003). Species belonging to the *Pseudo-nitzschia pseudodelicatissima* complex are known to be widely distributed in temperate to cold waters in the European Seas including the
Mediterranean Sea, where they form regular blooms (Álvarez et al., 2009; Caroppo, 2005; Lundholm et al., 2003). Single cells are 1.1 to 1.9 μm wide and can measure up to 80 μm in length. The cells are characterized by linear valves, tapering only near the apices, which are rounded. The cell frustules are delicate and joined by their ends to form stepped chains.

![Image of Pseudo-nitzschia calliantha 2-celled colony. Scale bar, 20 μm. (Picture by D. Sarno)](image)

**Figure 2.8** *Pseudo-nitzschia calliantha* 2-celled colony. Scale bar, 20 μm. (Picture by D. Sarno)

### 2.2.2 Copepods

#### 2.2.2.1 *Acartia clausi* Giesbrecht, 1889

*Acartia clausi* inhabits coastal waters where it occurs usually among the most abundant zooplankton species (Razouls et al., 2014). In the GoN it starts to increase in abundance in early spring with an extended peak from April to August (Fig. 2.5 A; Mazzocchi et al., 2012). Like most copepods of the sub-temperate and tropical regions, *A. clausi* is a continuous breeder; with the peak of its egg production rates occurring in spring–summer. In the Mediterranean, the copepod is reported to have 4-5 generations annually (Ianora, 1998). *A. clausi* is known to be an omnivorous feeder and exhibits switching behaviour between suspension and ambush-feeding modes depending on food availability (Kiorboe et al., 1996; Gismervik and Andersen, 1997).


2.2.2.2 *Centropages typicus* Kröyer, 1849

*Centropages typicus* is among the most common copepods of the temperate waters and is widely distributed both in neritic (e.g., Estrada *et al.*, 1985; Scotto di Carlo and Ianora, 1983) and offshore waters (e.g., Di Capua and Mazzocchi, 2004). In many coastal areas of the Mediterranean Sea and North Atlantic Ocean, it may account from 10% to 50% of total copepod number and constitute a significant part of the zooplankton biomass (Bonnet *et al.*, 2007; Mazzocchi *et al.*, 2007). In the GoN, its abundance starts increasing from early spring and reaches the major peak in April–June, sometimes followed by an occasional peak in October (Fig. 2.5 C; Di Capua and Mazzocchi, 2004; Mazzocchi and Ribera d’Alcalà, 1995; Ribera d’Alcalà *et al.*, 2004; Mazzocchi *et al.*, 2012).

It is the most fecund copepod species recorded so far in the Mediterranean Sea and North Atlantic Ocean with a very high egg production rate (~34 eggs female$^{-1}$ day$^{-1}$) (Halsband-Lenk *et al.*, 2001; Ianora, 1998; Ianora *et al.*, 1992, 2007). *C. typicus* breeds continuously during the year, even though there may be periods of higher and lower breeding intensity. An inverse relationship exists between individual egg production
rates and adult abundance, with maximum egg production rates from autumn to spring when population abundances were low, and minimum rates in summer, corresponding to an increase in adult female numbers (Ianora et al., 2007).

Under natural conditions, C. typicus is an omnivorous copepod and feed on a variety of organisms ranging from small algae, flagellates, dinoflagellates, eggs and nauplii of other copepods (reviewed by Calbet et al., 2007). It is known to exhibit ambush as well as suspension feeding modes (Cowles and Strickler, 1983; Tiselius and Jonsson, 1990), depending on the characteristics of the prey, with a selective preference for large motile prey (Calbet et al., 2007 and references therein).

Figure 2.10 Centropages typicus female. Total female length ~ 1.4 mm. (Picture courtesy of I. Di Capua).

2.2.2.3 Paracalanus parvus (Claus, 1863)

Paracalanus parvus is a cosmopolitan species and recorded in both oceanic and neritic zones (Razouls et al. 2014) and is one of the dominant copepods recorded in coastal Mediterranean regions (Abdel-Aziz and Dorgham, 2002; Scotto di Carlo and Ianora, 1983). In the GoN, it has a pronounced peak in summer (July-August) often coinciding with the peak of cladoceran Penilia avirostris (Fig. 2.5 E; Mazzocchi et al., 2012; Ribera d’Alcalà et al., 2004).
Chapter 2. Study site and target species

*P. parvus* is a broadcast spawner and has reproduction rates of 30-50 eggs female⁻¹ day⁻¹ (Ianora, 1998). It has a suspension feeding mode and feeds on small prey (Buskey, 1984; Price *et al.*, 1983; Tiselius and Jonsson, 1990). Field and laboratory studies have shown that this species is able to feed on small diatoms, ciliates and flagellates (Kleppel, 1993; Paffenhofer and Orcutt, 1986).

![Figure 2.11 Paracalanus parvus female. Total female length ~ 0.9 mm. (Picture courtesy of L. Di Capua).](image)

**2.2.2.4 Temora stylifera (Dana, 1849)**

*Temora stylifera* is one of the most abundant calanoid copepod species in coastal waters in the Mediterranean Sea. Its annual cycle has been studied in the inner GoN, where the population increases in late June–July, reaching maximum abundance in autumn, followed by a sharp decline in winter (Fig. 2.5 G; Di Capua and Mazzocchi, 2004; Mazzocchi and Ribera d’Alcalà, 1995; Mazzocchi *et al.*, 2006, 2012; Ribera d’Alcalà *et al.*, 2004).

The highest egg production and viability for this species is reported during the period of warmest-stratified water columns in August-October in association with phytoplankton blooms (Carotenuto *et al.*, 2006; Ianora, 1998). The reproductive biology of this species has been extensively studied, particularly, with regard to the effects of
diatoms on its fecundity, hatching success and larval development (Carotenuto et al.,
2006; Ianora et al., 2004; Mazzocchi et al., 2006; Turner et al., 2001).

The feeding ecology of this copepod is less explored, and the information on its
natural diet is scarce. In a grazing study carried out using natural food assemblage in the
northwest Mediterranean Sea, Broglio et al. (2004) reported *T. stylifera* feeding on both
phytoplankton and ciliates. Feeding studies in laboratory indicate that *T. stylifera* is
primarily herbivorous with some degree of omnivory (Paffenhöfer and Knowles, 1980).
The copepod is known to move in a smooth cruising motion which is associated with
creation of feeding currents that enable the copepod to efficiently capture phytoplankton
cells (Paffenhöfer, 1998).

![Figure 2.11 Temora stylifera female. Total female length ~ 1.5 mm. (Picture courtesy of I. Di Capua).](image)

As described above, the seasonal phases and the parallel development of phyto-
and zooplankton communities occur fairly regularly. The target species of copepods and
diatoms described above, due to their ample annual occurrence and their notable
numerical increase, are likely to be involved in predator-prey interactions. The diatom-
specific response of the copepods to the different diatoms can likely affect the
phytoplankton bloom dynamics.
CHAPTER 3.

Feeding rates and selectivity of target copepod species on bloom forming diatoms
3.1 Introduction

Copepods represent a high percentage of the herbivorous and omnivorous biomass in the pelagic ecosystem. In the Mediterranean Sea, copepods represent the major mesozooplankton group both in terms of abundance and biomass and are dominated by small-sized copepod species (Siokou-Frangou et al., 2010). Due to their high numerical abundance, they can have significant impact on the phytoplankton community through grazing. In general, grazing can have at least two, counteracting effects on phytoplankton dynamics: it may reduce algal standing crop, while nutrient regeneration (release of dissolved nutrients from damaged algae during zooplankton feeding) combined with excretion and egestion may stimulate algal growth (e.g., Bergquist and Carpenter, 1986; Moller, 2005; Urban-Rich, 1999).

Natural phytoplankton communities are composed of a highly diverse assemblage of phytoplankton species, and different species of copepods exploit different strategies to utilize them. Feeding in copepods is generally expressed in terms of feeding rates, which vary depending upon the copepod species and the prey. The relationship between food concentration and grazing rate typically follows a saturation curve, where the feeding rates occur over a broad range of food concentration until a critical concentration at which they become constant (Frost, 1972). The value for copepod ingestion rate, which is the amount of food ingested by a specimen in the unit of time, are known to be affected by the concentration, size and biochemical properties of the prey (Bämstedt et al., 2000a; Frost, 1972; Libourel and Michael, 1987; Price and Paffenhöfer, 1986). The feeding response of copepods can be therefore expected to vary depending on extreme high food and with diverse food types. Such a situation is typical during blooms, when the food availability increases several folds higher than the lower background values and the phytoplankton community is dominated by one or a few
species. Controlling a bloom at its onset will require the copepods to feed effectively on the blooming phytoplankton species when they are still at low concentration (Uye, 1986). Quantitative characterization of copepod feeding under different food environments is therefore of primary importance to understand the role of grazers in plankton dynamics.

Several methods can be employed to estimate copepod grazing on a natural plankton assemblage. The most common ones comprise measurements of respiration rates, radio isotope tracer techniques, the gut fluorescence method (Mackas and Bohrer, 1976), and the particle removal method, which compares incubations using natural communities with and without added grazers (Frost, 1972). When used simultaneously, these different methods may yield different results. These differences can be attributed to pigment destruction in guts (Head and Harris, 1996; McLeroy-Etheridge and Mcmanus, 1999; Tirelli and Mayzaud, 1998), which can lead to over estimation of grazing (Durbin and Campbell, 2007), stress due to experimental handling and manipulation of animals (all methods) and "bottle effect" during incubation (i.e., food removal method, Bamstedt et al., 2000b; Roman and Rublee, 1980). The choice of an appropriate method of course depends on the study organism and the objectives. The food removal method, for example, which uses microscopy, is relevant to obtain grazing estimated at the level of single cells or colonies of phytoplankton, which is not possible with other methods. Moreover, information on feeding selectivity, which is an important feature of copepod feeding, can also be obtained.

Copepods are capable of feeding selectively on a wide range of phytoplankton species based on their size (e.g., Cowles, 1979; Frost, 1972; Yang et al., 2010), concentration (Price and Paffenhöfer, 1986; Teegarden et al., 2001; Turner and Tester, 1989), motility (e.g. Jonsson and Tiselius, 1990; Wiadnyana and Rassoulzadegan,
1989), biochemical composition (Paffenhofer et al., 1995) and nutritional value (Cottonne et al., 2001). However, copepods also feed on some prey species or strains more than others despite similarities in size and motility (e.g. Demott, 1989; Teegarden, 1999). In temperate waters, Meyer-Harms et al. (1999) have shown that copepods select microzooplankton prey before and after the diatom spring bloom and graze preferentially on diatoms during the bloom. This type of "prey-switching" can potentially influence the population dynamics of plankton communities (Gismervik and Andersen, 1997; Kiørboe et al., 1996).

Considering the feeding response and selectivity and their possible implications on plankton dynamics, which differ according to the grazers and the prey properties, a species-based approach seems more appropriate to quantify the feeding. Also, estimating the selectivity of a copepod species, for example, when presented with multiple food types can compliment the better understanding of the role of these grazers in the food webs.

The main purpose of this study was to evaluate the feeding response of the target copepods to the bloom-forming diatom species occurring in the GoN. The selected diatoms were offered to the four copepod species during grazing experiments as monospecific diets and mixed diet to evaluate the species-specific feeding response which may impact the blooms. To test the hypothesis that the copepods feed differently in different phases of bloom, experiments were conducted at two food concentrations simulating the bloom and non-bloom conditions in the field.

The specific objectives were 1) to compare the feeding of target copepod species on different bloom forming diatoms in simulated non-bloom and bloom conditions and in monospecific and mixed diets and 2) to determine whether the copepods show any
selectivity for the solitary vs. colonial cells as well as amongst the different diatoms species.

3.2 Materials and methods

3.2.1 Diatom culturing

The four diatom species used as food for copepods in the experiments were isolated from the Gulf of Naples and cultured in the Laboratory of Ecology and Evolution of Plankton. Pseudo-nitzschia calliantha and Chaetoceros socialis were grown in F/2 medium (Guillard and Ryther, 1962), while, Leptocylindrus danicus and Leptocylindrus aporus were grown in K medium (Keller et al., 1987). The K medium includes selenium, nitrate and ammonium, increased chelation, reduced copper, and a moderate level of pH buffering as compared to F/2; it was found to be best suited for growing the diatoms of the genus Leptocylindrus from tests conducted in our laboratory (Nanjappa, 2012). Cultures were maintained in the exponential phase by periodic transfer into 50 ml polystyrene flasks with 30 ml of respective media. The stock cultures for the experiments were grown in 1-4 L flasks in 0.22 μm filtered media kept on shelves in a walk-in cold room under conditions of temperature and light:dark cycle as used in the respective experiments. Illumination on the shelves was provided by white fluorescent tubes and the irradiance ranged from 60 to 100 μmol photons m\(^{-2}\)s\(^{-1}\) as measured with a LICOR LI-185B Quantum Photometer. The flasks were gently swirled (3-4 times a day) to ensure proper mixing.

The growth rate of the diatom species was calculated from their respective growth curves (Table 3.1). The diatom growth rate was estimated by cell counts as

\[ k = \frac{\ln (C_t / C_0)}{t} \]
where $C_t$ and $C_0$ are the concentrations of diatoms at time $t$ and 0, respectively and $t$ is the incubation time. In all experiments, the diatoms were offered as diets to copepods during the exponential growth phase (4-8 days depending on species).

Prior to the experiments, subsamples from each diatom culture were examined under an inverted microscope for determining the cell concentration (cells L$^{-1}$) and measuring the cell size and chain length. The average cell size was calculated by measuring the cell length and width of at least 30 cells; the average cell volume was calculated based on the average size and converted into carbon content according to Menden-Deuer and Lessard (2000).

Table 3.1 Maximum growth rates (d$^{-1}$) of the four diatoms species estimated from cell counts at different temperatures.

<table>
<thead>
<tr>
<th>Diatom species</th>
<th>15°C</th>
<th>18°C</th>
<th>20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaetoceros socialis</td>
<td>1.77±0.17</td>
<td>2.79±0.30</td>
<td>2.87±0.25</td>
</tr>
<tr>
<td>Leptocylindrus aporus</td>
<td>1.38±0.09</td>
<td>1.93±0.02</td>
<td>2.12±0.03</td>
</tr>
<tr>
<td>Leptocylindrus danicus</td>
<td>1.77±0.08</td>
<td>1.28±0.03</td>
<td>1.42±0.04</td>
</tr>
<tr>
<td>Pseudo-nitzschia calliantha</td>
<td>1.56±0.01</td>
<td>1.82±0.34</td>
<td>2.56±0.04</td>
</tr>
</tbody>
</table>

For the estimation of actual per cell carbon content, a known volume (50-100 ml) of diatom culture was vacuum filtered in triplicates onto a 25 mm Whatman GF/F precombusted (450°C, 5h) glass fiber filter. After filtration, the filters were immediately frozen and stored at -20°C until further analysis. Samples were processed within 1–3 months of collection. Filters were fumed with concentrated HCl to remove inorganic C, dried, and analyzed at a Thermo Electron Corporation 1112 SERIES FLASHEA CHN elemental analyzer (Hedges and Stern, 1984). Cyclohexanone-2, 4-dinitrophenyl hydrazone was used as standard.
3.2.2 Copepod collection and acclimation

The copepods used for the experiments were collected from the coastal site st. LTER-MC (40°48.5'N, 14°15'E) in the inner Gulf of Naples by using a 200 µm Nansen net with 5 L non filtering cod end in vertical or oblique tows. In the laboratory, actively swimming adult females of the target copepod species were sorted from the zooplankton assemblage and acclimated for at least 24 hours to the temperature and photoperiod that simulated the in situ conditions. During the acclimation period, females were kept in 5 L glass jars and fed on natural plankton assemblage collected using Niskin bottles at depth of chlorophyll maximum from the same location as the copepods. Only adult females were used for the experiments to reduce the variability that may arise due to possible differences in feeding habits in different juvenile stages.

3.2.3 Experimental setup

Altogether, 20 grazing experiments were carried out, which included, for each of the four copepod species, 16 experiments with monospecific diets (Table 3.2) and 4 experiment with mixed diet (Table 3.3). The experimental conditions of temperature and light were chosen to simulate the average conditions recorded in situ during each respective season, which in turn were obtained from the environmental data collected at st. LTER-MC during the weekly sampling for the long-term time series. All the experiments with Acartia clausi and Centropages typicus were at 15°C and 12D:12N cycle, while those with Paracalanus parvus were conducted at 20°C at 14D:10N cycle and those with Temora stylifera were conducted at 18° C and 10D:14N cycle.

In all experiments, the diatoms were offered at two concentrations simulating bloom and non-bloom concentrations observed during the respective seasons at st. LTER-MC. The diatom concentrations for the two conditions were calculated starting
from the phytoplankton carbon measured at st. LTER-MC in the years 1984-2009 (dataset provided by Diana Sarno) during each season. The bloom and the non-bloom carbon values corresponded to the average of all the highest and lowest values of total phytoplankton carbon during each season, respectively (see Table 2.1 in Chapter 2). Food suspensions for the experiments were prepared by first counting the phytoplankton cell concentration and carbon per cell in the stock cultures and then adjusting the concentrations by dilution with 0.7 μm (GF/F) filtered seawater, to obtain the predetermined carbon concentrations for bloom and non-bloom conditions. For the experiments with mixed diet, each of the four diatom species used in the mixed suspension contributed equally to the total carbon.

For each grazing experiment, the food suspension was poured in 1 L glass bottles, with three replicates for start, control (without copepods) and treatment (with copepods) (18 bottles in total). Healthy and active females were sorted randomly from the acclimation beakers and added in number of 4-6 to each of the treatment bottles. The number of individuals was chosen based on the peak abundances of total copepods (3-8 copepods L⁻¹) recorded at st. LTER-MC in 1984-2009 (dataset provided by MG Mazzocchi). The bottles were carefully closed (sealed with plastic film to prevent air bubble formation) and mounted onto a slow moving plankton wheel (0.2 rpm). Experiments were run in the same conditions of temperature and light at which the copepods had been acclimated.

At the beginning of the experiments (t₀), sub-samples were collected from the start bottles for the cell counts (250 ml) and chl a analysis (250-500 ml). At the end of the experiments, the copepods from the treatment bottles were gently sorted with a wide bore pipette and collected in a graduated crucible along with a small volume of water (10-30 ml) and their general conditions, extent of gut fullness and mortality, if any, was
Chapter 3. Feeding rates and selectivity

reported. Sub-samples were collected from the control and treatment bottles for cell counts (250 ml) and chl $a$ analysis (250-500 ml). The remaining water from the treatment bottles was sieved through 10 $\mu$m mesh to collect the fecal pellets for later qualitative observations.

The copepods recovered from each experiment (from bloom as well as non-bloom experimental bottles) were pooled (25-30 individuals) and rinsed quickly with distilled water to remove the salt. The interstitial water was removed and the copepod pellet was placed into a pre-weighed tin cup and dried in an oven (60°C) for 24 h. These samples were then weighed on a microbalance to measure the individual biomass as dry mass (µg ind.$^{-1}$). The same copepod samples were later analyzed at the CHN Elemental Analyzer to measure the individual carbon content as µgC ind.$^{-1}$.

3.2.4 Cell counts and chlorophyll $a$ analysis

All sub-samples collected at the start and end of the experiments for cell counts were preserved with Lugol’s iodine solution (1%) and stored in cool and dark place until counted. Variable volumes of samples (2.97-50 ml), depending on cell concentration, were allowed to sediment in settling chambers and cells were counted at an inverted microscope (Utermöhl, 1958). In case the cell concentration was high, (i.e, in the bloom samples), the sample was settled in two replicates settling chambers and the cells were counted in replicate slides. Counts were performed by categories, from “single cell” to 2,3......n celled colonies either in random fields or 2-4 transects of the settling chamber at 20X or 40X magnifications. The selection of the random fields was done using pre-established fixed co-ordinates on a mechanical stage with a Vernier scale, which ensured a homogeneous pattern in counting. When counting the cells in transects, at least one vertical and one horizontal transect were considered. In both
cases, all the cells and colonies within the grid were counted. If a cells or colony crossed the top or the right side border of the grid, they were included in the counts, while, the cells or colonies which crossed the bottom and left border of the grid were excluded from the counts. In case a large colony had only 1 cell inside the grid, all the cells that belonged to that colony, even if they were beyond the border (top or right) were counted. This method ensured that each cell was counted properly as it was actually present in the sample as a single cell or a colony. Moreover, this method ensured consistency and statistical balance.

The sub samples for chl $a$ analysis were filtered on Whatman GF/F filters (25 mm diameter) and then extracted in 90% neutralized acetone at -20°C for 24 h. The analyses were performed using a SHIMADZU (mod. RF-5301PC) spectrofluorometer following Holm-Hansen et al. (1965). The instrument was calibrated with a solution of pure chl $a$, extracted from Anacystis nidulans (Sigma).

3.2.5 Feeding parameters, selectivity and daily ration

The clearance rate ($F$, ml cop.$^{-1}$ d$^{-1}$) and ingestion rate ($I$, cells cop.$^{-1}$ d$^{-1}$) were calculated based upon the microscope counts and the chl $a$ concentrations ($F$) according to Frost’s equations (Frost, 1972). In case of the mixed diets, the feeding rates were calculated on each diatom species separately as well as on the total cells. Ingestion rates in term of carbon were calculated only on the positive values.

Food selectivity was calculated on single and colonial cells in case of monospecific diets and on different diatom species in case of mixed diet, only when the ingestion rates were positive. For the monospecific diets, the prey preference was evaluated in two steps. In the first step, the selectivity on single or multi-celled colonies (with increasing number of cells forming the colony) was determined using Chesson’s
index of selectivity \( \alpha \) (Chesson, 1978, 1983), which was considered the best index suited to the present data set among the numerous ones proposed in the literature. Alpha \( \alpha_i \) relates the ingestion rates on the different food types to their availability according to the formula:

\[
\alpha_i = \frac{r_i/p_i}{\sum_{i=1}^{n} r_i/p_i}
\]

where \( r_i \) is the relative abundance of prey type \( i \) in the diet, \( p_i \) is the relative abundance of prey type \( i \) in the environment and \( n \) is the number of food types available. No selection occurs when \( \alpha_i = 1/n \) and food items are fed upon in the same proportions as their availability, when \( \alpha_i > 1/n \) selection is positive; when \( \alpha_i < 1/n \), selection is negative.

In the successive step, the cells were grouped into different size categories as <30 \( \mu \)m, 30-100 \( \mu \)m, 100-200 \( \mu \)m and >200 \( \mu \)m and the selectivity was evaluated using the (Vanderploeg and Scavia, 1979a, 1979b) selectivity index \( (E^*) \). This index had been suggested to be most suitable in cases where the food types are not equally abundant in a food complex (as reviewed by Lechowicz, 1982). In this case, \( E^* \) allowed the comparison of prey preference for each of the copepod species on the different size categories at two different concentrations of the monospecific diets. This index, is nothing but the Chesson’s \( \alpha_i \) (Chesson, 1978), normalized to numbers of available prey,

\[
E^* = \frac{[W_i - (1/n)]}{[W_i + (1/n)]}
\]

where \( W_i \) is Chesson’s (1978) coefficient:

\[
W_i = \frac{(r_i/p_i)}{\sum (r_i/p_i)}
\]

and \( n \) is the number of different categories, \( r_i \) is the relative proportion in the diet of a food category and \( p_i \) is the proportion of the same category in the environment. The
Chapter 3. Feeding rates and selectivity

Index can vary from –1 (greatest negative selection) to +1 (greatest positive selection) and 0 refers to neutral preference. Selectivity in the mixed diet was determined using the Chesson’s index of selectivity (α) (Chesson, 1978).

Of the two indices used, the Chesson’s α can be interpreted as the relative contribution of each food type to the diet if all food types were equally abundant and it does not vary with food density unless consumer behaviour also changes with food density. Therefore it can be used to detect such changes in behaviour. Values of α obtained from other experiments can be related and compared in a biologically reasonable way to provide a unifying link between clearance rates, attack rates, and consumer preference (Chesson, 1983). The Vanderploeg and Scavia Electivity index E* gives a measure of the predator’s perception of food’s value as a function of both its abundance and the abundance of other food types present in the environment. Both the indices can be used for rank order comparison of rates under different food environments.

The daily ration was expressed as the percentage contribution of the food carbon ingested per day to copepod body carbon (% body C day\(^{-1}\)). Daily ration was calculated only when the ingestion rates in terms of carbon were positive.

3.2.6 Statistical analysis

The grazing rates are presented as the average of the three replicate values (± stdev). In cases where one out of the three replicates has a negative value, only the positive values were considered for calculating the average. Similarly, one out of the three replicates has a positive value, the two negative values were considered for calculating the average. In cases where all the replicate grazing rates were negative, the values were not censored i.e. the negative values were neither deleted nor set to zero. In order to meet
the basic requirement of homogeneity of variances and normality, the values of F and I calculated based upon the cell counts were square root transformed before applying the statistical tests. One-way ANOVA was applied to compare the four diets at each of the two conditions. Student’s t-test was used to compare ingestion on one diet at bloom vs. non-bloom concentration.

The differences were considered significant at 0.01<p<0.05(*), 0.001<p<0.01 (**), and p<0.001 (***)). All the statistical analyses were carried out using Microsoft Excel and GraphPad Prism 4.03 software (GraphPad Software, San Diego, CA, USA),
Table 3.2. Characteristics of the diatom species offered as monospecific diets during the grazing experiments with the four copepod species *Acartia clausi*, *Centropages typicus*, *Paracalanus parvus* and *Temora stylifera*.

<table>
<thead>
<tr>
<th>Date</th>
<th>Diatom species</th>
<th>No. of cells in the colonies</th>
<th>Cell length (µm)</th>
<th>Cell biovolume (µm³)</th>
<th>Cell C content (from biovolume) (pg C)</th>
<th>Cell C content (from CHN) (pg C)</th>
<th>Cell N content (from CHN) (pg N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14/06/2012</td>
<td><em>C. socialis</em></td>
<td>1-17</td>
<td>7.68±1.54</td>
<td>317.03±0.55</td>
<td>30.92±7.29</td>
<td>15.13±0.41</td>
<td>3.03±0.15</td>
</tr>
<tr>
<td>03/06/2012</td>
<td><em>L. aporus</em></td>
<td>1-7</td>
<td>21.23±3.85</td>
<td>403.97±0.70</td>
<td>37.39±0.22</td>
<td>24.54±2.18</td>
<td>4.91±0.32</td>
</tr>
<tr>
<td>31/05/2012</td>
<td><em>L. danicus</em></td>
<td>1-16</td>
<td>31.38±9.12</td>
<td>2283.13±6.01</td>
<td>152.32±1.23</td>
<td>88.64±7.49</td>
<td>18.50±0.68</td>
</tr>
<tr>
<td>25/06/2012</td>
<td><em>P. calliantha</em></td>
<td>1-5</td>
<td>21.25±2.15</td>
<td>66.41±6.71</td>
<td>8.64±0.71</td>
<td>10.34±0.48</td>
<td>1.87±0.09</td>
</tr>
<tr>
<td>24/03/2012</td>
<td><em>C. socialis</em></td>
<td>1-10</td>
<td>7.50±1.51</td>
<td>331.38±66.61</td>
<td>31.75±5.21</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>22/05/2012</td>
<td><em>L. aporus</em></td>
<td>1-4</td>
<td>27.03±4.49</td>
<td>530.83±88.22</td>
<td>46.57±6.29</td>
<td>55.66±0.01</td>
<td>9.91±1.31</td>
</tr>
<tr>
<td>01/04/2012</td>
<td><em>L. danicus</em></td>
<td>1-18</td>
<td>38.31±7.39</td>
<td>1330.3±463.63</td>
<td>97.6±26.82</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>10/04/2012</td>
<td><em>P. calliantha</em></td>
<td>1-4</td>
<td>22.33±2.66</td>
<td>69.79±8.30</td>
<td>8.99±0.87</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>03/08/2012</td>
<td><em>C. socialis</em></td>
<td>1-8</td>
<td>10.00±1.89</td>
<td>698.77±183.64</td>
<td>58.31±12.81</td>
<td>16.29±0.90</td>
<td>3.27±0.26</td>
</tr>
<tr>
<td>19/07/2012</td>
<td><em>L. aporus</em></td>
<td>1-12</td>
<td>26.25±4.39</td>
<td>515.48±86.27</td>
<td>45.47±6.10</td>
<td>40.56±8.74</td>
<td>7.41±1.73</td>
</tr>
<tr>
<td>26/07/2012</td>
<td><em>L. danicus</em></td>
<td>1-15</td>
<td>34.63±9.01</td>
<td>1455.46±381.17</td>
<td>105.73±22.29</td>
<td>126.42±8.80</td>
<td>22.58±0.90</td>
</tr>
<tr>
<td>12/07/2012</td>
<td><em>P. calliantha</em></td>
<td>1-5</td>
<td>19.29±1.528</td>
<td>60.77±4.78</td>
<td>7.99±0.52</td>
<td>7.04±0.29</td>
<td>1.27±0.04</td>
</tr>
<tr>
<td>10/02/2012</td>
<td><em>C. socialis</em></td>
<td>1-21</td>
<td>8.00±0.00</td>
<td>402.18±0.00</td>
<td>37.25±0.00</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>19/12/2011</td>
<td><em>L. aporus</em></td>
<td>1-10</td>
<td>32.71±6.36</td>
<td>497.37±3.43</td>
<td>44.26±0.78</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>27/11/2011</td>
<td><em>L. danicus</em></td>
<td>1-18</td>
<td>36.69±7.93</td>
<td>3615.15±15.16</td>
<td>221.3±2.61</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>04/12/2011</td>
<td><em>P. calliantha</em></td>
<td>1-11</td>
<td>49.33±2.066</td>
<td>98.67±4.13</td>
<td>11.92±0.40</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Experimental dates are strictly in the seasonal order as shown in Fig. 2.4
Table 3.3. Characteristics of the diatom species offered as mixed diets during the grazing experiments with the four copepod species *Acartia clausi, Centropages typicus, Paracalanus parvus* and *Temora stylifera*.

<table>
<thead>
<tr>
<th>Date</th>
<th>Diatom species</th>
<th>No. of cells in the colonies</th>
<th>Cell length (µm)</th>
<th>Cell biovolume (µm³)</th>
<th>Cell C content (from biovolume) (pg C)</th>
<th>Cell C content (from CHN) (pg C)</th>
<th>Cell N content (from CHN) (pg N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20/06/2012</td>
<td><em>A. clausi</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>C. socialis</em></td>
<td>1-12</td>
<td>7.92±1.79</td>
<td>353.88±140.90</td>
<td>33.16±11.15</td>
<td>18.65±0.39</td>
<td>3.41±0.08</td>
</tr>
<tr>
<td></td>
<td><em>L. aporus</em></td>
<td>1-5</td>
<td>21.00±4.61</td>
<td>412.39±90.52</td>
<td>38.02±6.58</td>
<td>43.61±.78</td>
<td>7.69±0.35</td>
</tr>
<tr>
<td></td>
<td><em>L. danicus</em></td>
<td>1-13</td>
<td>30.94±5.76</td>
<td>1366.95±254.70</td>
<td>100.23±15.24</td>
<td>90.19±9.27</td>
<td>19.50±1.52</td>
</tr>
<tr>
<td></td>
<td><em>P. calliantha</em></td>
<td>1-5</td>
<td>20.36±1.66</td>
<td>63.62±5.18</td>
<td>8.35±0.55</td>
<td>14.83±1.26</td>
<td>3.14±0.38</td>
</tr>
<tr>
<td>26/05/2012</td>
<td><em>C. typicus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>C. socialis</em></td>
<td>1-13</td>
<td>7.92±1.79</td>
<td>389.74±195.08</td>
<td>36.32±14.40</td>
<td>28.14±0.69</td>
<td>5.50±0.15</td>
</tr>
<tr>
<td></td>
<td><em>L. aporus</em></td>
<td>1-7</td>
<td>24.77±5.30</td>
<td>486.47±104.04</td>
<td>43.47±7.46</td>
<td>29.98±1.31</td>
<td>5.43±0.30</td>
</tr>
<tr>
<td></td>
<td><em>L. danicus</em></td>
<td>1-9</td>
<td>37.78±7.65</td>
<td>2646.89±746.17</td>
<td>171.73±39.27</td>
<td>104.60±14.62</td>
<td>19.78±3.03</td>
</tr>
<tr>
<td></td>
<td><em>P. calliantha</em></td>
<td>1-3</td>
<td>20.00±2.04</td>
<td>62.50±6.38</td>
<td>8.23±0.68</td>
<td>10.29±0.31</td>
<td>1.44±0.03</td>
</tr>
<tr>
<td>20/12/2012</td>
<td><em>P. parvus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>C. socialis</em></td>
<td>1-7</td>
<td>8.50±1.61</td>
<td>464.43±218.51</td>
<td>41.17±15.98</td>
<td>22.13±1.41</td>
<td>3.92±0.00</td>
</tr>
<tr>
<td></td>
<td><em>L. aporus</em></td>
<td>1-8</td>
<td>23.91±5.32</td>
<td>469.59±104.48</td>
<td>42.09±7.57</td>
<td>29.95±1.34</td>
<td>5.59±0.45</td>
</tr>
<tr>
<td></td>
<td><em>L. danicus</em></td>
<td>1-10</td>
<td>32.96±9.65</td>
<td>2846.98±754.04</td>
<td>181.24±38.80</td>
<td>57.40±6.94</td>
<td>11.73±0.93</td>
</tr>
<tr>
<td></td>
<td><em>P. calliantha</em></td>
<td>1-3</td>
<td>48.65±5.16</td>
<td>152.04±16.14</td>
<td>16.91±1.46</td>
<td>5.83±0.16</td>
<td>1.18±0.02</td>
</tr>
<tr>
<td>01/08/2012</td>
<td><em>T. stylifera</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>C. socialis</em></td>
<td>1-10</td>
<td>7.50±0.00</td>
<td>401.68±120.39</td>
<td>37.00±8.84</td>
<td>16.98±4.48</td>
<td>2.96±0.83</td>
</tr>
<tr>
<td></td>
<td><em>L. aporus</em></td>
<td>1-8</td>
<td>29.79±5.48</td>
<td>500.91±163.17</td>
<td>44.17±11.85</td>
<td>39.65±2.62</td>
<td>6.05±0.53</td>
</tr>
<tr>
<td></td>
<td><em>L. danicus</em></td>
<td>1-13</td>
<td>29.26±10.03</td>
<td>1165.98±409.26</td>
<td>87.59±24.71</td>
<td>66.98±17.83</td>
<td>12.02±3.87</td>
</tr>
<tr>
<td></td>
<td><em>P. calliantha</em></td>
<td>1-3</td>
<td>28.96±2.49</td>
<td>180.99±15.56</td>
<td>19.49±1.35</td>
<td>25.61±3.82</td>
<td>2.91±0.11</td>
</tr>
</tbody>
</table>

Experimental dates are strictly in the seasonal order as shown in Fig. 2.4.
3.3 Results

3.3.1 Monospecific diets

3.3.1.1 Initial concentrations

The diatom concentration at the beginning of the experiments with monospecific diets reflected the seasonal differences recorded at sea, with values decreasing from spring to summer and autumn (Table 3.4).

In bloom conditions, the average initial concentrations of cells, chl \( a \), and carbon ranged from \( 0.48\pm0.04\times10^6 \) cells L\(^{-1}\) to \( 180.63\pm27.88\times10^6 \) cells L\(^{-1}\), from \( 3.05\pm0.11 \) μg chl \( a \) L\(^{-1}\) to \( 37.9\pm0.6 \) μg chl \( a \) L\(^{-1}\), from \( 48.81\pm3.60 \) μg C L\(^{-1}\) to \( 1549.79\pm239.1 \) μg C L\(^{-1}\), respectively (Table 3.4). In non-bloom conditions, the average initial concentrations of cells, chl \( a \) and carbon ranged from \( 0.05\pm0.01\times10^6 \) cells L\(^{-1}\) to \( 24.91\pm1.96\times10^6 \) cells L\(^{-1}\), from \( 0.32\pm0.01 \) μg chl \( a \) L\(^{-1}\) to \( 7.35\pm0.17 \) μg chl \( a \) L\(^{-1}\) and from \( 4.71\pm0.16 \) μg C L\(^{-1}\) to \( 213.79\pm16.83 \) μg C L\(^{-1}\), respectively (Table 3.4).

It is worth noting that the initial carbon concentrations measured from the CHN analysis differed from the expected values that were established before the experiments based on the cell biovolume and used for calculating the concentration of cultures to be added to attain the required carbon concentration at the start of the experiment (see Table 3.4). For example, in the experiment with \( C. \) typicus fed on a monospecific diet of \( P. \) calliantha, the final carbon concentration (1549.79 μg C L\(^{-1}\)) was nearly twice than the expected concentration of 800 μg C L\(^{-1}\). In another case of \( T. \) stylifera offered a monospecific diet of \( L. \) danicus, the final carbon concentration (48.81 μg C L\(^{-1}\)) was much less than the expected value of 160 μg C L\(^{-1}\). These differences between the expected and actual values highlight the overestimates based on the biovolume
estimates with respect to the instrumental analysis of cell carbon content. These differences were due to the different size and shape of the diatom species used as diets (see Table 3.2 and 3.3).

The mean carbon content of diatoms calculated based on the biovolume varied greatly for each experiment. For example, the biovolume of *L. danicus* varied between 1336 µm³ during the monospecific diet experiment with *T. stylifera* to 3516 µm³ in the experiment with *C. typicus*. This was due to the changing cell size of the diatoms, which were maintained as cultures for over a year while the experiments were conducted. It is a known fact the diatoms undergo cell-size reduction during cell division and as a result the mean cell size of a population decreases over successive generations (MacDonald-Pfizer rule) (MacDonald, 1986; Pfizer, 1986). Also, variable conditions of light and temperature at which the diatoms were grown could have contributed to the differences in the cell size of each diatom in different experiments. During this study, the attempt was to use the same strains of diatom cultures for all the experiments in order to eliminate any bias that may arise due to the chemical properties of different strains. However, in case of *P. calliantha*, new strain had to be used due to the extreme size reduction of the original strain.
Table 3.4 Initial concentrations of cells, chlorophyll $a$ and carbon content (average ± stdev) during the experiments at bloom and non-bloom conditions. The lowest and highest values of each parameter are highlighted in bold for each of the two conditions.

<table>
<thead>
<tr>
<th>Copepods</th>
<th>Diatoms</th>
<th>Cell concentration ((10^6 \text{ cells L}^{-1}))</th>
<th>Chlorophyll $a$ concentration ((\mu g \text{ chl } a \text{ L}^{-1}))</th>
<th>Carbon concentration ((\mu g \text{ C} \text{ L}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bloom</td>
<td>Non-bloom</td>
<td>Bloom</td>
</tr>
<tr>
<td>A. clausi (spring)</td>
<td>C. socialis</td>
<td>21.78±2.06</td>
<td>2.96±0.24</td>
<td>27.20±0.2</td>
</tr>
<tr>
<td></td>
<td>L. aorus</td>
<td>21.46±1.23</td>
<td>3.33±0.27</td>
<td>27.82±1.06</td>
</tr>
<tr>
<td></td>
<td>L. danicus</td>
<td>5.29±0.36</td>
<td>0.87±0.07</td>
<td>36.29±1.40</td>
</tr>
<tr>
<td></td>
<td>P. calliantha</td>
<td>104.07±13.40</td>
<td>13.45±1.36</td>
<td>37.9±0.60</td>
</tr>
<tr>
<td>C. typicus (spring)</td>
<td>C. socialis</td>
<td>29.06±0.91</td>
<td>3.38±0.14</td>
<td>20.37±0.54</td>
</tr>
<tr>
<td></td>
<td>L. aorus</td>
<td>15.92±0.06</td>
<td>2.16±0.07</td>
<td>22.24±0.60</td>
</tr>
<tr>
<td></td>
<td>L. danicus</td>
<td>7.32±1.31</td>
<td>1.01±0.58</td>
<td>34.78±0.58</td>
</tr>
<tr>
<td></td>
<td>P. calliantha</td>
<td>180.63±27.88</td>
<td>24.92±1.96</td>
<td>25.95±2.06</td>
</tr>
<tr>
<td>P. parvus (summer)</td>
<td>C. socialis</td>
<td>10.85±1.56</td>
<td>1.62±0.24</td>
<td>4.98±0.77</td>
</tr>
<tr>
<td></td>
<td>L. aorus</td>
<td>15.44±0.72</td>
<td>1.87±0.10</td>
<td>18.35±0.29</td>
</tr>
<tr>
<td></td>
<td>L. danicus</td>
<td>4.61±0.71</td>
<td>0.47±0.03</td>
<td>11.79±0.48</td>
</tr>
<tr>
<td></td>
<td>P. calliantha</td>
<td>46.40±5.77</td>
<td>5.80±0.17</td>
<td>11.03±0.60</td>
</tr>
<tr>
<td>T. stylifera (autumn)</td>
<td>C. socialis</td>
<td>3.61±0.42</td>
<td>0.31±0.01</td>
<td>3.92±0.06</td>
</tr>
<tr>
<td></td>
<td>L. aorus</td>
<td>2.19±0.12</td>
<td>0.24±0.04</td>
<td>3.05±0.11</td>
</tr>
<tr>
<td></td>
<td>L. danicus</td>
<td>0.48±0.04</td>
<td>0.05±0.00</td>
<td>3.66±0.64</td>
</tr>
<tr>
<td></td>
<td>P. calliantha</td>
<td>4.21±0.32</td>
<td>0.64±0.08</td>
<td>5.14±0.33</td>
</tr>
</tbody>
</table>
Chapter 3. Feeding rates and selectivity

3.3.1.2 Clearance rates

The clearance rates of the four copepod species on the different monospecific diets at two concentrations are shown in Table 3.5. At bloom concentrations, the positive clearance rates ranged between 14.05±1.88 ml cop⁻¹ d⁻¹ for *P. parvus* on *L. danicus* diet to 85.41±0.46 ml cop⁻¹ d⁻¹ for *T. stylifera* on *L. aporus* diet. The comparison of the clearance rates on the different diatom diets showed no significant difference for *C. typicus* and *A. clausi*, which had negative F for all diets. In contrast, the clearance rates for *P. parvus* were significantly different for all the diets, with positive rates on *C. socialis* (41.22±6.69 ml cop⁻¹ d⁻¹) and *L. danicus* (14.05±1.88 ml cop⁻¹ d⁻¹) and negative rates on *P. calliantha* and *L. aporus*. *T. stylifera* also showed significantly different rates on the different diatom diets.

At non-bloom concentrations, the clearance rate was highest for *T. stylifera* on *L. danicus* (272.08±33.38 ml cop⁻¹ d⁻¹) and lowest for *A. clausi* on *P. calliantha* (22.79 ml cop⁻¹ d⁻¹). The only case of negative clearance rates at non-bloom concentrations was for *C. typicus* on *P. calliantha*. All the copepod species except *A. clausi* showed significantly different clearance rates on the different diatom diets. *T. stylifera* had significantly higher clearance rates on the colonial *L. danicus*, while *C. typicus* and *P. parvus* had the highest clearance rates on *C. socialis* (54.72±7.45 ml cop⁻¹ d⁻¹) and *P. calliantha*, respectively (105.76±23.28 ml cop⁻¹ d⁻¹).

In the comparison between bloom and non-bloom concentrations, all the four copepod species showed significant differences in the clearance rates of at least one diatom diet (Table 3.5). The F values were significantly higher at non-bloom than at bloom concentrations for *A. clausi* only on *L. danicus*, and for *C. typicus* on *C. socialis* and *L. aporus*. *P. parvus* had significantly higher F on all monospecific diets except on *C. socialis*, while, *T. stylifera* showed significant difference in F only on *C. socialis* and *L. danicus*. 
Table 3.5. Clearance rates (F) of the target copepod species on different monospecific diatom diets at two different concentrations (bloom, non-bloom) calculated on cell concentrations (average± stdev). In cases where one out of the three replicates had a negative value, only the two positive values were considered for calculating the average and stdev is not reported. Similarly, when one out of three replicates was positive, the two negative values were averaged. The p values are reported significant at 0.01<p< 0.05(*), 0.001<p<0.01 (**), and p< 0.001 (***, bold).

<table>
<thead>
<tr>
<th>Copepods</th>
<th>Diatom species</th>
<th>F (cells) (ml copepod⁻¹ d⁻¹)</th>
<th>significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bloom</td>
<td>Non-bloom</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. socialis</td>
<td>-36.49</td>
<td>25.13</td>
<td>ns</td>
</tr>
<tr>
<td>L. aporus</td>
<td>-14.63±15.11</td>
<td>31.04</td>
<td>ns</td>
</tr>
<tr>
<td>L. danicus</td>
<td>-70.34±12.00</td>
<td>23.15±19.67</td>
<td>**</td>
</tr>
<tr>
<td>P. calliantha</td>
<td>-51.64±19.38</td>
<td>22.79</td>
<td>ns</td>
</tr>
<tr>
<td>A. clausi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. socialis</td>
<td>-19.67±11.82</td>
<td>54.72±7.45</td>
<td>**</td>
</tr>
<tr>
<td>L. aporus</td>
<td>-36.72±16.99</td>
<td>31.65±6.02</td>
<td>***</td>
</tr>
</tbody>
</table>
| L. danicus | -9.31±9.55     | 49.71±51.32                  |                 *
| C. typicus |                |                               |                    |                    |
| C. socialis | 41.22±6.69     | 52.17±20.66                  | ns                 |
| L. aporus | -17.42         | 34.56±0.00                   |                 *
| L. danicus | 14.05±1.88     | 68.31±2.66                   | ***                |
| P. calliantha | -21.56       | 105.76±23.28                 | **                 |
| P. parvus |                |                               |                    |                    |
| C. socialis | 57.81          | 163.97±0.84                  | **                 |
| L. aporus  | 85.41±0.46     | 85.58±23.69                  | ns                 |
| L. danicus | 50.82±19.69    | 272.08±33.38                 | **                 |
| P. calliantha | 82.99±33.54 | 112.90±11.17                 | ns                 |
| T. stylifera |                |                               |                    |                    |
| L. danicus | 50.82±19.69    | 272.08±33.38                 | **                 |
| P. calliantha | 82.99±33.54 | 112.90±11.17                 | ns                 |
| p         |                |                               |                    | ns **              |
Chapter 3. Feeding rates and selectivity

3.3.1.3 Ingestion rates

At bloom concentrations, the positive ingestion rates in terms of cells ranged between 2.6±0.9x10^4 cells cop⁻¹ d⁻¹ for T. stylifera fed on L. danicus to 50.1±7.2x10^4 cells cop⁻¹ d⁻¹ for P. parvus fed on C. socialis (Table 3.6). All the copepod species except A. clausi showed significant difference in the ingestion rates on the different diatom diets (Table 3.6 and Fig. 3.1 A, C, E, G). The ingestion rates of A. clausi and C. typicus were all negative. P. parvus had positive ingestion rates on C. socialis and L. danicus and negative ingestion rates on L. aporus and P. calliantha (Table 3.6). T. stylifera had the highest ingestion rates on P. calliantha followed by L. aporus, C. socialis and L. danicus.

At non-bloom concentrations, the ingestion rates were highest for P. parvus on P. calliantha (59.3±9.1x10^4 cells cop⁻¹ d⁻¹) and lowest for T. stylifera on L. danicus (0.9±0.1x10^4 cells cop⁻¹ d⁻¹). The comparison among ingestion rates on the four diatom diets showed that they differed significantly for all copepods (Table 3.6 and Fig. 3.1 B, D, F, H). The highest ingestion rates were always on P. calliantha for all the copepod species except for C. typicus, which had the highest ingestion on C. socialis. The lowest ingestion rates for all copepod species were always on the colonial L. danicus. It is worth noting that the only negative ingestion rates in the non-bloom conditions were for C. typicus fed on P. calliantha (Table 3.6).

The comparison between the two concentrations gave different results for the four copepod species (Table 3.6). A. clausi showed significant difference in ingestion only on L. danicus. C. typicus did not show any significant difference in the ingestion rates in bloom and non-bloom concentrations. The ingestion rates of P. parvus were significantly different at the two concentrations for all the diatom diets. T. stylifera fed at significantly higher rates at bloom concentration on all the diatom diets.
### Table 3.6. Ingestion rates (I) of the target copepod species on different diatom diets at two different concentrations (bloom, non-bloom) calculated on cell and carbon concentrations (avg. ± stdev). In cases where one out of the three replicates had a negative value, only the two positive values were considered for calculating the average and stdev is not reported. The p values are reported significant at 0.01<p<0.05(*), 0.001<p<0.01 (**), and p<0.001 (***, bold).

<table>
<thead>
<tr>
<th>Copepods</th>
<th>Diatom species</th>
<th>I (10^4 cells cop⁻¹ d⁻¹)</th>
<th>p</th>
<th>I (μg C cop⁻¹ d⁻¹)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bloom</td>
<td>Non-bloom</td>
<td>Bloom</td>
<td>Non-bloom</td>
<td>Bloom</td>
</tr>
<tr>
<td>C. socialis</td>
<td>-107.82</td>
<td>11.05</td>
<td>ns</td>
<td>negative</td>
<td>2.53</td>
</tr>
<tr>
<td>L. aporus</td>
<td>-39.17±41.69</td>
<td>11.25</td>
<td>ns</td>
<td>negative</td>
<td>2.76</td>
</tr>
<tr>
<td>L. danicus</td>
<td>-36.58±7.17</td>
<td>2.57±2.18</td>
<td>*</td>
<td>negative</td>
<td>2.28±1.94</td>
</tr>
<tr>
<td>P. calliantha</td>
<td>-627.97±259.87</td>
<td>28.43</td>
<td>ns</td>
<td>negative</td>
<td>2.94</td>
</tr>
<tr>
<td>A. clausi</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. Typicalis</td>
<td>-71.13±45.23</td>
<td>21.78±2.32</td>
<td>ns</td>
<td>negative</td>
<td>6.35±0.68</td>
</tr>
<tr>
<td>L. aporus</td>
<td>-55.04±27.66</td>
<td>5.86±1.04</td>
<td>ns</td>
<td>negative</td>
<td>2.32±0.41</td>
</tr>
<tr>
<td>L. danicus</td>
<td>-9.98±10.35</td>
<td>5.30±5.01</td>
<td>ns</td>
<td>negative</td>
<td>5.39±5.10</td>
</tr>
<tr>
<td>P. calliantha</td>
<td>-301.15±216.69</td>
<td>-57.09±42.02</td>
<td>ns</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>P. parvus</td>
<td>50.05±7.19</td>
<td>7.87±2.58</td>
<td>***</td>
<td>8.15±1.17</td>
<td>1.28±0.42</td>
</tr>
<tr>
<td>L. aporus</td>
<td>-37.84±2.40</td>
<td>7.99±3.53</td>
<td>**</td>
<td>-15.34±0.97</td>
<td>3.24±1.43</td>
</tr>
<tr>
<td>L. danicus</td>
<td>7.48±0.95</td>
<td>3.04±0.10</td>
<td>**</td>
<td>9.45±1.20</td>
<td>3.84±0.12</td>
</tr>
<tr>
<td>P. calliantha</td>
<td>-86.47±31.65</td>
<td>59.27±9.03</td>
<td>**</td>
<td>-6.09±2.23</td>
<td>4.18±0.64</td>
</tr>
<tr>
<td>P. stylifera</td>
<td>21.71</td>
<td>5.15±0.02</td>
<td>**</td>
<td>6.33</td>
<td>1.50±0.01</td>
</tr>
<tr>
<td>L. aporus</td>
<td>16.59±0.58</td>
<td>1.91±0.44</td>
<td>***</td>
<td>6.56±0.23</td>
<td>0.75±0.17</td>
</tr>
<tr>
<td>L. danicus</td>
<td>2.56±0.87</td>
<td>0.89±0.09</td>
<td>*</td>
<td>2.61±0.89</td>
<td>0.91±0.09</td>
</tr>
<tr>
<td>P. calliantha</td>
<td>34.78±10.99</td>
<td>6.36±0.51</td>
<td>**</td>
<td>4.72±1.49</td>
<td>0.86±0.07</td>
</tr>
</tbody>
</table>
Chapter 3. Feeding rates and selectivity

Figure 3.1 Ingestion rates of (A, B) *A. clausi*, (C, D) *C. typicus*, (E, F) *P. parvus* and (G, H) *T. stylifera* on different monospecific diatom diets measured as cell (A, C, E, G) and as carbon (B, D, F, H) at bloom and non-bloom concentration. Alphabetical (a, b, c) and numerical (1, 2, 3) symbols above the bars indicate statistical significance by post hoc pairwise comparison at bloom and non-bloom concentrations respectively. The bars with the same symbols represent no significant difference in grazing rates. Bars indicate average of three replicates ± stdev. Only positive values are shown.
Chapter 3. Feeding rates and selectivity

The ingestion rates in terms of carbon at the bloom concentration were highest for *P. parvus* fed on *L. danicus* (9.5 ± 1.2 μg C cop⁻¹ d⁻¹) and lowest for *T. stylifera* fed on *L. danicus* (2.6 ± 0.9 μg C cop⁻¹ d⁻¹) (Table 3.6). The rates were not calculated for *A. clausi* and *C. typicus* as the ingestion rates in terms of cells were negative. At the non-bloom concentrations, the highest rate was measured for *C. typicus* fed on *C. socialis* (6.4 ± 0.2 μg C cop⁻¹ d⁻¹), and the lowest rate for *T. stylifera* fed on *L. aporus* (0.8 ± 0.2 μg C cop⁻¹ d⁻¹) (Table 3.6). *P. parvus* and *T. stylifera* had significantly different carbon ingestion rates on the different diatom diets at bloom and non-bloom concentrations (Table 3.6; Fig. 3.1 F, H). Also, these two copepod species had higher carbon ingestion on all the diatom diets at bloom concentration than at non-bloom concentration (Table 3.6).

3.3.2 Mixed diet

3.3.2.1 Initial concentrations

In bloom conditions, the average initial concentrations of cells, chl *a*, and carbon ranged from 3.9 × 10⁶ cells L⁻¹ to 47.9 ± 6.3 × 10⁶ cells L⁻¹, from 4.3 ± 1.0 μg chl *a* L⁻¹ to 26.5 ± 0.7 μg chl *a* L⁻¹, from 140.4 ± 3.0 μg C L⁻¹ to 712.3 ± 75.6 μg C L⁻¹ μg C L⁻¹, respectively (Table 3.7). In non-bloom conditions, the average initial concentrations of cells, chl *a* and carbon ranged from 0.5 ± 1.0 × 10⁶ cells L⁻¹ to 6.3 ± 1.2 × 10⁶ cells L⁻¹, from 2.2 ± 1.0 μg chl *a* L⁻¹ to 3.5 ± 1.0 μg chl *a* L⁻¹ and from 18.7 ± 2.9 to 94.7 ± 10.6 μg C L⁻¹, respectively. Size differences among species (see Table 3.3) affected their respective contribution to the diet composition in terms of cell numbers and carbon (Table 3.7).
Table 3.7 Initial concentrations of cells, chlorophyll $a$ and carbon content average ± stdev) during the experiments at bloom and non-bloom conditions. The lowest and highest values of each parameter are highlighted in bold for each of the two conditions. The p values are reported significant at $0.01<p<0.05(*)$, $0.001<p<0.01 (**)$, and $p<0.001$ (***, bold).

<table>
<thead>
<tr>
<th>Copepods</th>
<th>Diatom Species</th>
<th>Cell concentration ($10^6$ cells L$^{-1}$)</th>
<th>Chlorophyll $a$ concentration (µg chl $a$ L$^{-1}$)</th>
<th>Carbon concentration (µg L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bloom</td>
<td>Non-bloom</td>
<td>Bloom</td>
</tr>
<tr>
<td><strong>A. clausi</strong> (spring)</td>
<td>C. socialis</td>
<td>6.47±1.00</td>
<td>0.92±0.15</td>
<td>167.95±25.97</td>
</tr>
<tr>
<td></td>
<td>L. aporus</td>
<td>2.85±0.10</td>
<td>0.62±0.06</td>
<td>116.28±4.26</td>
</tr>
<tr>
<td></td>
<td>L. danicus</td>
<td>0.81±0.15</td>
<td>0.10±0.00</td>
<td>76.93±14.13</td>
</tr>
<tr>
<td></td>
<td>P. calliantha</td>
<td>37.80±5.62</td>
<td>4.69±0.99</td>
<td>315.64±46.94</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>47.93±6.32</td>
<td>6.33±1.20</td>
<td>676.80±57.76</td>
</tr>
<tr>
<td><strong>C. typicus</strong> (spring)</td>
<td>C. socialis</td>
<td>5.98±0.87</td>
<td>0.77±0.17</td>
<td>168.20±24.46</td>
</tr>
<tr>
<td></td>
<td>L. aporus</td>
<td>4.21±0.37</td>
<td>0.60±0.20</td>
<td>126.10±11.18</td>
</tr>
<tr>
<td></td>
<td>L. danicus</td>
<td>0.97±0.19</td>
<td>0.14±0.07</td>
<td>101.01±19.86</td>
</tr>
<tr>
<td></td>
<td>P. calliantha</td>
<td>30.82±2.93</td>
<td>3.93±0.81</td>
<td>317.01±30.09</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>41.97±4.14</td>
<td>5.44±0.93</td>
<td>712.33±75.58</td>
</tr>
<tr>
<td><strong>P. parvus</strong> (summer)</td>
<td>C. socialis</td>
<td>1.02±0.25</td>
<td>0.34±0.21</td>
<td>40.92±9.91</td>
</tr>
<tr>
<td></td>
<td>L. aporus</td>
<td>1.68±0.09</td>
<td>0.56±0.03</td>
<td>60.65±3.20</td>
</tr>
<tr>
<td></td>
<td>L. danicus</td>
<td>0.43±0.11</td>
<td>0.12±0.03</td>
<td>52.52±12.94</td>
</tr>
<tr>
<td></td>
<td>P. calliantha</td>
<td>6.54±0.69</td>
<td>1.79±0.07</td>
<td>127.64±13.54</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>9.68±0.91</td>
<td>2.81±0.01</td>
<td>281.72±31.86</td>
</tr>
<tr>
<td><strong>T. stylifera</strong> (autumn)</td>
<td>C. socialis</td>
<td>0.77±0.11</td>
<td>0.11±0.02</td>
<td>28.28±3.99</td>
</tr>
<tr>
<td></td>
<td>L. aporus</td>
<td>1.04±0.10</td>
<td>0.12±0.02</td>
<td>43.51±4.11</td>
</tr>
<tr>
<td></td>
<td>L. danicus</td>
<td>0.38±0.07</td>
<td>0.06±0.01</td>
<td>29.32±5.76</td>
</tr>
<tr>
<td></td>
<td>P. calliantha</td>
<td>1.74±0.13</td>
<td>0.24±0.02</td>
<td>39.33±2.84</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>3.93±0.03</td>
<td>0.50±0.09</td>
<td>140.43±3.03</td>
</tr>
</tbody>
</table>
3.3.2.2 Clearance rates

At bloom concentrations, the total clearance rates in the mixed diets ranged between 10.8±12.1 ml cop⁻¹d⁻¹ for *P. parvus* and 50.3±3.0 ml cop⁻¹d⁻¹ for *T. stylifera* (Table 3.8). The clearance rates on the different diatom species in each diet were significantly different for *A. clausi* and *T. stylifera*. *A. clausi* had highest rates on *P. calliantha* and negative rates on the other diatoms. *T. stylifera* had significantly higher rates on the colonial diatoms *L. danicus* and *C. socialis* than on the non-colonial *L. aporus* and *P. calliantha*. *C. typicus* and *P. parvus* did not show any significant difference in the clearance rates on the different diatom species (Table 3.8).

At non-bloom concentrations, the rates ranged between 24.6 ml cop⁻¹d⁻¹ for *C. typicus* to 88.7±24.3 ml cop⁻¹d⁻¹ for *T. stylifera* (Table 3.8). *A. clausi* and *T. stylifera* had significantly higher clearance rates on *P. calliantha* (103.1 ml cop⁻¹ d⁻¹) and *L. danicus* (233.3±53.5 ml cop⁻¹ d⁻¹), respectively (Table 3.8). *C. typicus* and *P. parvus* did not show any significant difference in the clearance rates on the different diatom species.

The comparison of total clearance rates at the two concentrations showed that the rates were significantly higher at non-bloom concentration for *P. parvus* and *T. stylifera* while they did not show any significant difference in *A. clausi* and *C. typicus* (Table 3.8). When considering the single diatom species, *P. parvus* had negative clearance rates only on *P. calliantha* at the bloom concentration, and positive rates on all the diatom species at the non-bloom concentration. *T. stylifera* had significantly higher clearance rates on *C. socialis* and *L. danicus* diatoms than on *L. aporus* and *P. calliantha* at both concentrations (Table 3.8).
Table 3.8. Clearance rates (F) of the different copepod species on mixed diatom diets at two different concentrations calculated based on cell concentrations of each diatom species and total cells. The F values are presented as the average of the three replicate values (± stdev). In cases where one out of the three replicates had a negative value, only the two positive values were considered for calculating the average and stdev is not reported. Similarly, when one out of three replicates was positive, the two negative values were averaged. The p values are reported significant at 0.01<p<0.05(*), 0.001<p<0.01 (**), and p<0.001 (***, bold).

<table>
<thead>
<tr>
<th>Copepod species</th>
<th>Diatom species</th>
<th>Clearance rates (cells) (ml copepod⁻¹ d⁻¹)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bloom</td>
<td>Non-bloom</td>
</tr>
<tr>
<td>C. socialis</td>
<td></td>
<td>-3.53</td>
<td>32.58</td>
</tr>
<tr>
<td>L. aporus</td>
<td></td>
<td>-0.57</td>
<td>25.5±11.66</td>
</tr>
<tr>
<td>L. danicus</td>
<td></td>
<td>-30.81</td>
<td>-70.8±33.30</td>
</tr>
<tr>
<td>P. calliantha</td>
<td></td>
<td>47.2±24.84</td>
<td>103.12</td>
</tr>
<tr>
<td>All cells</td>
<td></td>
<td>35.25±19.87</td>
<td>71.03</td>
</tr>
<tr>
<td>C. socialis</td>
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<td>17.3±19.73</td>
<td>-44.2±33.14</td>
</tr>
<tr>
<td>L. aporus</td>
<td></td>
<td>15.21</td>
<td>-45.41</td>
</tr>
<tr>
<td>L. danicus</td>
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<td>-8.4±9.88</td>
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</tr>
<tr>
<td>P. calliantha</td>
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<td>17.7±29.17</td>
<td>37.7±7.32</td>
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<td>24.63</td>
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<tr>
<td>C. socialis</td>
<td></td>
<td>35.6±18.13</td>
<td>96.4±24.48</td>
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<tr>
<td>L. aporus</td>
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<td>26.13</td>
<td>54.4±19.70</td>
</tr>
<tr>
<td>L. danicus</td>
<td></td>
<td>30.3±19.71</td>
<td>80.69</td>
</tr>
<tr>
<td>P. calliantha</td>
<td></td>
<td>-2.62</td>
<td>64.9±5.80</td>
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<tr>
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<td></td>
<td>10.8±12.11</td>
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<tr>
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<td>63.7±16.69</td>
<td>174.0±11.43</td>
</tr>
<tr>
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<td>31.6±4.58</td>
<td>58.0±29.49</td>
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<tr>
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<td></td>
<td>154.2±24.29</td>
<td>233.2±53.47</td>
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<td>P. calliantha</td>
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<td>33.5±12.57</td>
<td>34.4±61.69</td>
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<tr>
<td>All cells</td>
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<td>50.2±3.01</td>
<td>88.7±24.26</td>
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</tbody>
</table>

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3.3.2.3 Ingestion rates

At the bloom concentrations, the highest ingestion rate was measured for *A. clausi* (172.1±29.7x10^4 cells cop^1 d^1) and the lowest one for *P. parvus* (12.8±14.0x10^4 cells cop^1 d^1) (Table 3.9). Only *A. clausi* showed significant difference in the ingestion rates on each diatom species that contributed to the mixed diet, with significantly higher rates on *P. calliantha* and negative ingestion rates on *C. socialis* and *L. danicus* (Fig. 3.2, A and Table 3.9). In terms of carbon, the highest ingestion rate was measured in *A. clausi* (14.4±7.1 μg C cop^1 d^1) and the lowest one in *P. parvus* (5.1±2.7 μg C cop^1 d^1) (Table 3.9). Only *A. clausi* and *T. stylifera* showed significantly differences in their carbon ingestion rates on the four diatom species. In case of *A. clausi*, *P. calliantha* contributed all the carbon (100%) to the total carbon ingested (Fig. 3.2 B). In the diet of *T. stylifera*, *L. danicus* accounted for most (46%) of the total carbon ingested, followed by *C. socialis*, *L. aporus* and *P. calliantha* (Fig. 3.2 H).

At the non-bloom concentrations, the highest and lowest ingestion rates were measured in *A. clausi* (29.7±27.5x10^4 cells cop^1 d^1) and in *T. stylifera* (4.8±1.1x10^4 cells cop^1 d^1), respectively (Table 3.9). In terms of carbon, the ingestion rates were highest for *P. parvus* (5.9±0.3 μg C cop^1 d^1) and lowest for *T. stylifera* (1.7±0.5 μg C cop^1 d^1). All the copepod species except *T. stylifera* showed significant difference in ingestion of the different diatoms, with highest ingestion rates on *P. calliantha* in terms of both cells and carbon (Fig 3.2 and Table 3.9).

The ingestion rates in terms of both cells and carbon were not significantly different in bloom vs. non-bloom concentrations, for all copepod species, with the only exception of *T. stylifera* that ingested significantly more at bloom than non-bloom concentrations (p<0.001) (Table 3.9).
Table 3.9. Ingestion rates (I) of target copepod species on mixed diatom diet at two different concentrations calculated in terms of cells and carbon. The I values are presented as the average of the three replicate values (± stdev). In cases where one out of the three replicates had a negative value, only the two positive values were considered for calculating the average and stdev is not reported. The p values are reported significant at 0.01<p<0.05(*), 0.001<p<0.01 (**), and p<0.001 (***, bold).

<table>
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<tr>
<th>Copepod species</th>
<th>Diatom species</th>
<th>I (10^4 cells cop^-1 d^-1)</th>
<th>p</th>
<th>I (µg C cop^-1 d^-1)</th>
<th>p</th>
</tr>
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<tbody>
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<td></td>
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<td>Non-bloom</td>
<td>Bloom vs. Non-bloom</td>
<td>Bloom</td>
</tr>
<tr>
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<td>1.96±0.08</td>
<td>ns</td>
<td>-0.70±0.51</td>
</tr>
<tr>
<td><em>L. aporus</em></td>
<td></td>
<td>-3.03±5.63</td>
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<td>-0.09±0.03</td>
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<tr>
<td><em>L. danicus</em></td>
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<td>-1.26±0.64</td>
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<td>-3.01±0.06</td>
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<tr>
<td><em>P. calliantha</em></td>
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<tr>
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<td>172.13±92.55</td>
<td>29.71±27.48</td>
<td>14.38±7.14</td>
<td>3.87±2.26</td>
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<td><em>C. socialis</em></td>
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<td><em>C. socialis</em></td>
<td>5.12±2.29</td>
<td>3.57±0.73</td>
<td>2.04±0.91</td>
<td>1.43±0.29</td>
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<td><em>L. aporus</em></td>
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<td>1.38±0.46</td>
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<td>1.82±0.45</td>
<td>0.67±0.02</td>
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<tr>
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<td>0.82±0.34</td>
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<td>0.34±0.14</td>
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<td>4.00±0.60</td>
<td>0.52±0.33</td>
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<tr>
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<td>1.74±0.55</td>
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* ns, ** p<0.01, *** p<0.001.
Figure 3.2. Ingestion rates of copepods on each diatom species in terms of cells (A, C, E, G) and carbon (B, D, F, H) expressed as % of total positive ingestion in mixed diet at bloom and non-bloom concentrations.
3.3.3 Daily ration

3.3.3.1 Monospecific diets

The daily rations for the copepods on the different monospecific diets are shown in Table 3.10. At bloom concentration, *P. parvus* had extremely high daily ration when fed on *C. socialis* (498.2±71.6 % body C day⁻¹) and *L. danicus* (419.2±53.1 % body C day⁻¹). In case of *T. stylifera*, the daily ration ranged from 25.7±8.7 % body C day⁻¹ (*L. aporus*) to 69.1±2.4 % body C day⁻¹ (*L. danicus*). The daily ration for *A. clausi* and *C. typicus* at bloom concentration could not be calculated due to the negative ingestion rates.

At the non-bloom concentration, *A. clausi* and *C. typicus* did not show any significant difference in the daily ration on the different diets. *P. parvus* showed the highest daily ration on *P. calliantha* (226.4±34.5% body C day⁻¹) and the lowest ration on *C. socialis* (78.3±25.7 % body C day⁻¹) diet. The daily ration also differed significantly among the different diets in the case of *T. stylifera* with highest daily ration on *C. socialis* (16.1±0.5 % body C day⁻¹) and lowest on *P. calliantha* (7.3±0.6 % body C day⁻¹) diets.

The daily ration for *T. stylifera* and *P. parvus* for each monospecific diet was significantly higher at bloom than non-bloom concentrations (Table 3.10).

3.3.3.2 Mixed diet

At bloom concentrations, the total daily ration from the mixed diets ranged from 64.0±1.1 % body C day⁻¹ for *T. stylifera* to 502.9±249.9 % body C day⁻¹ for *A. clausi* (Table 3.11). *T. stylifera* was the only species that showed significant difference in the daily ration from the different diatom species and acquired the highest carbon contribution (29.4%) from *L. danicus*. 
Table 3.10. Body carbon and daily ration of the target copepod species fed on different monospecific diets at bloom and non-bloom concentration (avg ± stdev). The daily ration was not calculated (-) for negative ingestion rates. The p values are reported significant at 0.01<p<0.05(*), 0.001<p<0.01 (**), and p<0.001 (***, bold). NA: Data not available

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<th>Copepod species</th>
<th>Copepod body carbon (µg C ind.⁻¹)</th>
<th>Diatom species</th>
<th>Daily ration (% body N day⁻¹) Bloom</th>
<th>Daily ration (% body C day⁻¹) Bloom</th>
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Table 3.11. Body carbon and daily ration of the target copepod species fed on mixed diet at bloom and non-bloom concentration (avg ± stdev). The daily ration was not calculated (-) for negative ingestion rates. The total ration is the sum of all the positive values of daily ration of individual diatom species. Negative values were subtracted. The p values are reported significant at 0.01<p<0.05(*), 0.001<p<0.01 (**), and p<0.001 (***, bold).

<table>
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<tr>
<th>Copepod species</th>
<th>Copepod body carbon (µg C ind⁻¹)</th>
<th>Diatom species</th>
<th>Daily ration (% body N day⁻¹)</th>
<th>Daily ration (% body C day⁻¹)</th>
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<td>29.4±4.4</td>
<td>4.6±2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P. calliantha</td>
<td>6.33±2.20</td>
<td>1.36±0.00</td>
<td>10.3±3.6</td>
<td>2.3±2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>40.70±3.38</td>
<td>6.95±3.07</td>
<td>64.0±1.1</td>
<td>15.2±0.7</td>
</tr>
</tbody>
</table>
At non-bloom concentrations, the daily ration ranged from 15.2±0.8 % body C day\(^{-1}\) for *T. stylifera* to 448.3±20.4 % body C day\(^{-1}\) for *P. parvus*. Only *A. clausi* and *C. typicus* had significantly different daily rations from the different diatoms, *P. calliantha* contributing the highest in both cases. The daily ration for all the copepod species fed on mixed diet was significantly higher at bloom than non-bloom concentration, except for *P. parvus* (Table 3.11)

### 3.3.4 Selectivity

#### 3.3.4.1 Selectivity on single cells vs. colonies in monospecific diets

In order to assess the influence of food morphology and size on copepod feeding, the selectivity index \(\alpha\) was calculated for single cells and multi-celled colonies of each diatom species offered as monospecific diets at bloom and non-bloom concentration. In case of the copepods *A. clausi* and *C. typicus* the selectivity could be evaluated only at the non-bloom concentration as the feeding rates at the bloom concentration were negative.

*Acartia clausi* preferred multi-celled colonies with number of cells ranging between 2-9 cells, but the selectivity varied according to the diatom species (Fig. 3.3). While the negative selectivity on the single cells of the four diatoms was conspicuous, the longer colonies of *C. socialis* and *L. danicus* with \(\geq 10\) cells were also negatively selected (Fig. 3.3 A, C). Effect of cell concentration on selectivity was diatom species specific. In case of *L. aporus*, the selectivity was inversely related to the cell concentration; for *P. calliantha*, the only positive selectivity was measured at ~4000 cells ml\(^{-1}\) (Fig. 3.3 D). There was no clear relationship between concentration and selectivity pattern for *C. socialis* and *L. danicus*. In terms of size, *A. clausi* showed positive selectivity only on the 30-100 µm and 100-200 µm size categories of *L. aporus* and *L. danicus*, respectively (Fig. 3.7 C).
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Figure 3.3 Chesson's index values ($\alpha$) calculated for feeding selectivity patterns of *A. clausi* on single cells and multi-celled colonies of different diatom species at non-bloom concentrations. Green line corresponds to cell concentration. Bars indicate average of three replicates ±stdev. The dotted line indicates $\alpha$ value for non-selective feeding corresponding to $1/n$ classes, $n$ is number of prey categories. Values above the line indicate positive prey selection for the particular prey category, while values below the line indicate negative prey selection.
Centropages typicus showed mostly positive selectivity on multi-celled colonies, ranging from short colonies of 2 cells in L. aporus to larger/longer colonies of 7-11 cells in L. danicus and C. socialis (Fig. 3.4). Positive selectivity on single cells was observed only in case of C. socialis (Fig. 3.4 A). Although there was no clear relation between the selectivity pattern and the cell concentration, most of the positive $\alpha$ values were measured at intermediate cell concentration for each diatom diet (Fig. 3.4). When the cells of the different diatoms were grouped into different size classes, the selectivity was observed on the <30 µm and >200 µm size category classes (Fig. 3.7 D).

Paracalanus parvus showed positive selectivity on both single cells and multi-celled colonies of different diatoms (Fig. 3.5). This wide range of selectivity was also evident when considering the different size-classes (Fig. 3.7 A, E). At bloom concentration, the copepod showed a strong positive selectivity for the colonies of 6-7 cells (C. socialis and L. danicus) and a low preference for smaller colonies (2-4 cells) and single cells of C. socialis (Fig. 3.5 A, D), without any effect of cell concentration on selectivity. In terms of size, the selectivity was on <30 µm size category (C. socialis) and 100-200 µm (L. danicus) (Fig. 3.7 A). At non-bloom concentration, only multi-celled colonies were positively selected for the predominantly solitary diatoms L. aporus and P. calliantha (Fig. 3.5 C, F). For the predominantly colonial diatoms, the selectivity varied from single cells, small 2-celled colonies of C. socialis and larger 8-celled colonies of L. danicus (Fig. 3.5 B, E). The selectivity shifted from negative to positive with decreasing cell concentration for L. aporus and P. calliantha. Conversely, the selectivity became negative with decreasing cell concentration for C. socialis. In case of L. danicus, most of the positive selectivity values were associated to a wide range of cell concentration of ~15-115 cells ml$^{-1}$, with no relation between cell concentration and selectivity.
Figure 3.4 Chesson’s index values ($\alpha$) calculated for feeding selectivity patterns of *C. typicus* on single cells and multi-celled colonies of different diatom species at non-bloom concentrations. Green line corresponds to cell concentration. Bars indicate average of three replicates ±stdev. The dotted line indicated $\alpha$ value for non-selective feeding corresponding to 1/n classes, n is number of prey categories. Values above the line indicate positive prey selection for the particular prey category, while values below the line indicate negative prey selection.
Figure 3.5 Chesson’s index values ($\alpha$) calculated for feeding selectivity patterns of *P. parvus* on single cells and multi-celled colonies of different diatom species at bloom (A, D) and non-bloom (B, C, E, F) concentrations. Green line corresponds to cell concentration. Bars indicate average of three replicates ± stdev. The dotted line indicated $\alpha$ value for non-selective feeding corresponding to $1/n$ classes, $n$ is number of prey categories. Values above the line indicate positive prey selection for the particular prey category, while values below the line indicate negative prey selection.
In general, the selectivity was positive on <30 \( \mu m \) (\textit{C. socialis}) and 100-200 \( \mu m \) (\textit{L. danicus} and \textit{P. calliantha}), while it was close to neutral selection on the 30-100 \( \mu m \) size category (Fig. 3.7 E).

\textit{T. stylifera} showed a wide range of selective ingestion ranging from single cells to multi-celled colonies in the four diatom species at both concentrations (Fig. 3.6). At the bloom concentration, the selectivity was positive on colonies of more than 3 cells (\textit{L. aporus} and \textit{P. calliantha}) up to 13 cells (\textit{C. socialis} and \textit{L. danicus}) (Fig. 3.6 A, C, E, G). At the non-bloom concentration, the copepod showed positive selection on single cells (\textit{L. danicus} and \textit{P. calliantha}) and multi-celled colonies of 2-11 cells of the four diatoms (Fig. 3.6 B, D, F, H). In terms of size, only the positive selectivity was restricted to 30-100 \( \mu m \) (\textit{C. socialis}) and 100-200 \( \mu m \) (\textit{L. aporus} and \textit{P. calliantha}) at the bloom concentration (Fig. 3.7 B). While, at the non-bloom concentration, the <30 \( \mu m \) cells and colonies (\textit{L. danicus} and \textit{P. calliantha}) were also positively selected along with the 30-100 \( \mu m \) (\textit{L. aporus}, \textit{L. danicus} and \textit{P. calliantha}) and 100-200 \( \mu m \) (\textit{L. danicus}) size fraction.

For each monospecific diet, there was no clear difference in the selectivity pattern for each diatom species at the two concentrations, except for \textit{P. calliantha}. In this case, \textit{T. stylifera} showed concentration-dependent positive selection only on the 4-5 celled colonies at the bloom concentration, whereas single cells and 2-4 celled colonies were positively selected at the non-bloom concentration. Considering the size, \textit{T. stylifera} showed different pattern of selective ingestion on the different size categories of all the diatom species except \textit{C. socialis} at the two concentrations (Fig. 3.7 B, F). For \textit{L. danicus} and \textit{P. calliantha}, the selectivity increased to a wider range of size categories at non-bloom than at bloom concentration, whereas for \textit{L. aporus}, the positive \( \alpha \) value
on the 100-200 μm at bloom concentration changed to neutral selectivity value of 0 at non-bloom concentration.

3.3.4.2 Selectivity on diatom species in mixed diets

For *A. clausi* the selection was positive only on *P. calliantha* at bloom concentration and on *C. socialis* and *P. calliantha* at non-bloom concentration (Fig. 3.8 A). *C. typicus* showed positive selectivity on the colonial *C. socialis* at bloom concentration, and the predominantly solitary *P. calliantha* at non-bloom concentration (Fig. 3.8 B). *P. parvus* selected positively both the colonial diatoms *C. socialis* and *L. danicus* at the bloom concentration and only the colonial *C. socialis* at non-bloom concentration (Fig. 3.8 C). *T. stylifera* showed a general preference for the predominantly colonial diatoms over the predominantly solitary diatoms in the mixed diet. At the bloom concentration, *L. danicus* was the only positively selected diatom, while at the non-bloom concentration, this species was positively selected along with *C. socialis* (Fig. 3.8 D).

In summary, for the monospecific diets, all the copepod species showed selective feeding on single cells as well as multi-celled colonies, although, there were differences in the patterns depending upon the diatom species and their concentrations. The general pattern of size-selectivity varied ranging from being non-selective to being highly selective. At the same time, the selectivity of size-classes of cells or colonies of the same diatom differed at the two concentrations indicating variable feeding response at different food concentrations. The most evident difference was observed for *P. parvus* and *T. stylifera*, both species being more selective on the diatom species as well as size categories at non-bloom concentration than bloom concentration. For the mixed diet, each copepod showed a variable selectivity, selecting different diatoms at each concentration.
Figure 3.6 Chesson's index values ($\alpha$) calculated for feeding selectivity patterns of *T. stylifera* on single cells and multi-celled colonies of different diatom species at bloom (A, C, E, G) and non-bloom (B, D, F, H) concentrations. Green line corresponds to cell concentration. Bars indicate average of three replicates ±stdev. The dotted line indicated $\alpha$ value for non-selective feeding corresponding to $1/n$ classes, $n$ is number of prey categories. Values above the line indicate positive prey selection for the particular prey category, while values below the line indicate negative prey selection.
Figure 3.7 Electivity ($E^*$) values for each copepod species with respect to size classes of diatoms offered as monospecific diets in (A, B) bloom and (C, D, E, F) non-bloom concentration. 0 means neutral selection (no selective grazing), positive numbers indicate positive selection (preference) and negative numbers correspond to negative selection (avoidance).
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Figure 3.8 Chesson’s index values ($\alpha$) calculated for the target copepods on predominantly solitary (L. aporus and P. calliantha) and predominantly colonial (C. socialis and L. danicus) diatom species in the mixed diet at bloom and non-bloom concentrations. Bars indicate average of three replicates ± stdev. Red dotted line corresponding to the value of 0.25 (=1/n classes, n =4, n is number of prey classes) indicates neutral selection. Values above 0.25 for prey classes indicate positive prey selection for the particular prey, while values below 0.25 indicate negative prey selection.
3.4 Discussion

3.4.1 Acartia clausi

Acartia clausi feeding rates measured during the present study for the different monospecific diatom diets at non-bloom concentration fall within the ranges reported for this species and its congeners in other laboratory and field studies (Table 3.12). Despite a wide range in initial carbon concentration offered at the non-bloom concentrations, to the copepod during this study (45-139 μg C L\(^{-1}\)), the copepod showed similar carbon ingestion rates for the different diets. However, the feeding rates differed significantly in terms of cells ingested. The cell ingestion was a function of cell concentration, and, therefore, the rates were highest for the most abundant P. calliantha, and lowest for L. danicus. This response of A. clausi implies adjustment of feeding to optimize energy gains. The copepod ingested the smaller cells at higher rates in contrast to lower ingestion of larger cells (L. danicus) to maximize the carbon ingestion. This feeding performance also indicates ability of the copepod to adapt to varying food condition.

In simulated bloom conditions, the copepods were offered the different diatoms at concentrations ranging from 329-1076 μg C L\(^{-1}\). At these high levels of carbon, the feeding rates were negative for all diets and not significantly different. However, the full guts of the copepods and presence of fresh fecal pellets at the end of the experiment, showed that the copepods were feeding actively. Such high concentrations of food are available during the natural phytoplankton blooms at st. LTER-MC. Feeding of A. clausi in laboratory conditions had also been previously reported at high food concentrations of up to 1000 μg C L\(^{-1}\) (Deason, 1980). Negative feeding rates are not uncommon in grazing studies carried out with bottle experiments using natural diet, and can occur due to an increased abundance of prey items in the presence of grazers versus

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controls without grazers (Nejstgaard et al., 2001). It is possible that in the bloom bottles, the diatom cells were nutrient-stressed due to high cell concentration, which in turn reduced their growth rates. The presence of grazers could have favored the release of nutrients through sloppy feeding or increased respiration and release of urea and ammonia (Lehman, 1980; Roman and Rublee, 1980). Another potential cause for the increase of the cell numbers can be the grazing of copepods on microzooplankton, which releases the overall grazing pressure on phytoplankton (trophic cascade) in the experimental bottles (as discussed in Nejstgaard et al., 2001). However, the present study was based only on copepods and diatoms; therefore, the effect of trophic cascade can be excluded, but the possibility of nutrient limitation in the control bottles cannot be ruled out. Some authors suggested adding artificial nutrients to the bottles to avoid this problem (e.g., Roman and Rublee, 1980). However, this problem could not be predicted in this study, given the fact that the filtered sea water used for all the experiments (from st. LTER-MC) is only seldom nutrient depleted (Ribera d’Alcalà et al., 2004). The cell concentrations used to simulate the bloom condition were quite high, although comparable to the natural phytoplankton abundances at st. LTER-MC (Ribera d’Alcalà et al., 2004). At such high densities and an incubation time of 24 hours, it is highly possible that the nutrient were limited or depleted at some point affecting the algal growth in the control bottles.

The feeding rates on the various diatom species in the mixed diet were significantly different at both bloom and non-bloom concentrations and varied from highly positive (P. calliantha) to strongly negative (L. danicus). These results confirm that the feeding response is complex and may not be dependent on a single factor but various aspects such as prey size or abundance (e.g. Frost, 1972; Kiørboe et al., 1996). Despite these differences, the rates did not vary significantly between the two
concentrations, implying that the concentration of food was a less important factor affecting the feeding rates for *A. clausi* when the diet was diversified. This is further confirmed by the similar carbon ingestion rates observed at the two concentrations, suggesting the ability of *A. clausi* to modify its feeding to benefit from mixed diet even at lower food concentrations. Such feeding plasticity and the ability to exploit diverse food sources can have important implication on the survival and reproductive success of *A. clausi* in a given environment (Calliari and Tiselius, 2005).

In general, *A. clausi* showed a Holling type III sigmoidal functional response over the wide range of cell and carbon concentrations. The ingestion rates saturated at the high bloom concentrations while the clearance rates peaked at 4696 cells ml⁻¹ (*P. calliantha*) and declined at both very high and very low cell concentrations. Similar response has been also been reported previously for *A. clausi* (Gismervik and Andersen, 1997) and for *A. tonsa* (Kiorboe et al., 1996). In case of *A. tonsa*, the saturation of ingestion at 500 cell ml⁻¹ for the diatom *Thalassiosira weissflogii* (Kiorboe et al., 1996). *Acartia* spp. are known to reduce their clearance rates for algae below some threshold food abundance (e.g., Deason, 1980; Paffenhofer and Stearns, 1988) For diatoms, the clearance rate appears to decreases when the cell abundance is below 100 to 500 cells ml⁻¹, or 4 to 40 μg C L⁻¹ (Gismervik and Andersen, 1997). The feeding threshold at lower food concentrations may be due to few chemoreceptors, and an inability of the copepod to change the flow field in order to re-route the cells toward the mouth (Paffenhofer and Stearns, 1988).

*A. clausi* showed consistent positive selectivity only on the colonies of 30-200 μm length over a wide size range of different diatoms ranging between ~8 μm (*C. socialis*) to > 200 μm (*L. danicus*) offered as monospecific diets at non-bloom concentration. These results are in accordance with those of Nival and Nival (1976) and Katechakis *et*
al., (2004) who found a lower size limit of 7-7.5 μm to cell ingestion for A. clausi in natural food assemblage as well as laboratory cultures. Also, the upper size limit of 200 μm for A. clausi in this study is close to 210 μm and ~250 μm size limit as suggested by Katechakis et al. (2004). For the mixed diets, the copepod showed a higher degree of selectivity when feeding at the non-bloom concentration than at bloom concentration. Amongst the four diatom species, P. calliantha was positively selected at both concentrations, while, C. socialis was selected along with P. calliantha at the non-bloom concentration. These results are contrary to the expected size-based selectivity for larger prey observed for copepods (e.g., Frost, 1977; Runge, 1980). Instead, the present results indicate that A. clausi selected the most abundant prey, which can be explained by “peak tracking”, when the copepod feeds efficiently on the dominant food taxa corresponding to the peaks of biomass (Richman et al., 1977). This behaviour has been demonstrated for A. clausi and other copepod genera and may not necessarily be related to the cell size (e.g., Katechakis et al., 2004; Perissinotto, 1992; Schnack, 1983a). In a grazing experiment with natural food assemblage in the NW Mediterranean, the selectivity of A. clausi always matched the peak of available food particles and was interpreted as an active selection of ≥70 μm diatoms, which were the dominant food taxa corresponding to the peaks (Katechakis et al., 2004).

The increased selectivity at non-bloom concentration could be aimed at compensating the energy requirements, indicating a change in foraging behaviour between two extreme food environments. A. clausi is known to switch its feeding behaviour between ambush and suspension mode, which can enable it to maximize ingestion (e.g., Gismervik and Andersen, 1997; Kiorboe et al., 1996). The positive selectivity for C. socialis may be explained by its larger colonies and spines, which may generate more mechanical signals. For an ambush feeder like Acartia clausi, such a
diatom colony can be more detectable than cylindrical diatoms like *Leptocylindrus* species. The copepod might have therefore compensated suspension feeding on the more abundant *P. calliantha*, with ambush feeding on *C. socialis* at the non-bloom concentration.

The daily rations of *A. clausi* on the monospecific diets at the non-bloom condition fall within the range reported by other studies (see Table 3.12). In the mixed diet, *A. clausi* ingested 135 % and 503 % of body carbon at non-bloom and bloom concentration, respectively. The highest daily ration value at the bloom concentration is similar to those reported by Deason (1980) for *A. clausi* when feeding at higher concentrations of the diatom *Skeletonema costatum* (1000 μg C L⁻¹). Fessenden and Cowles (1994) also reported similarly high ration (578% of body carbon) for *A. longiremis*. In the present study, the maximum contribution to the total daily ration at both concentrations was from *P. calliantha*, followed by *C. socialis* at non-bloom concentration, which reflects their selection accompanied by higher ingestion.

In summary, *A. clausi* showed an opportunistic feeding, with concentration-dependent ingestion and selectivity, which can also be more beneficial as the encounter rates for more abundant species is higher. The carbon ingestion can be maximized in this way by feeding on the more abundant species and by spending less energy to feed on less abundant species. This can be a situation during a bloom when the concentration of a particular diatom increases. *A. clausi*, with its ability to adapt its feeding performances to the food type and concentration, may have a potential impact on the bloom dynamics.
Table 3.12 Feeding rates and daily ration of *Acartia clausi* and its congeners in different food environments compiled from literature.

<table>
<thead>
<tr>
<th>Species and stage</th>
<th>Study</th>
<th>Food</th>
<th>Filtration rates (ml cop$^1$d$^{-1}$)</th>
<th>Ingestion rates (µg C cop$^1$d$^{-1}$)</th>
<th>Daily ration % body C d$^{-1}$</th>
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<td><em>T. pseudonana</em> or <em>T. flaviatilis</em></td>
<td>---</td>
<td>0.2-5.7</td>
<td>-</td>
<td>Donaghay and Small, 1979</td>
</tr>
<tr>
<td><em>A. clausi</em> female</td>
<td>Onagawa bay, Japan</td>
<td>Natural</td>
<td>3.5-24.0</td>
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<td>Ayukai, 1987</td>
</tr>
<tr>
<td><em>A. clausi</em> female</td>
<td>Villefranche, France</td>
<td>Natural</td>
<td>34-630</td>
<td>--</td>
<td>--</td>
<td>Wiadnyana and Rassoulzadegan, 1989</td>
</tr>
<tr>
<td><em>A. tonsa</em> females</td>
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<td><em>Strobilidium spiralis</em></td>
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<td>--</td>
<td>---</td>
<td>Jonsson and Tiselius, 1990</td>
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<tr>
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<td>--</td>
<td>0.85</td>
<td>21</td>
<td>Rodriguez and Durbin, 1992</td>
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</table>
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<th>Food</th>
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<th>Ingestion rates (µg C cop⁻¹ d⁻¹)</th>
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<th>Feeding Rate</th>
<th>Selectivity</th>
<th>Reference</th>
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<td>6.3-188</td>
<td>Rollwagen Bollens, G and Penry, D, 2003</td>
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<td>0-38</td>
<td>0-14</td>
<td>--</td>
<td>Katechakis et al., 2004</td>
</tr>
<tr>
<td>A. clausi</td>
<td>--</td>
<td>Laboratory algal cultures</td>
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<td>1.7-2.1</td>
<td>--</td>
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<td>55-92</td>
<td>Dutz and Peters, 2008</td>
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<td>0.03-6.1</td>
<td>8-168</td>
<td>Fileman et al., 2010</td>
</tr>
<tr>
<td>A. tonsa</td>
<td>Humbolt current, Chile, Natural</td>
<td>--</td>
<td>7.2-26.0</td>
<td>--</td>
<td>Aguilera et al., 2011</td>
</tr>
</tbody>
</table>
3.4.2 Centropages typicus

The clearance rates of *Centropages typicus* obtained at non-bloom concentration for the different monospecific diets varied significantly among diets, with highest rates on *C. socialis*. Overall, the positive values of clearance rates in this study fall within the range found in the literature (Table 3.13). In laboratory studies, the copepod had shown a wide range of clearance rates (0-1221 ml cop⁻¹ d⁻¹) depending on the prey (as reviewed by Calbet *et al.*, 2007). In a feeding experiment with *C. typicus* by Tomasini and Mazza (1979), the clearance rates increased from 7-17 ml cop⁻¹ d⁻¹ to 40 ml cop⁻¹ d⁻¹ with increasing prey size (3.1- 19.4 μm). Similarly, in the present study, the clearance rates were higher for the larger (linear dimension) colonial *C. socialis* and *L. danicus* than the smaller solitary *L. aporus*, which was offered at an intermediate concentration with respect to the two colonial species. The clearance rates were thus a function of both cell size and concentration. *C. typicus* also showed a sigmoidal functional response to the variation in cell concentration. The clearance rates on *P. calliantha*, which was offered at higher cell concentration as compared to the other diets, were negative. The rates were negative at the bloom concentration, when the monospecific diets were offered at 7.3 - 180.6 x 10⁶ cells L⁻¹.

The ingestion rates in terms of cells for the monospecific diets varied significantly at the non-bloom concentration and showed similar pattern as for the clearance rates. The highest cell ingestion rates were for *C. socialis* which was offered at 3.4 cells x 10⁶ L⁻¹ while, at a higher cell concentration of 24.9 cells x 10⁶ L⁻¹ for *P. calliantha*, the rates were negative. The difference in the cell ingestion rates did not reflect the carbon ingestion rates, which were similar for all the diets. The ingestion rates at the bloom concentration were negative for all the diets, presumably due to reasons similar to those mentioned above for *A. clausi*. The cell ingestion rates for the mixed diet varied
significantly only at non-bloom concentration, with highest rates on the most abundant *P. calliantha*. Unlike the monospecific diets, the cells ingestion rates in the mixed diet corresponded to the carbon ingestion rates, which also varied significantly with highest carbon ingestion for *P. calliantha*. The total carbon ingestion values for the mixed diets were not significantly different at the two concentrations. The estimated carbon ingestion rates for *C. typicus* in this study ranged between 2.3 - 6.4 μg C L⁻¹ for monospecific diets and 1.8 - 9.4 μg C L⁻¹ for the mixed diets and are consistent with those reported by other studies for *C. typicus* and its congeners (Table 3.13).

*Centropages typicus* showed selectivity on both single cells and colonies ranging between ~7.5 μm (*C. socialis*) measuring and > 200 μm (*L. danicus*) which were offered as monospecific diets. Laboratory studies have reported *C. typicus* feeding upon a wide size range of organisms including small algae (Tomasini and Mazza, 1979), appendicularian juveniles and eggs (López-Urrutia *et al.*, 2004), nauplii of other copepods (Titelman, 2001). The optimum of prey-size for *C. typicus* seems to be on particles >10 μm (Calbet *et al.*, 2007), but regarding the upper size limit, this copepod can ingest prey larger than its own size like yolk-sac fish larvae (Turner *et al.*, 1985). Selectivity for larger cells (both diatoms as well as non-diatom prey) has also been reported for *C. typicus* as well as its congeners *C. chierchiae* (e.g., Miralto *et al.*, 1995; Vincent and Hartmann, 2001). In the mixed diet experiments of the present study, the copepod selected different diatoms at the two concentrations. At the bloom concentration, the copepod showed positive selectivity only on the colonial *C. socialis*, which was present at an intermediate cell concentration, similar to *L. aporus*, but different than the *P. calliantha* and *L. danicus*. Selectivity dependent on food concentration has previously been described for *Centropages brachiatus* (Cowles, 1979). However, the cell concentration cannot be considered as the only factor...
influencing selectivity, since, *L. aporus* which was available at concentration similar to *P. calliantha* was not selected. Copepods can select their food particles individually, based on several factors; prey size (Frost, 1972), motility (Tiselius and Jonsson, 1990) and nutritional quality (Barofsky *et al.*, 2010), to name a few. Along with these characteristics, the most important difference between the different diatom species offered during the present study was their shape. Both *C. socialis* and *L. aporus* have a basic cylindrical shape, but they are very different in length/size. Moreover, *C. socialis* is characterised by long spines/setae and forms long fan-shaped or spherical colonies. These differences may manifest in dissimilar perception of the diatoms by the copepod and therefore affect their selectivity and ingestion. At the non-bloom concentration, the copepod showed positive selectivity only on the most abundant *P. calliantha*, which was also ingested at high rates. Such type of selectivity can be explained by the optimal foraging theory, whereby the copepod selects the most abundant prey during low food availability (Charnov, 1976). This theory states that a prey should be ignored if its energy value relative to the time it takes to consume the prey is not profitable compared to alternative prey in the given food environment. This can be profitable when food is scarce, like during a non-bloom condition in nature. In fact, *P. calliantha* comprised the major part of the total carbon ingested at the non-bloom concentration. Finally, the different preference for the diatoms at the two concentrations indicates different foraging behaviour when feeding in different food environments.

The daily ration of *C. typicus* obtained from the different monospecific diets and mixed diets fall within the ample range (1.2-184% body C cop$^{-1}$ d$^{-1}$) reported for *C. typicus* as well as for its congeneric species (Table.3.13). The higher ration at bloom concentration indicates the ability of *C. typicus* to exploit the richer food environment. *C. typicus* is a common and abundant species in the coastal regions, whereas its higher
abundance in the open ocean is associated with spring blooms (Carlotti et al., 2007; Mazzocchi et al., 2007). This distribution pattern related to high food availability indicates a low adaptability of *C. typicus* to fluctuations in food availability (Calbet et al., 2007). The higher ration indicates that the copepod can benefit during the periods of high food availability by feeding on the most abundant prey. In the Gulf of Naples, *C. typicus* is a dominant copepod during spring, which is characterized by high biomass represented by a diverse phytoplankton assemblage, dominated by different diatoms. Such diverse food environments together with a constant supply of food may explain why *C. typicus* prevails during the spring.
Table 3.13 Feeding rates and daily ration of *Centropages typicus* and its congeners in different food environments compiled from literature.

<table>
<thead>
<tr>
<th>Species and stage</th>
<th>Study</th>
<th>Food</th>
<th>Filtration rates (ml cop$^{-1}$ d$^{-1}$)</th>
<th>Ingestion rates (µg C cop$^{-1}$ L$^{-1}$)</th>
<th>Daily ration % body C d$^{-1}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. typicus</em></td>
<td>Laboratory</td>
<td><em>Chaetoceros pseudocurvisetus</em></td>
<td>0.1-22</td>
<td>-</td>
<td>-</td>
<td>Tomasini and Mazza, 1978</td>
</tr>
<tr>
<td>female</td>
<td>(13°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. typicus</em></td>
<td>Laboratory</td>
<td><em>Skeletonema costatum</em></td>
<td>1.6-29</td>
<td>0.02-0.46</td>
<td>-</td>
<td>Tomasini and Mazza, 1978</td>
</tr>
<tr>
<td>female</td>
<td>(12.5°C)</td>
<td><em>Lauderia borealis</em></td>
<td>1.1-40</td>
<td>----</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>females</td>
<td>Laboratory</td>
<td><em>I. galbana (3.6)</em></td>
<td>0.2-17</td>
<td>0.007-2.16</td>
<td>-</td>
<td>Tomasini and Mazza, 1978</td>
</tr>
<tr>
<td></td>
<td>(13°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. typicus</em></td>
<td>Laboratory</td>
<td><em>Thalassiosira weissflogii</em></td>
<td>19.2</td>
<td>-</td>
<td>-</td>
<td>Dagg, 1983</td>
</tr>
<tr>
<td>female</td>
<td></td>
<td><em>Cyclotella cryptica</em></td>
<td>6.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Chapter 3. Feeding rates and selectivity

<table>
<thead>
<tr>
<th>Species</th>
<th>Environment</th>
<th>Food Source</th>
<th>Feeding Rate</th>
<th>Ch.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. typicus</em></td>
<td>New York Bight</td>
<td>Phytoplankton</td>
<td>48-96</td>
<td>12</td>
<td>Smith and Lane, 1988</td>
</tr>
<tr>
<td><em>C. typicus</em></td>
<td>Laboratory</td>
<td><em>S. sulcatum</em> + <em>Prorocentrum micans</em></td>
<td>349–1221</td>
<td>80</td>
<td>Wiadnyana and Rassoulzadegan, 1989</td>
</tr>
<tr>
<td><em>C. typicus</em></td>
<td>Mediterranean</td>
<td>Natural phytoplankton</td>
<td>124–149</td>
<td>24-58</td>
<td>Pagano et al., 1993</td>
</tr>
<tr>
<td><em>C. typicus</em></td>
<td>Laboratory (15°C)</td>
<td><em>Thalassiosira weissflogii</em></td>
<td>--</td>
<td>4.50</td>
<td>Bonnet and Carlotti, 2001</td>
</tr>
<tr>
<td><em>C. typicus</em></td>
<td>Laboratory (15°C)</td>
<td><em>Hymenomonas elongata</em></td>
<td>--</td>
<td>0.94</td>
<td>Bonnet and Carlotti, 2001</td>
</tr>
<tr>
<td><em>C. typicus</em></td>
<td>Laboratory (15°C)</td>
<td><em>Strombidium sulcatum</em></td>
<td>--</td>
<td>1.95</td>
<td>Bonnet and Carlotti, 2001</td>
</tr>
<tr>
<td><em>C. typicus</em></td>
<td>Laboratory</td>
<td><em>H. elongata + S. sulcatum</em></td>
<td>--</td>
<td>3.25</td>
<td></td>
</tr>
<tr>
<td><em>C. typicus</em></td>
<td>Laboratory</td>
<td><em>T. weissflogii + S. sulcatum</em></td>
<td>--</td>
<td>5.95</td>
<td></td>
</tr>
<tr>
<td><em>C. typicus</em></td>
<td>Laboratory</td>
<td><em>Calanus</em> eggs</td>
<td>175–260</td>
<td></td>
<td>Sell et al., 2001</td>
</tr>
<tr>
<td><em>C. typicus</em></td>
<td>Laboratory</td>
<td><em>Temora longicornis</em> nauplii</td>
<td>442</td>
<td></td>
<td>Titelman, 2001</td>
</tr>
<tr>
<td><em>C. typicus</em></td>
<td>Laboratory</td>
<td><em>Acartia tonsa</em> nauplii</td>
<td>146</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>Environment</td>
<td>Food Type</td>
<td>Feeding Rate</td>
<td>Selectivity</td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>----------------------</td>
<td>-------------------------</td>
<td>--------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td><em>C. typicus</em> copepodites</td>
<td>Mediterranean</td>
<td>Phytoplankton &gt;5 μm</td>
<td>44</td>
<td>1.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(12.5°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. typicus</em> copepodites</td>
<td>Mediterranean</td>
<td>Phytoplankton &lt;5 μm</td>
<td>18</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(12.5°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. typicus</em> copepodite</td>
<td>Mediterranean</td>
<td>Ciliates</td>
<td>79</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(12.5°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. typicus</em> copepodite</td>
<td>Mediterranean</td>
<td>Ciliates</td>
<td>76</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(18°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. hamatus</em> females</td>
<td>Laboratory</td>
<td>Mixture of ciliates and</td>
<td>--</td>
<td>12.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. weissflogii</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ditylium brightwellii</em></td>
<td></td>
<td></td>
<td>19-159</td>
<td>2.4-18.8</td>
<td></td>
</tr>
<tr>
<td><em>C. chierchiae</em> females</td>
<td>Laboratory</td>
<td><em>Phaeodactylum tricornutum</em></td>
<td>51-75</td>
<td>2.1-29.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(8-24°C)</td>
<td><em>Gymodinium spp.</em></td>
<td>33-88</td>
<td>0.2-17.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Rhodomonas baltica</em></td>
<td>24-121</td>
<td>0.1-20.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Broglio et al., 2004
Saage et al., 2009
Garrido et al., 2013
Chapter 3. Feeding rates and selectivity

3.4.3 Paracalanus parvus

The mean clearance and ingestion rates of Paracalanus parvus on the different diatoms offered as monospecific and mixed diets estimated in this study fall within the range known for this calanoid species (Table 3.14). Checkley (1980) reported similar values for P. parvus during laboratory feeding experiment with the diatom Thalassiosira fluviatilis (99 ml cop⁻¹d⁻¹) and Ditylium brightwellii (102 ml cop⁻¹d⁻¹). During the present study, the most significant difference for the monospecific diets was observed at the bloom concentration when the rates changed from positive to negative with increase in the cell concentrations. Accordingly, the copepod had negative rates on the more abundant L. aporus and P. calliantha. Vargas and González (2004) observed a similar response of P. parvus when feeding on natural food assemblage, when the clearance rates decreased for diatoms and nanoflagellates as their respective concentrations increased. This relationship between the clearance rate and the food concentration is similar to the model proposed by Marin et al. (1986), wherein the clearance rates decrease above a certain critical concentration of food. Paffenhofer (1988) also reported a decrease in the clearance rates for P. parvus when feeding on the diatom Thalassiosira weissflogii with an increase in its concentration.

The grazing rates varied significantly among diets at the non-bloom concentrations; however, in contrast to the bloom concentrations, the rates increased with increasing cell concentration. A similar trend was also observed in the mixed diet. Moreover, the rates were higher at the non-bloom concentration than at the bloom concentration. This difference in the feeding response of P. parvus from bloom to non-bloom concentration is likely due to the difference in the perceptions of the cell at two extreme food environments. Copepods are known to be able to increase their clearance rates as food levels decreases by elevating their food perception performance, which can
eventually result in the clearance rates increase (Paffenhöfer and Lewis, 1990). *P. parvus* is a suspension feeding copepod that creates feeding current, it is likely that the range and distance at which phytoplankton cells can be detected by the *P. parvus* increases at a lower food concentration, leading to an increase in the clearance rates (Paffenhöfer and Stearns, 1988).

Ingestion rates in terms of cells also followed a similar pattern like the clearance rates, in both monospecific and mixed diets, at bloom and non-bloom concentrations. The ingestion in terms of carbon for the monospecific diets was higher at the bloom concentration, with higher values for the *C. socialis* and *L. danicus*. The carbon ingestion rates for the mixed diet however did not show any significant difference at the two concentrations, although the contribution of each diatom to the total carbon varied from bloom to non-bloom. Over the range of carbon concentration considered, the ingestion can be described as a Holling type 2 response (Holling, 1959), indicating saturation of feeding at a lower threshold of carbon. The main factor responsible for saturating in the functional response is the digestion and gut transit time of ingested particles (Tiselius *et al.*, 2013). This may be true in case of food with limited nutritional value as the selectivity by the copepods would be tuned toward rapid assessment of the quality of frequently encountered particles.

The selectivity of the different diatoms in the present study did not reflect in the carbon ingestion rates at both concentrations. In general, the copepod showed positive selectivity on <30 μm up to 200 μm size category. The <30 μm size category included mostly colonies of more than 2 cells. This selection matches the optimum particle size for *P. parvus*, which is considered of the order of 20-40 μm calculated as 2-5% of the cephalothorax length (Bergreen *et al.*, 1988). The species of the genus *Paracalanus* are also known to collect small particles (< 5 μm) passively (Price *et al.*, 1983). The smallest
cells in the present experiments were the single cells of *C. socialis* measuring ~8.5 μm. It is possible that the copepod also ingested these cells which lead to the higher cell and carbon ingestion for this diatom species.

The daily ration of *P. parvus* was extremely high, with highest carbon ingestion rates corresponding to 498% of its body carbon for monospecific diets and 448% of its body carbon for the mixed diet. These values are higher than those reported previously for *P. parvus* females, which ingested daily a maximum of nearly 360% of its body weight when fed on the diatom *Thalassiosira fluviatilis* (Checkley, 1980). Interestingly, the daily ration from the mixed diets was similar at both concentrations. The relatively low level of satiation concentration for *P. parvus* at the non-bloom concentration may be due to its ability to adapt to conditions in which food availability is limited (Schnack-Schiel, 1982). In a study carried out in Marennes-Oleron Bay (France) during an algal spring bloom, *P. parvus* showed a negative correlation between the grazing activity and algal concentration (Sautour and Castel, 1999). In the Gulf of Naples, *P. parvus* reaches its peak abundance during summer, when phytoplankton blooms are comprised by small-sized, mainly solitary forms of diatoms (Ribera d’Alcalà *et al.*, 2004). The diatom species *L. aporus* used in the present experiments is one of the dominant diatoms of the summer phytoplankton assemblage, for which *P. parvus* has shown an evident feeding response both as monospecific diet as well as when fed in mixed diet. At the same time, other species of diatoms, morphologically different from *L. aporus*, were also ingested and selected. This indicated that *P. parvus* is able to feed on a variety of diatoms, irrespective of their size or shape, a trait which can be necessary to meet energy requirements. The ability to selectively consume diverse phytoplankton (Checkley, 1980) and capture very small cells efficiently, coupled with facility to maintain fecundity may explain why *P. parvus* is a successful coastal species.
Table 3.14 Feeding rates and daily ration of *Paracalanus parvus* in different food environments compiled from literature.

<table>
<thead>
<tr>
<th>Species</th>
<th>Region</th>
<th>Food type</th>
<th>Filtration rates</th>
<th>Ingestion rates</th>
<th>Daily ration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Thalassiosira fluviatilis</em></td>
<td>45-99</td>
<td></td>
<td></td>
<td>(Checkley, 1980)</td>
</tr>
<tr>
<td><em>P. parvus</em> female</td>
<td>Laboratory</td>
<td><em>Ditylium brightwellii</em></td>
<td>102</td>
<td>--</td>
<td>360</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Gonyaulax polyedra</em></td>
<td>51</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. parvus</em></td>
<td>South California</td>
<td>Natural Dinoflagellate</td>
<td>4.4-8.6</td>
<td>--</td>
<td>--</td>
<td>(Fiedler, 1982)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Gymnodinium</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. parvus</em> C5</td>
<td>South California</td>
<td>Natural Dinoflagellate</td>
<td>2.8-6.4</td>
<td>--</td>
<td>--</td>
<td>(Fiedler, 1982)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Gymnodinium</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. parvus</em> female</td>
<td>southern Benguela, South</td>
<td>Natural</td>
<td>1.1-4.4</td>
<td>1.5-1.2</td>
<td>19-33</td>
<td>(Peterson <em>et al.</em>, 1990)</td>
</tr>
<tr>
<td><em>P. parvus</em> female</td>
<td>Marennes-Oleron Bay,</td>
<td>Natural</td>
<td>--</td>
<td>--</td>
<td>7-15</td>
<td>(Sautour and Castel, 1999)</td>
</tr>
<tr>
<td><em>P. parvus</em> female</td>
<td></td>
<td>Dinoflagellates</td>
<td>35-146</td>
<td>0.03-0.41</td>
<td>--</td>
<td>(Suzuki <em>et al.</em>, 1999)</td>
</tr>
</tbody>
</table>
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**P. parvus**

<table>
<thead>
<tr>
<th>NW Mediterranean</th>
<th>Ciliates</th>
<th>23-58</th>
<th>0.06-0.68</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5 μm Phyto</td>
<td></td>
<td>1-39</td>
<td>0.03-0.65</td>
</tr>
<tr>
<td>&gt; 5 μm Phyto</td>
<td></td>
<td>6-33</td>
<td>0.37-0.82</td>
</tr>
</tbody>
</table>

(Broglio et al., 2004)

**P. parvus**

<table>
<thead>
<tr>
<th>Mejillones Bay, Chile</th>
<th>Natural</th>
<th>0-300</th>
<th>0.8-5.7</th>
<th>27-154</th>
</tr>
</thead>
</table>

(Vargas and González, 2004)
3.4.4 Temora stylifera

The clearance rates and ingestion rates of *Temora stylifera* on the different diatoms in both monospecific and mixed diets showed a wide range and were comparable to the range of values reported for the same species (Broglio *et al*., 2004; Schnack, 1983b) and its congener *T. longicornis* (Arendt *et al*., 2005; Wang and Conover, 1986) (Table 3.15). At the non-bloom concentration, *T. stylifera* showed a significant difference in the feeding rates on the different diatoms offered as monospecific diets. Both the clearance and the cell ingestion rates were a function of cell size and initial carbon concentrations of the diatoms. The highest rates were therefore observed on the larger colonial *L. danicus*, which was offered at a lower concentration than the other smaller diatoms offered at relatively higher concentrations. Such kind of expected feeding response between feeding rates and size and concentration of cells has been reported earlier for *T. stylifera* as well as other copepod species (e.g. Frost, 1972; Paffenhöfer, 1988; Schnack, 1983b). Also at the bloom concentration, *T. stylifera* showed a similar pattern in the cell ingestion as seen at the non-bloom concentration, although the rates for each species were at least 2-fold higher at the bloom concentration. In general the rates for *T. stylifera* in the present study varied significantly among the different diatoms species as well as from bloom to non-bloom concentrations. The results therefore show that the copepod can exhibit wide flexibility by modulating its feeding depending on the food environment.

The ingestion rates in terms of carbon reflected the size of the diatoms most of the times in both monospecific as well as mixed diets, when the carbon ingested was higher for the larger colonial *C. socialis* and *L. danicus* as compared to the solitary smaller species. For the mixed diet, all the four diatom species were ingested at similar rates, however the positive selectivity was restricted only to the two colonial diatom species,
Chapter 3. Feeding rates and selectivity

despite their lower availability. Preference for larger cells in the congener *T. longicornis* has been previously reported (Tackx and Rijswijk, 1990; Vincent and Hartmann, 2001). The observed selectivity for the larger cells can be due to the particle retention efficiency of the copepod, which is 100% for >10\(\mu\)m cells (ESD) as compared to ~25% for cell of 5\(\mu\)m (Dam, 1986). In this study, *T. stylifera* showed positive selectivity mainly on the cells and colonies ranging between >30 \(\mu\)m to 200 \(\mu\)m for the monospecific diet experiments. The <30 \(\mu\)m size category was selected only at the non-bloom concentration. This may be due to the general low availability of larger size category of cells or colonies as compared to the smaller size category at the non-bloom concentration. Such a selectivity for smaller cells at lower food concentrations has also been reported for *T. longicornis* (Dam and Peterson, 1991; Gentsch *et al.,* 2008). The observed difference in the selectivity at the two food concentrations indicate that *T. stylifera* can potentially affect the size structure of the phytoplankton community in the natural environment through its selective feeding. The effect in this case would be more pronounced at lower food concentrations, or during a non-bloom situation. Also, it implies that food limitation is strongly dependent on the size structure of the phytoplankton assemblage (Dam and Peterson 1991).

The selectivity of the larger diatoms species also reflected in their higher contribution to the daily ration. The highest daily ration values of 69% body C d\(^{-1}\) and 67.7% body C d\(^{-1}\) were for *C. socialis* and *L. danicus*. The ration from these two species was also higher in the mixed diets. Although the smaller *L. aporus* and *P. calliantha* were also ingested at both concentrations, the selectivity for larger cells indicate that *T. stylifera* behaves as an opportunistic feeder, consuming more of the cells that were available at the highest concentrations in mixed diet, but, at the same time showed selection on larger-sized cells.
3.5 Conclusions

During the present study, all the four copepod species showed a wide range of feeding responses to the different food environments represented by different bloom-forming diatom species and two contrasting food concentrations.

- Despite the variable grazing rates and selectivity patterns shown by the copepods, it was evident that the diversity in size of the diatoms did not limit their ingestion.
- The response of each copepod was diatom-specific but it differed when the diatoms were offered as monospecific diets as compared to when they were offered together as mixed diets.
- In general, the feeding rates varied as a function of the cell concentration (A. clausi, P. parvus) or as a combined effect of cell concentration and size (C. typicus, T. stylifera).
- The effect of food concentration on the grazing rates was evident only for P. parvus and T. stylifera.
- A. clausi, C. typicus and T. stylifera obtained a higher daily ration when feeding at higher food concentration, while, for P. parvus the daily ration was similar at different food concentrations.

Overall, feeding of the copepods is more complex than expected even in simplified laboratory conditions and is susceptible to a combination of various factors related to the food environment.
CHAPTER 4.

Behavioural interactions of target copepod species with bloom-forming diatoms: Observations at small scale
Chapter 4. Behavioural interactions

4.1 Introduction

The behaviour of an individual animal is a reflection of its interactions with the environment. Consequences of small-scale interactions at the population level can have far-reaching effects on the whole ecosystem. In the marine system, copepod grazing, i.e. their feeding on phytoplankton, is one of the most important processes that shape the planktonic food webs and can be influenced directly or indirectly by individual behaviour. Copepod species differ appreciably in swimming patterns and speed, feeding behavior, and morphology of feeding appendages. Swimming and feeding in copepods are tightly coupled and their modes can be interdependent. The motion behavior and speed of a copepod are subject to morphological and biological constraints such as size, array of sensory structures, gender or developmental stage. However, the swimming modes can be modulated depending on several environmental conditions, including food quality and quantity, and in relation to the encounter rates with prey (Gerritsen and Strickler, 1977). Analyzing the individual behaviour thus becomes crucial in evaluating the interaction of a particular copepod species with its food environment and ultimately its role in the food web. In the last three decades, the attention to small-scale processes in plankton has increased thanks to the availability of proper techniques and studies on copepod behaviour have flourished (Table 4.1).

Copepods had been considered traditionally as rather passive feeders, using the maxillary setae as a mechanical filtering sieve (Marshall, 1973), until, direct observations with high-speed micro-cinematography revealed an array of activities involved in food capture and handling (Alcaraz et al., 1980). Further information pertaining a complex feeding behavior involving cephalic appendages (Koehl and Strickler, 1981, Price et al., 1983) and its quantification using frame motion analysis (Cowles and Strickler, 1983) increased our understanding of the wide variety of
mechanisms that copepods use to detect, pursue, capture, and reject prey (Price, 1988). Since then, improved direct observation techniques have allowed to investigate copepod behavior at the scales proper of these small crustaceans including capturing food (e.g., Cowles and Strickler, 1983; van Duren et al., 2003; Kiørboe et al., 2009; Tiselius et al., 2013), locating a mate (Doall et al., 1998; Bagøien and Kiørboe, 2005; Kiørboe, 2008) and avoiding predators (Buskey et al., 2002; Buskey and Hartline, 2003; Bradley et al., 2013). While most of the early studies related to feeding behavior described the capture mechanisms in tethered copepods (e.g., Koehl and Strickler, 1981; (Price and Paffenhöfer, 1986a); Paffenhöfer and Stearns, 1988), successive studies analyzed the feeding activities in free swimming animals in relation to motion (e.g. Tiselius and Jonsson, 1990; Saiz and Alcaraz, 1992; van Duren and Videler, 1995; Caparroy et al., 1998).

Copepods are not passive filter feeders but rather depend on perceiving prey in order to capture them (Kiørboe, 2013). This applies in case of all the three different characteristic food searching strategies used by copepods as reviewed by Kiørboe (2011a): i) cruising through the water and capturing individual prey, ii) generating a feeding current and retrieving prey either by directly intercepting it or by straining/filtering the prey out of the feeding current, iii) adopting an ambush mode to passively encounter motile prey and capture them by active attacks. Switching between feeding strategies is common among some copepod species that change feeding modes depending upon different quality and quantity of food items to maximize energy gain (e.g., Price and Paffenhöfer, 1986; Price, 1988; Kiørboe et al., 1996; Meyer-Harms et al., 1999).

A cruise feeding copepod swims actively to encounter prey detected by fluid signal (Kiørboe, 2011a). Cruising increases encounter rate (Gerritsen and Strickler,
Chapter 4. Behavioural interactions

1977), and may reduce the rate of fluid deformation ahead making it possible to overtake the escaping prey in a hydrodynamically quieter way (Kerfoot, 1978). Upon remote detection of the prey, the copepod either i) uses feeding currents generated during swimming (using the cephalic appendages) to capture the prey (Strickler, 1982; Hwang and Strickler, 2001) or ii) it lunges towards its prey and captures the prey (Doall et al., 2002). Feeding on particles while cruising has been described in several calanoid copepods like *Centropages typicus*, *C. hamatus* (Tiselius and Jonsson, 1990), *Euchaete elongata* (Yen, 1985) *Labidocera trispinosa* (Greene and Landry, 1985; Landry and Fagerness, 1988) and *Metridia longa* (Kjellerup and Kiørboe, 2011). The use of a laminar flow feeding current to entrain prey has been reported in the cruising predatory copepod *Euchaeta rimana* while in a hovering position (Yen et al., 1991; Fields and Yen, 2002; Doall et al., 2002). Recent studies have demonstrated that short-distance detection using hydromechanical cues and not direct interception is involved in the capture of motile as well as non-motile prey in the cruising copepod *M. longa* (Kjellerup and Kiørboe, 2011). A non-motile prey was detected only after the copepod had swum past the prey and the prey was in a ventral or latero-ventral position to the copepod. Instead, the motile prey was detected in front of the copepod close to the antennules. This was also observed in the small, fast swimming calanoid *Clausocalanus furcatus*, which captures only the prey within the close vicinity of its head (Uttieri et al., 2008).

During suspension feeding, the copepod generates a feeding current by rapid vibration of the cephalic appendages, which scan the water for food items (e.g., Alcaraz et al., 1980; (Strickler, 1985; Vanderploeg and Paffenhofer, 1985; Price and Paffenhofer, 1986a, b). Typical suspension feeders like calanoids *Neocalanus cristatus* and *Calanus pacificus* (Frost et al., 1983) have finely-setose second maxillae and
maxillipeds which generate the feeding current and deflect the prey to the second maxillae (Price et al., 1983). The feeding current propels the animals forward and draws food towards the mouth appendages. Prey is perceived remotely by using chemical signals, and the feeding current is redirected such that the detected prey comes within reach of the second maxillae that capture the prey (Andrews, 1983; Kiørboe, 2011a; Koehl and Strickler, 1981). For this reason, these feeding currents have been termed 'scanning currents' (Childress et al., 1987). Several authors have studied the flow patterns (e.g., van Duren et al., 2003; Jiang et al., 2002a, b), fluid mechanical aspects (Alcaraz et al., 1980), morphology of the flow field (e.g., Strickler, 1982; Tiselius and Jonsson, 1990; Yen and Strickler, 1996), and prey capture modes (Bruno et al., 2012; Bundy and Vanderploeg, 2002) in relation with the feeding currents. The analysis of the flow field around a copepod that generates a feeding current showed a conical stream tube extending from the 'capture area' of the copepod's feeding appendages (Jiang et al., 2002b) with chemo- and mechanoreceptors receiving input from fairly well-defined locations (Yen, 2000). A steady laminar flow was observed along the body of T. longicornis with highest velocity and acceleration rates measured around the tip of the feeding appendages (Van Duren et al., 2003). A holographic Particle Image Velocimetry analysis of the flow field of a Diaptomus minutus female showed a recirculating pattern of the feeding current that required the animal to hop occasionally in order to sample a new water volume (Malkiel et al., 2003).

The ambush predator copepods detect the prey remotely though their hydromechanical signals and initiate an attack jump (Jonsson and Tiselius, 1990; Svensen and Kiørboe, 2000; Jiang and Paffenhöfer, 2008). Prey detection in active ambush-feeding copepods has been studied in details in copepods of the genera Acartia (Jonsson and Tiselius, 1990) and Oithona (Svensen and Kiørboe, 2000). Direct high-
speed video observations of prey attack and capture in small free-swimming *Acartia tonsa* and *Oithona davisae* females showed that the attacks are successful and feasible because of their rapidity and precision (Kiørboe *et al.*, 2009), as also observed in *O. plumifera* (Paffenböfer and Mazzocchi, 2002). For the two copepod species studied, Kiørboe *et al.* (2009) found that a typical attack was instigated when the prey was within approximately 0.2 mm from the copepod antennules. The prey was approached with a very rapid jump (at speeds of about 100 mm s⁻¹). Due to this rapid forward lunge of the copepod, the prey remains almost unaffected until the feeding basket opens and the prey is accelerated toward the mouth. The feeding appendages then reverse, thus enclosing the prey.

The calanoid copepods of interest for the present study, i.e., *Acartia clausi*, *Centropages typicus*, *Paracalanus parvus* and *Temora stylifera* are common species in marine coastal regions. In the Gulf of Naples, they occur with peak abundance in different seasonal periods (see Chapter 2). Therefore, these species interact with different phytoplankton communities and bloom-forming species.

*Acartia clausi* is known to show a switching behaviour between suspension and ambush-feeding modes depending on food availability (Kiørboe *et al.*, 1996; Gismervik and Andersen, 1997). A jump–sink behaviour has been commonly observed in *A. tonsa* (Jonsson and Tiselius, 1990; Tiselius, 1992) and *A. clausi* (Tiselius and Jonsson, 1990; Saiz and Alcaraz, 1992). Jonsson and Tiselius (1990) reported two different types of feeding behaviors for *A. tonsa* at different food concentrations: a more raptorial feeding behavior at lower food concentration (spending most of the time in passive sinking), and a typical suspension feeding at higher food concentration.

*Centropages typicus* displays a flexible behavioural repertoire which includes a raptorial component (Cowles and Strickler, 1983) and a passive sinking (Tiselius and
Jonsson, 1990). This species is known to exhibit both the ambush as well as suspension feeding modes, depending on the characteristics of the prey, with a selective preference for large motile prey, such as ciliates or dinoflagellates, enhanced under moderate intensities of turbulence (Tiselius and Jonsson, 1990). The 2nd maxillae in *C. typicus* are prehensile, with the setae on the basal segments serving as a filter but those on the endopod acting as a grasping organ (Anraku and Omori, 1963). Both mechano- and chemoreceptors are used for prey detection in *C. typicus*. These structures are also reported to be involved in particle handling (mechanoreceptors), speed increasing of the feeding current and food recognition (chemoreceptors) (Calbet *et al.*, 2007; Poulet and Gill, 1988).

*Paracalanus parvus* has a suspension feeding behaviour associated to a slow cruising motion (Price *et al.*, 1983; Buskey, 1984; Wong, 1988) and allocates most of its time to movement of mouthparts. The feeding appendages comprise a non-differentiated maxilla and display a monotonous, suspension-feeding behaviour suitable for catching small, non-motile prey (Tiselius and Jonsson, 1990). The feeding currents result in an upwards thrust, and feeding bouts are interspersed with times of sinking, which give the copepod an almost constant net vertical position (Tiselius and Jonsson, 1990).

Recently, high-speed high-resolution video filming of *P. parvus* has been used to describe in detail how it detects, captures and handles the prey (Tiselius *et al.*, 2013). The copepod showed a highly regular motion of mouth appendages to create a feeding current, which was mainly generated by the motion of the antennae and the maxillipede. Prey particles were detected always within close proximity, or when touched with the setae of appendages. Upon detection, the capturing motion started when the particle was within the sweeping volume of the setae and in most cases the particle was within 5 μm.
The prey was captured actively and always started with a wide sweeping of the maxillipeds. The endite and endopod setae of the second maxilla and the epipod setae of the first maxilla were active in handling the cells. The maxilla moved at twice the frequency as compared to the other appendages and was responsible to bring the cell towards the mouth.

*Temora stylifera* can be considered as primarily suspension feeder, as reported for the congeneric species *T. longicornis* (Tiselius and Jonsson, 1990) and *T. turbinata* (Hwang and Turner, 1995). It is known to move continuously in a smooth manner while creating a feeding current (e.g., Paffenhöfer *et al*., 1996; Paffenhöfer and Loyd, 1999). The nauplii of *T. stylifera* are also known to move their appendages continuously to produce a feeding current and a screw-like swimming trajectory (Jiang and Paffenhöfer, 2004). In case of *T. turbinata*, the feeding current helps the copepod to cruise through the water by continuously pulling it forward (Wu *et al*., 2010).

The existing studies on feeding behaviour of the target copepod species have thus examined different aspects of prey perception, capture, and handling and revealed the complex and diverse feeding mechanism like prey switching and selectivity based on prey properties and abundance. The behavioural flexibility allows the copepods to modify the efficiency of prey searching by modulating the time allocated to swimming, sinking and feeding. Selecting the best feeding strategy and modifying motility in response to the ambient food type and concentration is necessary to balance the gain and losses of energy.

Due to their different feeding strategies, it is likely that the target copepod species impact diatom standing stock in different ways. Moreover, their feeding behavior may also contribute to differentiate their impact on the bloom dynamics. In the present study, the individual feeding behaviour of the target copepod species was investigated at small
scale using a high-speed camera and the role of the feeding interactions were considered in the context of diatom bloom scenario. In particular, the feeding response and different components of motion behaviour of copepods in presence of different bloom-forming diatom species were examined. The final goal was to relate the small-scale interactions at individual level to higher population level and explaining the role of the species feeding behaviour in the bloom dynamics. The quantitative and qualitative results acquired from this study should also help interpreting some of the results obtained from the grazing experiments presented in Chapter 3.

The main questions addressed in this chapter are:

1) What is the effect of feeding on different bloom-forming diatom species on the motion pattern of the target copepods?

2) To what extent does diatom morphology influence feeding at bloom concentration?

The copepods may show different behavioral modalities when feeding on different blooming species (e.g., large or chain forming diatoms versus small single-celled ones), which may in turn affect the feeding rates. The four diatom species of interest for the present study exhibit morphological differences pertaining to size, shape and colonial occurrence. As reported in Chapter 2 with more details, the two closely related species used in this study, *Leptocylindrus aporus* and *Leptocylindrus danicus*, have a basic cylindrical shape, but, vary in cell size and type of occurrence. *L. aporus* is a predominantly solitary species, occasionally forming short colonies, while, *L. danicus* forms long colonies of up to 20 cells. *Chaetoceros socialis* has also a cylindrical shape and forms curved chains, but the corners of each cell have long spines which intertwine with the spines of other cell, that group together to form spherical colonies. *Pseudonitzschia calliantha* cells are needle-shaped and form small stepped-chains by aligning tip-to-tip.
### Table 4.1. Studies on feeding behavior of different copepod species based on direct video observations using different cinematographic techniques listed in chronological order.

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4.2 Materials and methods

4.2.1 Copepod collection and acclimation

The behavioural study was conducted on three of the four target copepod species, i.e., *Acartia clausi*, *Centropages typicus* and *Temora stylifera*. The species *Paracalanus parvus* was not investigated in this case because of the low population abundance in that year. On the other hand, the individual feeding behavior of this species had been recently analyzed by Tiselius *et al.* (2013).

The copepods used for the video observations were collected during April-August 2013 from the coastal site LTER-MC (40°48.5'N, 14°15'E) in the inner Gulf of Naples by using a 200 μm Nansen net with 5L non filtering cod end in vertical or oblique tows (Table 4.2). Actively swimming adult females of the target copepod species were sorted in the laboratory from the zooplankton samples and acclimated for at least 24 hours to temperature and photoperiod that simulated the *in situ* conditions, as it was done for the grazing experiments presented in Chapter 3. During the acclimation period, females were kept in 5 L glass jars and fed on natural plankton assemblage collected using Niskin bottles at depth of chlorophyll maximum from the same location as the copepods.

4.2.2 Experimental conditions

For the experiments, healthy adult female copepods were selected and transferred into FSW in a 500 ml glass beaker and transported to the video–recording room. They were again acclimated in FSW for 1 h to the temperature and light condition used for recording, which were the same as those of the respective grazing experiments (Table 4.2). The experiment with *C. typicus* and *A. clausi* were carried out at 16 °C, while those with *T. stylifera* were carried out at 20 °C.
A known number of females were then transferred into either a small cuvette or a larger flask for video observations. The types of observational container and the copepod density were chosen after several trials and tests and represented a compromise between several factors that had to be considered for allowing the video recording, i.e., the size and swimming modes of the copepod species, and the need of keeping in focus single individuals for a sufficient time lapse. The results of these preliminary tests indicated that a larger flask (corning 70 x 48 x 20 mm, 75 ml) was suited for recording *T. stylifera*, taking into consideration its relative size and swimming behavior to attain the appropriate density for filming. For *A. clausi* and *C. typicus*, the smaller cuvette (Hellma 101-QS Suprasil Quartz, 45x12.5x12.5 mm, 4.5 ml) was more suited for recording.

In the cuvette, the density was 1-2 copepods ml\(^{-1}\), while in the flask the density was \(~1\) copepod ml\(^{-1}\). This copepod density was much higher (\(~350\) times) than the total copepod abundance ever recorded at LTER-MC, but, it was justified by the need to increase the probability of having the copepods in focus and to ensure sufficient numbers of trajectories for analysis.

During the video recording, copepods were fed on monospecific diets of the diatoms *Chaetoceros socialis*, *Leptocylindrus aporus*, *Leptocylindrus danicus* and *Pseudo-nitzschia calliantha*, which were cultured as described in section 3.2.1 (Chapter 3) and offered at bloom concentrations (Table 4.2). A new set of copepods and diatoms was used every 30 min of recording (always followed by acclimation as described above prior to recording). This change was necessary to eliminate the interference of the fecal pellets produced by the copepods. Changing the diet also ensured that the copepods were not feeding in a changed food environment (chains of colonial diatoms
were prone to mechanical breakage due to physical contact of the copepods during swimming).

**Table 4.2** Overview of the experiments performed to record the feeding behavior of three target copepod species in different food conditions.

<table>
<thead>
<tr>
<th>Copepods</th>
<th>Container</th>
<th>Date</th>
<th>Diatoms</th>
<th>Diatom conc. (10^6 cells L^-1)</th>
<th>Carbon conc. (µg C L^-1)</th>
<th>No. of cells in colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acartia clausi</em></td>
<td>cuvette</td>
<td>12/06/2013</td>
<td><em>Leptocylindrus aporus</em></td>
<td>29.8±7.5</td>
<td>702.65</td>
<td>1-3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13/06/2013</td>
<td><em>Leptocylindrus</em></td>
<td>14.7±4.8</td>
<td>598.32</td>
<td>1-10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20/06/2013</td>
<td><em>Pseudo-nitzschia</em></td>
<td>78.6±7.9</td>
<td>715.54</td>
<td>1-3</td>
</tr>
<tr>
<td><em>Centropages typicus</em></td>
<td>cuvette</td>
<td>12/05/2013</td>
<td><em>Chaetoceros socialis</em></td>
<td>24.3±5.4</td>
<td>762.33</td>
<td>1-11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12/05/2013</td>
<td><em>Leptocylindrus aporus</em></td>
<td>30.6±4.3</td>
<td>746.15</td>
<td>1-5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>09/05/2013</td>
<td><em>Leptocylindrus</em></td>
<td>15.5±5.9</td>
<td>727.21</td>
<td>1-14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24/05/2013</td>
<td><em>Pseudo-nitzschia</em></td>
<td>40.8±4.7</td>
<td>812.59</td>
<td>1-4</td>
</tr>
<tr>
<td><em>Temora stylifera</em></td>
<td>flask</td>
<td>28/08/2013</td>
<td><em>Chaetoceros socialis</em></td>
<td>3.27±0.4</td>
<td>134.28</td>
<td>1-18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21/08/2012</td>
<td><em>Leptocylindrus aporus</em></td>
<td>2.44±0.1</td>
<td>122.89</td>
<td>1-7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>29/08/2013</td>
<td><em>Leptocylindrus</em></td>
<td>1.85±0.1</td>
<td>118.62</td>
<td>1-17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22/08/2013</td>
<td><em>Pseudo-nitzschia</em></td>
<td>4.17±0.6</td>
<td>146.35</td>
<td>1-5</td>
</tr>
</tbody>
</table>

### 4.2.3 Video equipment and setup

The video recording set-up (Fig. 4.1) used to study the small-scale feeding behavior of the target copepods was assembled at Stazione Zoologica Anton Dohrn, Naples (SZN). It consists of a high-speed (HS) camera system mounted on a stereozoom microscope and a light source (Fig 4.2). The whole set-up was based in a temperature-controlled room to simulate the *in situ* conditions.
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Figure 4.1 Overview of the experimental setup used for recording the small-scale feeding behavior of target copepods with a high-speed camera.

Figure 4.2 A close view of the different components of the recording set-up positioned linearly A) camera head, B) microscope C) observational containers with copepods D) light source.
The HS camera was a Photron Fastcam MC2 (512 x 512 pixel resolution at frame rates up to 2,000 fps) with a 2 GB memory (8 seconds recording time at 1,000 fps). The camera was connected with a cable to a personal computer (PC) run on Windows XP operation system. The PC was assembled on a 054KM3 Intel motherboard, 4GB DDR3 RAM and has a storage space of 1.5 TB. The camera head was mounted on an Olympus SZ series (SZ40) stereomicroscope. The feeding events were recorded at different magnifications between 10X-30X which allowed observing the details of copepod feeding activity. The realized field of view (in the center of the observational container far from the walls) was therefore variable and ranged between 100 mm² at 10X and 11 mm² at 30X with a spatial resolution of 20.45-6.49 μm/pixel for the different magnification levels.

The camera was controlled through a Gigabit Ethernet PC interface using a Photron FASTCAM Viewer (PFV) software, which allowed (1) setting camera options, i.e., recording and display settings (2) recording of the videos to the camera memory (3) saving the video sequences recorded on the camera to the PC.

The set up used for recoding the behaviour is shown in Fig. 4.2. A modified microscope stage with control knobs which allowed an X (right and left) and Y (up and down) movement was used for placing the container with the copepods to be filmed. This arrangement was useful to fine adjust the position of the container every time the magnification was changed. Illumination was provided by a 50 watt halogen bulb placed opposite to the observational container. The microscope equipped with the camera head, the stage and the light source were all secured on a 60 x 90 cm Kinetic System inc. Vibro-Plane 9100 table to avoid mechanical vibrations (Fig. 4.1). A monochrome monitor was used simultaneously for live viewing of the videos during recording (Fig. 4.1).
4.2.4 Video recording and analysis

The copepods were recorded only when occurring in the center of the observational container, far from the walls, at frame rates of 250 or 500 frames s⁻¹ (520 x 520 pixels) at different magnifications between 10 and 30X. The camera was manually triggered whenever the copepod appeared in focus and filmed at a pre-set frame rate. The videos were saved as AVI files for analysis.

Only the video sequences with copepods clearly in focus were considered for further analysis. Each of the selected video sequences was analyzed frame by frame using the software ImageJ (National Institutes of Health, USA) to acquire quantitative data of parameters related to copepod swimming and feeding behaviour.

For the three copepod species studied, the response to different monospecific diets was estimated in terms of the proportion of the time allocated towards different behaviors related to motion, i.e., feeding, grooming and other (escape response or any other behavior). In case of T. stylifera, the response was also estimated through changes in the frequency of appendage beating in presence of different diets. All the three copepods tested, showed suspension feeding mode, i.e. created feeding currents with appendage movement. However, each copepod species showed a characteristic feeding behaviour, different from the others. For A. clausi, brief bouts of appendage movement accounted to feeding. In case of C. typicus, the copepod created feeding current while swimming, hence the time allotted to feeding refers to swimming and appendage movement together. While, for T. stylifera, time spend in appendage movement which generated a feeding current is equivalent to feeding.

The following behaviors were identified when the copepod was in motion: swimming as an active movement using the cephalic appendages; sinking as a downward passive displacement due to gravity; hovering, when the copepod remained
stationary in the water with minimal or no movement of appendages; *jumping* as a very rapid movement of the copepod between two successive points.

*Grooming* was described for all the copepods and its frequency in different diets was reported. Other behaviours included mainly *escape reactions*, when the copepod propelled itself forward at a high speed using its swimming legs for rapid acceleration. Since this behaviour did not appear to be directly linked to feeding, it was omitted from further discussion. Motion, feeding and grooming were reported for the copepod species studied; however, not all the movements pertaining motion and not all the parameters related to feeding were always exhibited by the three copepod species.

The pixel to micrometer conversion factor was obtained by recording a graduated transparent graph millimeter paper on the different observational containers and at the different magnifications used for recording. The distance (in μm) between the graduated lines on the paper was measured in pixels using Image J software.

### 4.2.5 Data analysis and statistics

The behavioural parameters mentioned in the previous section were first quantified for each diet in terms of frames, which were later converted to milliseconds. The time spent by copepods in motion, feeding, grooming and other states for each diet offered were calculated in terms of percentages of total time for which the copepods were in focus. Percentage data on the copepod behavioral time budgets for different diets was arcsine transformed (Sokal and Rohlf, 1995) and further tested with One-way ANOVA. The analysis was then completed by a post-hoc multiple comparison test (Tukey test) to identify the statistically different groups. The values of beat frequency are reported as average with standard error. The difference in beat frequencies among the diets was also tested with One-way ANOVA followed by post-hoc Tukey test.
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The frequencies and duration of the feeding bouts jump, and grooming were compared with One-way ANOVA. The significance level for all the analysis performed was always set at 5%. The statistical analyses were conducted using Graphpad Prism 4 software.

4.3 Results

The individual feeding behaviour of *Acartia clausi*, *Centropages typicus* and *Temora stylifera* feeding on different diatom diets was analyzed in 232 videos recorded with high speed camera over a total recording time of 680.3 s. The details of recording are presented in Table 4.3.

**Table 4.3** Details of recording for each copepod species fed on different monospecific diets.

<table>
<thead>
<tr>
<th>Species</th>
<th>Diet</th>
<th>No. of video clips analyzed</th>
<th>Duration of each video clip (s) (avg ± stdev)</th>
<th>No. of individuals recorded</th>
<th>Total recording duration (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. clausi</em></td>
<td><em>C. socialis</em></td>
<td>22</td>
<td>3.7 ± 2.2</td>
<td>26</td>
<td>66.01</td>
</tr>
<tr>
<td></td>
<td><em>L. aporus</em></td>
<td>8</td>
<td>4.6 ± 2.3</td>
<td>13</td>
<td>30.43</td>
</tr>
<tr>
<td></td>
<td><em>L. danicus</em></td>
<td>25</td>
<td>3.7 ± 2.2</td>
<td>29</td>
<td>92.23</td>
</tr>
<tr>
<td></td>
<td><em>P. calliantha</em></td>
<td>26</td>
<td>3.2 ± 1.8</td>
<td>32</td>
<td>74.61</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>81</strong></td>
<td></td>
<td></td>
<td><strong>263.28</strong></td>
</tr>
<tr>
<td><em>C. typicus</em></td>
<td><em>C. socialis</em></td>
<td>14</td>
<td>2.2 ± 1.5</td>
<td>16</td>
<td>33.38</td>
</tr>
<tr>
<td></td>
<td><em>L. aporus</em></td>
<td>9</td>
<td>1.4 ± 0.7</td>
<td>11</td>
<td>12.04</td>
</tr>
<tr>
<td></td>
<td><em>L. danicus</em></td>
<td>17</td>
<td>2.8 ± 1.7</td>
<td>23</td>
<td>41.90</td>
</tr>
<tr>
<td></td>
<td><em>P. calliantha</em></td>
<td>16</td>
<td>8.2 ± 4.5</td>
<td>16</td>
<td>45.10</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>56</strong></td>
<td></td>
<td></td>
<td><strong>132.42</strong></td>
</tr>
<tr>
<td><em>T. stylifera</em></td>
<td><em>C. socialis</em></td>
<td>22</td>
<td>4.2 ± 1.6</td>
<td>30</td>
<td>99.66</td>
</tr>
<tr>
<td></td>
<td><em>L. aporus</em></td>
<td>22</td>
<td>5.4 ± 2.4</td>
<td>40</td>
<td>161.69</td>
</tr>
<tr>
<td></td>
<td><em>L. danicus</em></td>
<td>26</td>
<td>5.8 ± 1.7</td>
<td>59</td>
<td>183.32</td>
</tr>
<tr>
<td></td>
<td><em>P. calliantha</em></td>
<td>25</td>
<td>7.6 ± 3.0</td>
<td>35</td>
<td>235.56</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>95</strong></td>
<td></td>
<td></td>
<td><strong>680.23</strong></td>
</tr>
</tbody>
</table>
4.3.1 Acartia clausi

Over a total recording time of 263.28 s from 81 videos (Table 4.3), A. clausi, showed a suspension feeding behaviour and distributed its time between motion, feeding and grooming activities (refer to video 4.1). The copepod was mostly observed in motion (94%); the time allocated to grooming (4%), and feeding (2%) was much less. The motion behaviour was mostly comprised of hovering (87%), followed by jumps (11%) and sinking (2%). During hovering, the copepod remained with A1 perpendicular to the body axis. The hovering was frequently interrupted by jumps made by flapping of the thoracic legs and then folding of the A1.

![Graph of A. clausi activities](image)

**Figure 4.3** Average time allocated to different activities by A. clausi fed on different monospecific diets at bloom concentration.

![Graph of motion behaviors](image)

**Figure 4.4** Average time allocated to different motion behaviors by A. clausi fed on different monospecific diets at bloom concentration.
The copepod did not show any significant difference in the total time allotted to different behaviors when feeding on different diets (Fig. 4.3). Also, the time allotted to the different motion activities was similar across all diets. Sinking behaviour was only observed for the copepod for the *L. danicus* diet (Fig. 4.4). The only significant difference observed was in the duration and frequencies of jumps. The highest jump frequency was for *L. aporus* (229 jumps min⁻¹), while the lowest was for *C. socialis* (119 jumps min⁻¹). Instead, the jump duration was highest for *P. calliantha* (42 ms) and lowest for *L. aporus* (34 ms) (Table 4.4).

**Table 4.4** Swimming behaviour of *A. clausi* reported as percentage of time allocated to swimming, jumps and grooming. The duration and frequency for jumps (avg. ± SE) are computed by averaging the values from each video.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Swimming Bout</th>
<th>Jump</th>
<th>Grooming</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Duration ms</td>
<td>Frequency min⁻¹</td>
<td>Duration ms</td>
</tr>
<tr>
<td><em>C. socialis</em></td>
<td>230.3±65.7</td>
<td>25.9±3.0</td>
<td>35.1±1.0</td>
</tr>
<tr>
<td><em>L. aporus</em></td>
<td>75.0±19.0</td>
<td>22.0±10.1</td>
<td>34.2±2.2</td>
</tr>
<tr>
<td><em>L. danicus</em></td>
<td>112.3±23.4</td>
<td>57.1±11.9</td>
<td>35.2±1.9</td>
</tr>
<tr>
<td><em>P. calliantha</em></td>
<td>90.4±20.9</td>
<td>55.6±17.8</td>
<td>41.9±2.4</td>
</tr>
</tbody>
</table>

The feeding behaviour could be attributed to occasional brief feeding bouts characterized by appendage movement while in hovering phase, which probably created feeding current. The occurrence of these feeding bouts was too rare across all the diets to provide enough frames for estimating the beat frequency. The behaviour could be however described as suspension feeding distinguished by the intermittent beating of cephalic appendages (A2 and maxillae) and the swimming legs (Fig. 4.5). The average duration of feeding bouts ranged between 75-230 ms, but the values did not vary significantly for the different diets (Table 4.4). In addition to motion and feeding, *A.*
*A. clausi* also displayed grooming during all diets except for *L. danicus*. Grooming, involved cleaning of the A1, as they were drawn between the swimming legs and the mouth appendages, a process which lasted for ~829 ms on average (Fig. 4.6). The copepod did not show any significant difference in the duration and frequency of grooming for the different diets (Table 4.4).

Figure 4.5 Appendage movements of *A. clausi* during a feeding bout. The sequence of images is extracted from high-speed video recorded at 500 fps at different time intervals.
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Figure 4.6 Typical grooming behaviour of *A. clausi* showing different stages involved in cleaning of the antennulae. The sequence of images at different time intervals was extracted from the high-speed video recorded at 500 fps.

4.3.2 *Centropages typicus*

The behaviour of *C. typicus* was analyzed over a total recording time of 132 seconds (Table 4.3). For all the diets, the copepod showed a suspension feeding mode involving feeding current. In case of *C. typicus*, swimming and feeding are closely linked and therefore, the time allotted to feeding is considered to be equivalent to the time allotted to swimming and appendage movements. Motion and feeding together comprised 88% of total time budget, while grooming made up the remaining 12%. Of the total time spend in motion, 49% was allocated to sinking, followed by 44% to swimming and 7% to jumps. The division of the time for each of these activities
changed according to the diets; however, there was no significant difference in the time allotted to swimming and sinking across all the diets (Fig. 4.7).

![Figure 4.7 Average time allocated to different motion behaviors by *C. typicus* fed on different monospecific diets at bloom concentration.](image)

The time allotted to jumps varied significantly according to the diets, with the highest for *C. socialis* (10.3%) and lowest for *P. calliantha* (3.4%). There was however no significant difference in the duration and frequency of jumps (Table 4.5). Swimming was due to the rhythmic beating of cephalic appendages (A2, maxillae and maxillipeds). The beating of the appendages not only caused displacement of the copepod, usually in a straight path, but, also generated a feeding current. On a few occasions, the copepod also created feeding current while in a brief stationary position. Feeding, in this case, is therefore assumed to be equivalent to the bouts of swimming together with the feeding currents when the copepod was in stationary position. The swimming bouts were interrupted by periods of sinking and jumps and grooming. During sinking, the movement of the appendages ceased and the copepod remained motionless. The jumps were caused by flapping of the A1 and the swimming legs (refer to video. 4.2).
Table 4.5 Duration and frequency of swimming bout, jumps and grooming of *C. typicus* for different diets reported as avg. ± SE.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Swimming Bout</th>
<th>Jump</th>
<th>Grooming</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Duration</td>
<td>Frequency</td>
<td>Duration</td>
</tr>
<tr>
<td></td>
<td>ms</td>
<td>min⁻¹</td>
<td>ms</td>
</tr>
<tr>
<td><em>C. socialis</em></td>
<td>623.7±55.2</td>
<td>94.77±11.4</td>
<td>68.0±4.0</td>
</tr>
<tr>
<td><em>L. aporus</em></td>
<td>446.4±104.3</td>
<td>173.9±48.2</td>
<td>56.0±5.5</td>
</tr>
<tr>
<td><em>L. danicus</em></td>
<td>901.7±48.5</td>
<td>133.6±40.2</td>
<td>52.5±3.2</td>
</tr>
<tr>
<td><em>P. calliantha</em></td>
<td>1345.6±238.7</td>
<td>119.3±26.5</td>
<td>39.6±5.2</td>
</tr>
</tbody>
</table>

The duration of swimming/feeding bouts ranges between 446 ms (*L. aporus*) and 1346 ms (*P. calliantha*). The frequency of bouts was highest for *L. aporus* and lowest for *C. socialis* (Table 4.5). In spite of this variation in the values of both duration and frequency of swimming bout and jumps, there was no significant difference among diets. This may be due to the high standard error value due to the high degree of variability in the values for each individual, which were used to calculate the mean, and to the variable sample size. Grooming consisted of the A1 been drawn down to the body and “cleaned” by the brushing action of the cephalic appendages (Fig. 4.8). The duration of grooming ranged between 524 ms (*L. aporus*) to 714 ms (*L. danicus*). The frequency of grooming was significantly different among the diets; it was highest for *L. aporus* (83.5 min⁻¹) and lowest for *P. calliantha* (29.2 min⁻¹) (Table 4.5).
Figure 4.8 Typical grooming behaviour of *C. typicus* showing different stages involved in cleaning of the antennules. The sequence of images at different time intervals was extracted from the high-speed video recorded at 500 fps.
4.3.3 *Temora stylifera*

The different behaviours of *T. stylifera* females fed on monospecific diatom diets were analysed based on 95 video clips corresponding to 680.23 seconds of recording time (Table 4.3). The videos showed that this species acquires food particles via suspension feeding mode. The feeding current was generated by the continuous synchronized beating of all the cephalic appendages except the antennules (A1s), while in a stationary or hovering position. The long bouts of feeding were interrupted briefly by motion and grooming (refer to video 4.3). The copepod occasionally cruised through the field of view. The feeding current also caused the copepod to move, but at a very slow speed, either in straight path or in small smooth loops. The motion behavior comprised swimming and jumps. Grooming was consistently observed for all the diets (Fig. 4.9). Grooming initiated with the copepod drawing the A1s anteriorly between the swimming legs and the mouthparts along the length of the body. The maxillipeds then extend outwards from the other appendages to grab the A1s. At this point, the swimming legs moved backward along the A1s and the A1s are then pulled under the maxillipeds (cleaned). The A1s and the swimming legs then return to their normal positions. The whole process occurred over an average time of 205±76.4 ms.

Feeding accounted for the majority (88.1 %) of the copepod behavioural repertoire in all the diets. Motion (9.3 %), including slow swimming (cruising?) and jumps, grooming (2.1%) and other activities (0.5 %) made the remaining time budget, across all diets. The time allotted to these different behaviors varied significantly among diets (Fig. 4.10).
Figure 4.9 Typical grooming behaviour of *T. stylifera* showing different stages involved in cleaning of the antennulae. The sequence of images at different time intervals was extracted from the high-speed video recorded at 500 fps.
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Figure 4.10 Average time allocated by *T. stylifera* to different activities (feeding, swimming, grooming and others) when fed on different monospecific diets at bloom concentration.

Figure 4.11 Average time allocated to different motion behaviors by *T. stylifera* fed on different monospecific diets at bloom concentration.
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The time allotted to swimming and jumps during motion did not vary significantly among the different diets (Fig. 4.11). However, the jumps duration and frequency varied significantly (Table 4.6). The copepod showed less frequent, but longer duration jumps for *P. calliantha* as compared to the more frequent, short duration jumps for other diets. Similarly, the copepods did not show any significant difference in the time allotted to feeding for the different diets, but, the duration and the frequency of the feeding bout varied significantly (Fig. 4.10 and Table 4.6).

**Table 4.6** Duration and frequency of swimming, jumps and grooming for *T. stylifera* (avg. ± SD) on the different diets.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Swimming bout</th>
<th>Jump</th>
<th>Grooming</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Duration ms</td>
<td>Frequency min⁻¹</td>
<td>Duration ms</td>
</tr>
<tr>
<td><em>C. socialis</em></td>
<td>1335.2±1078.5</td>
<td>70.9±57.4</td>
<td>49.6±10.7</td>
</tr>
<tr>
<td><em>L. aporus</em></td>
<td>1977.9±1411.8</td>
<td>47.2±41.8</td>
<td>47.4±13.8</td>
</tr>
<tr>
<td><em>L. danicus</em></td>
<td>1248.1±1045.6</td>
<td>73.9±48.3</td>
<td>46.4±13.6</td>
</tr>
<tr>
<td><em>P. calliantha</em></td>
<td>3656.1±3201.5</td>
<td>28.3±24.9</td>
<td>58.4±15.7</td>
</tr>
</tbody>
</table>

The duration of feeding bout was more than twice longer for *P. calliantha* than for *C. socialis* and *L. danicus*. Inversely, the feeding bout frequency was much higher for *C. socialis* and *L. danicus* than for *P. calliantha*. To compare the feeding behaviour of the copepods in different diets, the beat frequencies of antennae or the mandibular palps of at least 6 individuals was measured for each diet. The copepods showed significant difference in the beat frequencies when feeding on the different diets. The highest beat frequency was reported for *L. danicus* (57.4 Hz) and the lowest for *L. aporus* (41.4 Hz) (Fig. 4.12).

Similarly, the copepods also showed significant difference in the total time allotted to grooming (Fig. 4.10). This difference can be attributed to the change in the
mean duration of grooming for the different diets. The duration of grooming was longest for *P. calliantha* whereas it was shortest for *L. danicus*. The frequency of grooming was however similar for all the diets (Table 4.11)

![Beat frequencies of the feeding appendages of *T. stylifera* when fed on different diatom diets (avg + standard error).](image)

**Figure 4.12** Beat frequencies of the feeding appendages of *T. stylifera* when fed on different diatom diets (avg + standard error).

### 4.4 Discussion

#### 4.4.1 *Acartia clausi*

The small-scale behavior of *A. clausi* in presence of monospecific diatom diets can be described as mostly passive (hovering), frequently interrupted with jumps and brief periods of feeding bouts. This is in accordance with the free-swimming behaviors of this species as previously observed with other video equipment at different scales (Rosenberg, 1980; Saiz and Alcaraz, 1992). Feeding was characterized by occasional feeding bout with brief movements of the appendages. The pattern of appendage movement observed can be related to suspension feeding mode (Kiorboe *et al.*, 1996). Such kind of feeding has been previously reported for adults (Rosenberg, 1980) and copepodites (Takahashi and Tiselius, 2005) of *A. clausi*, and the congeneric *A. tonsa*
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(Jonsson and Tiselius, 1990; Saiz, 1994). The generation of feeding current in *A. clausi* is however different from other copepods, as it also involves the swimming legs in supplement to the "scoop-net' filtering action of the maxillae (Rosenberg, 1980). Kiorboe (2011b) reported a similar behaviour in *A. tonsa*, when the swimming legs move collectively backwards and forwards coordinated with the opening and closing of the feeding appendages, while the copepod is almost stationary, generating a rather fluctuating feeding current. The copepods of the genus *Acartia* are also capable of raptorial feeding behaviour and can switch between modes depending upon the prey type and concentration (e.g., Gismervik and Andersen, 1997; Jonsson and Tiselius, 1990; Kiorboe *et al.*, 1996; Saiz and Alcaraz, 1992).

During the present observations, the copepod spent most of its time in hovering for all the diets, followed by grooming and feeding, and the different diets did not affect the time budget for the different behaviours. These results are in agreement with those obtained from three-dimensional observations by Bianco (2010), who found no change in the behaviour of *A. clausi* with change in food condition. The time allotted to feeding was low (0.4-4.2%), and similar to what reported for *A. clausi* (5%) feeding on suspension of *T. weissfogii* (Tiselius and Jonsson, 1997). Similar response was observed for *A. clausi* fed on a suspension of diatoms, when the copepod spent less time (7%) in feeding at higher food concentration compared to low food concentration (Piontkovski and Petipa, 1976; Takahashi and Tiselius, 2005). However, different and contrasting results were also found for the same calanoid species. Rosenberg (1980) reported a feeding time of 31% and 44% of total time when feeding on natural particles and *Skeletonema costatum*, respectively. Higher values of 30% were reported for the congeneric *A. tonsa* (Jonsson and Tiselius, 1990). Similarly, Saiz (1994) reported much higher range (78-99%) of feeding time for *A. tonsa*, decreasing overall from low food
concentration to high food concentration. In the present study, the food quantity, which varied among diets, did not seem to affect the feeding time when offered at high bloom concentrations. These results are in agreement with those from the grazing experiments, when the copepod did not show any significant difference in feeding rates for the different diets.

The most significant difference in the copepod motion was observed for the jumps when both the jump frequency and the time allotted to jumps was significantly higher for the predominantly solitary *L. aporus* and *P. calliantha* as compared the colonial *C. socialis* and *L. danicus*. Although this response appears to be species-specific, it can also be related to the respective concentrations of the diatoms. Leising and Franks (2002) reported a similar response for *A. clausi* fed on variable concentrations of *Rhodomonas* sp. The authors found a significant increase in the time allocated to jumps as well as the frequency of jumps in presence of food as compared to the control without food. They suggested that such change in the copepod behavior in response to the presence of higher food resources is consistent with an area-restricted-search foraging strategy which allows the copepods to find and remain within patches of food. Tiselius (1992) also proposed this foraging strategy which involves the copepod finding an area of higher food resource, and stay within that area by modulating its motion behaviour, thus leading to higher resource intake. Changing the behaviour by varying the shape of the swimming path, speed of swimming and frequency of jumps, in response to high food concentrations has been observed for *A. clausi* (Piontkovski and Petipa, 1976) and *A. tonsa* (Tiselius, 1992) as well as other copepod species like *T. longicornis* (Tiselius and Jonsson, 1990), *Oithona davisae* (Uchima and Hirano, 1988), and *Clausocalanus furcatus* (Uttieri et al., 2008).
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The response of *A. clausi* to the high food concentration as for *L. aponus* and *P. calliantha* by increasing the frequency of jump seems consistent with the above observations. Such strategy may also be of ecological importance to exploit a rich food scenario like during a phytoplankton bloom, especially for a copepod like *A. clausi*, which does not depend on food reserve (lipid) (Kerambrun, 1987).

### 4.4.2 *Centropages typicus*

For all the diets tested, this copepod species showed a suspension feeding mode, with feeding current created while swimming with the help of cephalic appendages. In general, the motion behaviour was dominated by swimming and sinking with a small fraction of time allotted to jumps. Grooming also comprised an important part of the copepod behavioural repertoire. These observations agree with those of Cowles and Strickler (1983), who first described the different activities that make up the swimming behaviour of *C. typicus* (slow swimming, resting breaks, escape reactions, and grooming). Bianco (2010) also found a similar behaviour for *C. typicus*, where the copepod spent nearly 75% of its time in swimming. The author also reported a significant increase in the average swimming duration and a decrease in sinking in the presence of food. Motion behaviour, dominated by slow swimming and break (sink) was also reported for *C. hamatus* (Costello *et al.*, 1990; Hwang *et al.*, 1993). Slow swimming is not only a mean to perceive the food environment, but is also enhances particle encounter (Kiørboe and Saiz, 1995; Poulet and Gill, 1988). Encounter rates in turn depend on the velocity differences between the copepod and the prey, for which motility is a prerequisite. A copepod can generate a velocity difference by swimming in different pattern, modifying its motion behaviour or generation of feeding current (Kiørboe and Jiang, 2012; Kiørboe and Saiz, 1995). *Centropages* also creates feeding
current, which helps to detect potential prey. The sinking state, when the copepod sinks passively, allows the copepod to capture non-motile preys (algae) and exploit deeper water. Sinking can be therefore an effective way to increase feeding efficiency with less energy cost (Moison et al., 2009).

This species is known to display a flexible behavioral repertoire with both the ambush as well as suspension feeding modes depending on the characteristics of the prey (Tiselius and Jonsson, 1990). In general, *C. typicus* and other species of the same genus have consistently displayed selection for large motile prey in natural and laboratory feeding studies (e.g. Calbet et al., 2007; Caparroy et al., 1998; Paffenhofer and Knowles, 1980; Turner and Tester, 1989). This apparent preference for large phytoplankton reflects the appendage morphology of the copepod and has been linked to the distance between maxillary setae (Tomasini and Mazza, 1979). Regardless of the difference in the morphology and concentration of the diatom species used in this study, the copepod always exhibited suspension feeding. Also, the time allotted to feeding as well as the length and frequencies of the swimming bouts were not affected by the different diets. These results contrast to those of Caparroy et al. (1998), Bianco (2010) and Cowles and Strickler (1983) who found a change in the foraging effort with changing food environment. Cowles and Strickler (1983) found a relationship between the frequency and duration of slow swimming bouts and rests to the characteristics of the food and its abundance in *C. typicus*. Similar observations were made in studies on *C. velificatus* and *C. hamatus*, when the time allocated to slow swimming (creation of feeding currents), as well as the frequency and amplitude at which cephalic appendages beat were directly related to the concentration of food, and inversely to the time at rest and the duration of swimming bouts (Tiselius and Jonsson, 1990). However, the comparison of the above results with other studies must be done with caution since the
food environment used in the other studies were different. While, Caparroy et al. (1998) and Cowles and Strickler (1983) used dinoflagellates *Strombidium sulcatum* and, Tiselius and Jonsson (1990) used nauplii as food. In case of Bianco (2010), the experiment were carried out with the diatom *Skeletonema pseudocostatum* and phytoflagellates, both not comparable in size with the diatoms used in the present study.

The only difference observed in the present study in *C. typicus* motion in relation to the different diets was the change in the parameters of jumps. The copepod jumped less frequently and allotted the least of its time to jumps for *P. calliantha*, as compared to the other diets. The increase in the jump frequency could be a response to the higher cell concentration of *P. calliantha*. In a study to investigate the relationship between the swimming of *C. velificatus* and encounter rates at a high food concentration, Bundy et al. (1993) reported alternate bouts of swim-sink, with vertical upward movements (jumps) after sinking, in order to search more efficiently a given water volume. This observation may also be applied to *C. typicus* in this current study while feeding on a high concentration of *P. calliantha* cells. This can further be validated by the fact that the copepod allotted more time to sink (62%) accompanied with relatively longer swimming/feeding bouts for *P. calliantha* as compared to the other diets.

### 4.4.3 *Temora stylifera*

*T. stylifera* spent most of the time in a suspension feeding mode, creating feeding currents by continuous beating of the feeding appendages in a hovering position. Such kind of suspension feeding has been previously reported for several calanoid copepods across many genera including *Temora* (Frost et al., 1983; Jonsson and Tiselius, 1990; Koehl and Strickler, 1981; Tiselius and Jonsson, 1990). A 3D motion analysis of *T. stylifera* has also shown that the copepod spends nearly 99% of its time in slow swimming, generating feeding current (Bianco, 2010). van Duren and Videler, (1995)
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reported that *T. longicornis* spent nearly 100% time spend in slow cruising or in a stationary position generating feeding current to collect food particles. The metachronal beating pattern of the cephalic appendages in *T. longicornis* involves the mandibular palps and the maxillules which produce the feeding current, while the maxillae and the maxillipeds appear to aid in filtering or grasping the particles from the feeding current (van Duren *et al.*, 2003). The hovering position is known to increase the efficiency of feeding by about 50%; the feeding current balances gravity and the copepod is almost stationary in the water, a strategy suitable when feeding on small non-motile prey (Strickler, 1982; Tiselius and Jonsson, 1990).

The beating of appendages in copepods not only generates a quasi-steady feeding current, but also provides propulsion (Jiang *et al.*, 2002b). In several other calanoid copepods, which generate feeding current, the specific motion pattern of the appendages has been attributed to the biramous nature of their feeding appendages (i.e., antennae, mandibles and maxillules) (Gauld, 1966; Paffenhöfer and Lewis, 1990). The highly precise and clearly timed coordinated beating of the endopodites and exopodites of the feeding appendages generate the feeding current, while the characteristic asymmetry in the propulsion and recovery stroke of the feeding appendages aid the copepod in swimming (Strickler, 1984). The time allocated to the beating of the mouth parts are therefore closely linked with the feeding and motion behaviour. Food availability (Jonsson and Tiselius, 1990) and concentration (Paffenhöfer and Loyd, 1999) are important factors that are known to influence the appendage beating and motion behaviour in copepods.

*T. stylifera* showed a suspension feeding mode when fed on the different diets. The different diets did not affect the total time allocated by this species to feeding; however, there were significant differences in the length and frequency of the feeding
bouts as well as beat frequency of the feeding appendages. These differences indicate that the food environment is a critical factor which can affect the small-scale behavior in *T. stylifera*. In this study, the copepods used more frequent but short-duration feeding bout when feeding on the colonial diatoms *C. socialis* and *L. danicus*, instead of less frequent but long-duration feeding bouts for the predominantly solitary *L. aporus* and *P. calliantha*. This pattern can be attributed to both the respective concentration and the morphology of the diatoms offered, which may result into different encounter rates. At low concentrations, with large cells, the copepod might require a wider screening of the environment to increase the encounter rates to acquire more cells to feed and therefore, results in an extended period of swimming bouts. Copepods can modify both their feeding and swimming behaviour by varying the flow field of the feeding current by changing the beating of the mouth appendages and changing the path and speed of swimming respectively, to increase the prey encounter (e.g., Bundy and Vanderploeg, 2002; van Duren and Videler, 1995; Tiselius and Jonsson, 1990).

*T. stylifera* indeed showed significant difference in the total time allotted to motion when feeding on the different diets. The total time spent in motion was significantly higher for *C. socialis* as compared to other diets. These differences were mainly due to the variable share of time between swimming and jumps for each species. Similar response of change in motion, in relation to food concentration was also reported in *T. longicornis* by van Duren and Videler, (1995) who recorded low moving speed at low and very high food concentrations, while higher moving speed at intermediate food concentration. These authors associated this copepod behaviour with the optimal foraging theory, i.e., speed increases with food until a threshold of food concentration, beyond which increasing speed does not allow to acquire more food. Although the swimming speed was not measured in the present experiments due to the
short recording time and two-dimensional nature of the videos, it is highly possible that the copepods responded to the different diets not only by allotting variable time to motion, but also by increasing or decreasing the swimming speed. Bianco (2010) observed a similar increase in the swimming speed of *T. stylifera* in presence of food with respect to food absence. In a feeding experiment in presence of the ciliate prey *Strobilidium* sp., *T. turbinata* responded by increasing its swimming, which was hypothesized as an adaptation to increase the encounter rate (Wu *et al.*, 2010).

The duration and frequency of jumps in *T. stylifera* were also significantly affected by the different diets, might be related to the concentration of the cells in the diets. The lowest jump frequency was observed for *P. calliantha* which had higher cell concentration than the other diets. This may suggest that high food concentrations, which result in higher encounter rates, decreases the motility of copepod. Such kind of response to food environment has also been observed in copepods of the genus *Acartia*, which changed their jump frequency depending on the availability and concentration of food (Tiselius, 1992; Tiselius and Jonsson, 1997). Such a change may be even more relevant in case of a hovering copepod like *T. stylifera*. When a copepod generates feeding current while in a hovering position, particles which are rejected remain in the water below the copepod, and are not entrained into the feeding current again (Strickler, 1982). The particles can be recirculated and brought toward the mouth again due to combination of feeding currents and sinking. The jump helps the copepod to relocate to a new (0.5 mm) upward position where its mouthparts are just below the stagnation point of the previous recirculation zone, thus, allowing it to explore new volume of water for food (Malkiel *et al.*, 2003).

*T. stylifera* spend an important fraction of time in grooming during the present observations, with variable duration for the different diets. The main function of the
grooming is to clean the antennae or the body from excess particles or debris (Strickler, 1984). The cephalic appendages of copepods are covered with mechano- and chemoreceptors, therefore, cleaning becomes crucial in order to maintain their sensory performance (Paffenhöfer and Loyd, 2000 and references therein). Under a stressful environment such as, for example, at high food concentrations, increased number of cells can interfere with the sensory apparatus and reflect in higher grooming events (Costello et al., 1990; Cowles and Strickler, 1983; Rosenberg, 1980). The longest duration of grooming for T. stylifera was in fact observed for P. calliantha, which was offered at a relatively higher cell concentration than the other diatoms.

4.5 Conclusions

The use of HS camera allowed us to study various components of the feeding related behaviour of the copepods A. clausi, C. typicus and T. stylifera and revealed the significance of the various, extremely fast processes at small-scale. The set-up used in this study was a compromise between important factors like the container volume, density of animals and light conditions, which are all critical when recording at a high magnification and high frame rates (Dur et al., 2011). However, the high number of trajectories/ videos, with independent observations of several individuals had provided with a substantial dataset for assessing the copepod behavioural response to different diatom diets.

- All the three examined copepod species showed a suspension feeding mode when feeding on the different diatom diets. Although the feeding mode was same across all the different diets, each copepod species showed plasticity and modulations of the different components of the behavior in different food conditions.

- The most significant effect of food on individual copepod behaviour was observed for T. stylifera, who showed a species-specific response to the different diets by
changing the beat frequency of the feeding appendages. This species also varied the
time budget for different activities and modulated the frequency and duration of the
motion and feeding parameters.

- Subtle but significant changes in movement of feeding appendages, such as duration
  of feeding bouts and proportion of time allotted to different behaviours can in turn
  explain the different feeding rate on the different diatom diets in the grazing
  experiments. These modulations at small-scale can be attributed to the food
  environment, i.e., cell concentration and specific morphology of the cells.

- In case of *A. clausi* and *C. typicus*, the change in the behaviour was manifested only
  for the jumps frequency and time allotted to jumps respectively. However, the
  overall motion behaviour did not change significantly according to the different
  diets.

- The low time spend in feeding for *A. clausi* might explain the low values of feeding
  rates, as observed in the grazing experiments.
Chapter 5.

Grazing impact of key copepod species on seasonal phytoplankton blooms
Chapter 5. Grazing Impact

5.1 Introduction

The plankton dynamics in the oceans is driven by complex interactions among physical, chemical and biological processes. Physical variables such as light, temperature, and turbulence, as well as nutrient availability are generally considered the main factors directly affecting the structure and function of phytoplankton communities (Margalef, 1978). The influence of these factors, often referred to as ‘‘bottom-up’’ control, affects the amount of phytoplankton available to fuel marine food webs. While these processes certainly affect the growth rates of phytoplankton and structure the community, the loss processes that can assert ‘‘top-down’’ controls also play an equally important role (Banse, 1994; Dagg et al., 2009; Irigoien et al., 2005; Strom et al., 2001; Takahashi et al., 2008). Microzooplankton grazing is the largest source of mortality for marine phytoplankton contributing significantly to the loss of autotrophic biomass (Banse, 1994; Calbet, 2001; Calbet and Landry, 2004; Schmoker et al., 2013). It has been also shown to affect seasonal dynamics of phytoplankton communities across a broad range of ocean regions and habitats (Mariani et al., 2013). Broadly, zooplankton have at least two, counteracting effects on phytoplankton: grazing can reduce algal standing crop, and at the same time can stimulate algal growth through nutrient release from damaged algae during zooplankton feeding, combined with excretion and egestion (Banse, 1995; Bergquist and Carpenter, 1986)

Several studies have asserted the important role of grazers in controlling not only the phytoplankton seasonal cycle but also the occurrence and development of a bloom (e.g. Bathmann et al., 1990a; Behrenfeld, 2010; Greve et al., 2004; Irigoien et al., 2005; Maar et al., 2004; Schlüter et al., 2012; Wiltshire and Manly, 2004). However, the grazing impact varies seasonally and reflects the high degree of variability in the spatial distribution of consumers and of primary production levels in the region (e.g. Landry et
al., 1994; Perissinotto, 1992; Froneman et al., 2000; Tan et al., 2004). Moreover, the degree of grazing pressure depends to a large extent on the structure of phytoplankton community in terms of prevailing groups, i.e., large diatoms or small flagellates (e.g., Gifford et al., 1995; Sommer et al., 2005) and the structure of zooplankton community (e.g., Hansen et al., 2000; Bernard et al., 2012).

Both micro- and mesozooplankton may be significant consumers of phytoplankton but their relative predation impact is still a subject of debate (Dolan and McKeon, 2004). Microzooplankton grazing is generally considered as exerting the main predatory pressure consuming 59-74% of primary production in most marine ecosystems (Calbet and Landry, 2004; Schmoker et al., 2013), whereas mesozooplankton, and in particular copepods, consume in general 10-40% of the primary production on a global scale (Calbet, 2001). Several studies in different environments have highlighted the high grazing pressure exerted on the primary production by the protist-dominated fraction of pelagic consumers (Calbet and Landry, 2004; Dagg, 1995; Fonda Umani et al., 2005; Gifford, 1988; Landry et al., 1997; Paranjape, 1984; Strom et al., 2001).

Studies that investigated simultaneously both microzooplankton and mesozooplankton grazing on phytoplankton found that mesozooplankton have lower grazing pressure but can be a significant source of mortality for protozoan grazers and other microzooplankton (Liu and Dagg, 2003; Lonsdale et al., 1996; Sipura et al., 2003). In temperate latitudes, mesozooplankton grazing rarely exceeds 30% of either phytoplankton biomass or production even when taking into account only phytoplankton fractions which are more likely consumed by copepods (Dam et al., 1993; Huskin et al., 2001). Some studies have reported a high impact of copepod grazing, which matches or even exceeds the daily primary productivity (Bathmann et
Chapter 5. Grazing Impact

The ample variability reported in the grazing impact can be related to numerous factors including abundance, distribution and species composition and the size of the local phytoplankton community. The smaller size classes of mesozooplankton communities (200-500μm), due to their higher ingestion rates and high numerical abundance have been shown to have a larger impact on phytoplankton standing stock compared to the larger size classes (Morales et al., 1991).

The role of zooplankton grazing is of particular importance in oligotrophic and more stable marine ecosystems (Calbet, 2001). Several studies have highlighted an increased importance of mesozooplankton grazing in biogeochemical cycles with increasing oligotrophy of the system (Dam et al., 1995; Calbet, 2001). For example, in the oligotrophic South Atlantic Gyre, the mesozooplankton feeding impact on primary production was three times higher than that in the highly productive Northwest Atlantic upwelling region (Calbet et al., 2009). Even though a large proportion of the primary production in oligotrophic systems is dominated by pico-sized autotrophs (Agawin et al., 2000), grazing impact of mesozooplankton can be considerable (Calbet et al., 2009). This is due to the ability of certain mesozooplankton groups like cladocerans, appendicularians and other tunicates to feed on the small prey (Atienza et al., 2006; Katechakis et al., 2002, 2004; López-Urrutia et al., 2003).

Studies on the impact of zooplankton grazing in highly productive and dynamic environments, like those in upwelling regions, report quite variable and contrasting results. Coastal upwelling is usually intermittent and does not provide optimal conditions for mesozooplankton populations to develop in sufficient abundance to have a persistent impact on primary producers (Kiorboe, 1993). In the most productive Northwest African upwelling region of the Atlantic, a poor zooplankton–phytoplankton
coupling was reported both in terms of biomass and grazing impact, while the protozoan community represented by ciliates and dinoflagellates was well developed and most likely exerted an important grazing impact on phytoplankton populations (Huskin et al., 2001). Similar low mesozooplankton control on phytoplankton has also been reported in areas with peaks of production (Dam et al., 1995; Calbet, 2001; Huskin et al., 2001; Bode et al., 2003). In contrast to these results, high mesozooplankton grazing impact has been reported in upwelling regions of the equatorial Pacific, which suggests that the relative stability of this region, opposite to coastal intermittent upwelling, allows for the development of zooplankton populations well coupled with the primary producers (Landry et al., 1997; Roman and Gauzens, 1997; Calbet et al., 2009).

Numerous studies have examined the role of copepods as grazers and, although highly variable, the results of these studies suggest that copepods may at times consume 100% of the daily phytoplankton production (Hansen et al., 2000; Pakhomov and Perissinotto, 1997). In a study carried out in the Pearl River estuary (China), more than 76% of the chlorophyll standing stock or up to 104% of the daily phytoplankton production was removed by copepods in summer (Tan et al., 2004). Some studies concluded that copepods grazed daily most of the primary production during upwelling events (Braun et al., 1990; Valde’s et al., 1990b; Varela et al., 1991; Tenore et al., 1995), while it has been shown that losses due to copepod grazing are of minor importance during blooms following upwelling phenomena (Barquero et al., 1998; Batten et al., 2001; Halvorsen et al., 2001a, b). The low grazing impact in these studies was due to the copepods grazing on other food sources in addition to phytoplankton, for example, smaller zooplankton or detritus (Batten et al., 2001; Calbet, 2001; Halvorsen et al., 2001a, b). The role of grazers in highly productive and dynamic environments, like during upwelling and blooms, is highly debated (Franks, 2001). Uye (1986)
suggested that grazing by copepods can be important during the initial stage of algal blooms. Some studies in non-upwelling areas have suggested that zooplankton can consume most of the primary production during periods when phytoplankton growth decays and the abundance of zooplankton increases, thus contributing to terminate the bloom (Lenz et al., 1993). Tsuda et al., (2006) have similarly reported a low grazing impact by copepods on diatom blooms induced by iron addition in the eastern subarctic Pacific, but increased impact on the declining period of the bloom.

In the Gulf of Naples, the seasonal blooms are important features of phytoplankton dynamics and their occurrence and composition is well documented (Ribera d'Alcalà et al., 2004; Zingone et al., 1995, 2010). The annual cycle of autotrophic biomass is characterized by a first peak in winter, a second and major one in late spring-summer and a third one in autumn (Ribera d'Alcalà et al., 2004). The peaks in phytoplankton are followed by an increase in zooplankton abundance that is dominated, throughout the year, by copepods, which are therefore important grazers of the primary producers. However, the potential impact of copepod feeding on phytoplankton blooms has not been investigated yet in the Gulf of Naples. Estimating the grazing impact on diatoms may provide insights into the mechanisms of the dynamics of algal blooms in this typical Mediterranean coastal embayment. This chapter presents estimates of the copepod grazing impact based on the feeding rates of the target copepod species reported in Chapter 3, and the diatom and copepod standing stocks recorded at st. LTER-MC in the years 1984-2009. The availability of a rich planktonic dataset such as the MareChiara long-term time series has allowed to explore the ample range of variability of plankton composition and abundance along the seasonal and interannual scales; it may therefore provide a reliable scenario of the role of copepod grazing on the development and decline of diatom blooms in coastal waters.
5.2 Material and methods

The diatom biomass and copepod abundance utilized for estimating the grazing impact are those collected at st. LTER-MC in the period 1984-2009 (phytoplankton dataset provided by D. Sarno and mesozooplankton dataset provided by M.G. Mazzocchi). Details on the sampling and analytical methods applied to the LTER-MC investigations are reported elsewhere (e.g., Mazzocchi et al., 2011; Ribera d’Alcalà et al., 2004).

5.2.1 Phytoplankton abundance and standing stock

In brief, phytoplankton samples were collected at the surface with Niskin bottles and the cell identification and counts were performed at an inverted microscope. The carbon content of the different phytoplankton species was calculated from mean cell biovolume based on linear measurements (Menden-Deuer and Lessard, 2000). For the target diatom species, the carbon content were not estimated for *Leptocylindrus aporus* and *Pseudo-nitzschia calliantha* in the natural phytoplankton sample collected at st. LTER-MC. Therefore, the carbon content of all the species of *Leptocylindrus* and *Pseudo-nitzschia* reported in the dataset were grouped together. The total carbon content for the target diatom species is thus the sum of the carbon content of *Chaetoceros socialis*, *Leptocylindrus* spp. and *Pseudo-nitzschia* spp. The total diatom carbon was the sum of the carbon content of all the diatom cells in the phytoplankton assemblage.

Three seasons have been considered in the present study and examined separately, i.e., late winter-spring (March-June), summer (July-September) and autumn (October-December). For each season, the carbon values of each considered taxonomic category were separated into bloom and non-bloom conditions. The bloom and non-bloom values corresponded to all the values above and below a set threshold of total phytoplankton
carbon concentration of 400 $\mu$gC L$^{-1}$ (spring), 250 $\mu$gC L$^{-1}$ (summer) and 80 $\mu$gC L$^{-1}$ (autumn), respectively (see Table 2.1 in Chapter 2). These carbon values, which refer to the surface sample, were multiplied by the averaged depth of mixed layer in each season (Mazzocchi et al., 2011) to estimate the total diatom carbon available to the copepods (standing stock) and are reported as mg C m$^{-2}$. At st. LTER-MC, the mixed layer depth shows a wide variation over an annual scale, which also results in the different vertical extension of the bloom. The moving zooplankton thus experience variable distribution of food in different seasons.

5.2.2 Copepod abundance

The abundance of the grazer community was calculated from mesozooplankton samples collected by vertical tows with a Nansen net (200 $\mu$m) in the 0-50 m layer (see Mazzocchi et al., 2011 for further methodological details). The grazing impact was estimated for three grazer categories, i.e., females and total population (females+males+juveniles) of the target copepod species (Acartia clausi, Centropages typicus, Paracalanus parvus, and Temora stylifera), and total copepods. The aim was to assess the grazing impact of the most abundant copepod grazers which co-occurred with the phytoplankton assemblage. Therefore, the abundance values of the three grazer categories corresponded to zooplankton samples collected at the same time as the selected phytoplankton sample representing bloom and non-bloom conditions. The abundance values for all the three grazer categories are reported as number of individuals in the unit area (ind. m$^{-2}$).

5.2.3 Grazing impact

The four target copepod species occur throughout the year in the inner Gulf of Naples but peak in succession from spring through autumn, i.e., A. clausi and C. typicus in
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spring, *P. parvus* in summer and *T. stylifera* in autumn, and therefore interact with
different phytoplankton communities and bloom-forming species (Ribera d’Alcalà *et al.*, 2004). The grazing impact of each species was hence calculated only for the season
in which their population growth and peaks occur.

In the first step, grazing rates in carbon units (µg C cop⁻¹ d⁻¹) of each copepod
species were multiplied by the abundances of each grazer category to obtain the daily
ingestion rates of total copepod assemblage in the integrated water column (community
ingestion rate) (mg C m⁻² d⁻¹). Since the natural phytoplankton assemblage comprises
several species, the grazing rates used for this calculation were those obtained from the
mixed diet experiments. The mixed diet in this study weakly represented the diversity of
food items available to the copepods under laboratory conditions. The calculations were
carried out separately for bloom and non-bloom concentrations, applying the rates for
the respective concentrations (Chapter 3, section 3.3.2).

While calculating the potential community ingestion rates for the total population
(females+males+juveniles) and total copepods category, the grazing rates of females
from the experiment were applied to all individual in those categories. For the total
population category, it was assumed that all individuals in the population of a copepod
species have similar ingestion rates under the same environmental conditions), i.e, the
grazing rates of females were applied to males and juveniles. Similarly, for the total
copepod category, the rates of females were applied to all individuals of the copepod
population.

Finally, the grazing impact i.e., the phytoplankton carbon removed per day (% d⁻¹)
of each grazer category was calculated by realting their respective community ingestion
rates with the phytoplankton standing stock in the water column.
The comparison of grazing impacts and other parameters were assessed at the 5% significance level either by a Student’s t-test or One-way ANOVA.

5.3 Results

5.3.1 Diatom standing stock

The total diatom biomass in the mixed layer at st. LTER-MC during the period 1984-2009 was highest during spring corresponding to 5149.5±5236.1 mg C m\(^{-2}\) and 927.8±832.0 mg C m\(^{-2}\), in bloom and non-bloom conditions, respectively (Table 5.1). The high standard deviation observed for diatom biomass reflects the large temporal variation observed in the diatom biomass at the coastal site within each season and at an interannual scale. The contribution of diatoms was considerable in all the seasons, with the highest percentage during summer, followed by spring and autumn.

Table 5.1 Biomass of total diatoms during bloom and non-bloom conditions in different seasons expressed in terms of carbon over the mixed layer depth and average contribution of bloom-forming diatoms and total diatoms to total diatom and total phytoplankton biomass respectively. Values are reported as avg ± stdev.

<table>
<thead>
<tr>
<th>Season</th>
<th>Mixed layer depth (m)</th>
<th>Total diatom carbon (mg C m(^{-2}))</th>
<th>Contribution of total diatoms to total phytoplankton biomass</th>
<th>Contribution of target bloom-forming diatoms to total diatoms biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bloom</td>
<td>Non-bloom</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Spring</td>
<td>10</td>
<td>5149.5±5236.1</td>
<td>927.8±832.0</td>
<td>53.6±24.6</td>
</tr>
<tr>
<td>Summer</td>
<td>7</td>
<td>3558.7±2502.1</td>
<td>470.2±369.1</td>
<td>62.8±23.0</td>
</tr>
<tr>
<td>Autumn</td>
<td>35</td>
<td>4374.3±2868.3</td>
<td>420.53±560.4</td>
<td>42.3±29.2</td>
</tr>
</tbody>
</table>

The average seasonal cycle of the summed target diatoms, including *C. socialis* and species of genus *Leptocylindrus* spp. and *Pseudo-nitzschia* spp. showed a large
variation, with biomass values ranging from 53.3 to 917.9 mg C m\(^{-2}\) (Fig. 5.1). The standing stock increased rapidly from winter to a spring peak in April, which was followed by a slower decrease until June. From July, the standing stock showed a second increase attaining the highest value in August, and then a gradual decrease till the end of the year. *Leptocylindrus* was by far the most important taxon on annual basis, and dominated in biomass from May till November. *C. socialis* was the most important species until April. *Pseudo-nitzschia* contributed the least to the total standing stock; its biomass was highest in March-May and in September-October (Fig. 5.1).

![Average seasonal cycles of the target diatom taxa at st. LTER-MC during the period 1984-2009.](image)

**Figure 5.1** Average seasonal cycles of the target diatom taxa at st. LTER-MC during the period 1984-2009.

### 5.3.2 Copepod abundance

The abundance of the total population and only females of the target copepod species differed significantly during the different seasons (Table 5.2). The abundance of total population in bloom condition ranged between 229.5±305.3 ind. m\(^{-3}\) (*P. parvus*) to 563.0±464.8 ind. m\(^{-3}\) (*C. typicus*), while in the non-bloom condition, it ranged between
104.8 ±179.0 (*T. stylifera*) to 509.0±688.6 ind. m⁻³ (*P. parvus*). During spring, the abundance of *A. clausi* was higher as compared to *C. typicus* in both conditions. The abundance of females also showed a similar pattern to the total population at both conditions. The contribution of females to the total population was highest for *P. parvus*.

**Table 5.2** Abundance (ind. m⁻³) of total population (females+males+ juveniles) and only females of the target copepod species during bloom and non-bloom conditions. Values are reported as avg. ± stdev.

<table>
<thead>
<tr>
<th>Season</th>
<th>Copepod species</th>
<th>Total population of target copepod species (ind. m⁻³)</th>
<th>Total females of target copepod species (ind. m⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bloom</td>
<td>Non-bloom</td>
</tr>
<tr>
<td>Spring</td>
<td><em>A. clausi</em></td>
<td>411.8±523.7</td>
<td>235.8±297.5</td>
</tr>
<tr>
<td>Spring</td>
<td><em>C. typicus</em></td>
<td>229.5±305.3</td>
<td>162.1±191.5</td>
</tr>
<tr>
<td>Summer</td>
<td><em>P. parvus</em></td>
<td>563.0±464.8</td>
<td>509.0±688.6</td>
</tr>
<tr>
<td>Autumn</td>
<td><em>T. stylifera</em></td>
<td>268.2±349.7</td>
<td>104.8±179.0</td>
</tr>
</tbody>
</table>

**5.3.3 Community grazing rates**

The abundance of each grazer category combined with the individual ingestion rates of their respective females yielded community ingestion rates which ranged from 0.8±1.3 to 66.9±146.5 mg C m⁻² d⁻¹ for the only female category and from 8.9±15.5 to 295.5±376.5 mg C m⁻² d⁻¹ for the total population(Table 5.3).

At the bloom condition, the rates were significantly higher for *A. clausi*, for both grazer categories, while, at the non-bloom condition, the rates were significantly higher for *P. parvus*. All the copepod species, except *P. parvus*, showed significantly higher rates at bloom condition as compared to the non-bloom condition.
Table 5.3. Individual daily ingestion rates of the target copepod species from incubation experiments (see Chapter 3, section 3.3.3.2) and community ingestion rates (mg C m\(^{-2}\) d\(^{-1}\)) in the 0-50 m layer at st. LTER-MC for the different grazer categories in bloom and non-bloom conditions. Values are reported as avg ± stdev.

<table>
<thead>
<tr>
<th>Copepod species</th>
<th>Individual ingestion rates (µg C cop(^{-1}) d(^{-1}))</th>
<th>Community ingestion rates integrated in the 0-50 m layer (mg C m(^{-2}) d(^{-1}))</th>
<th>Total population of target copepod species</th>
<th>Only females of target copepod species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bloom</td>
<td>Non-bloom</td>
<td>Bloom</td>
<td>Non-bloom</td>
</tr>
<tr>
<td>A. clausi</td>
<td>14.4</td>
<td>3.8</td>
<td>295.5±376.5</td>
<td>45.6±57.6</td>
</tr>
<tr>
<td>C. typicus</td>
<td>9.1</td>
<td>1.8</td>
<td>104.7±139.2</td>
<td>15.16±17.9</td>
</tr>
<tr>
<td>P. parvus</td>
<td>5.1</td>
<td>5.9</td>
<td>140.6±119.4</td>
<td>150.9±204.2</td>
</tr>
<tr>
<td>T. stylifera</td>
<td>8.7</td>
<td>1.7</td>
<td>116.7±152.1</td>
<td>8.9±15.5</td>
</tr>
</tbody>
</table>

5.3.4 Grazing impact

The grazing impact of the target copepod species on the standing stock of the selected bloom-forming diatoms is presented in Figure 5.2. During late winter-spring (March-June), A. clausi females grazed daily from 20.8 to 454.0 % of the biomass of the target diatoms while the total population grazed from 75.5 to 1238.9 % (Fig. 5.2A).

The grazing impact showed an increase with increasing diatom standing stock in the initial phase of the bloom (March- April), but a decrease during the peak of the bloom (May-June) (Fig. 5.2A). The grazing impact of C. typicus for the same seasonal period was much lower, ranging between 8.6 to 28.6% d\(^{-1}\) for the females and from 66.5 to 418.4% d\(^{-1}\) for the total population (Fig. 5.2B). The grazing impact of both copepod species was highest in April and lowest in May. The impact of grazing was highest during the initial phase of the bloom and decreased as the bloom progressed (Fig. 5.2B).

During summer, the grazing impact of P. parvus females ranged from 84.4 to 451.9 % d\(^{-1}\), while for the total population, it ranged from 248.2 to 1137.7% d\(^{-1}\) (Fig. 5.2
Figure 5.2 Standing stock of bloom forming *C. socialis*, *Leptocylindrus* spp. and *Pseudo-nitzschia* spp. (considered together) and grazing impact of the target copepod species (as females only and as total population) as % of total biomass removed daily during spring (A, B), summer (C) and autumn (D).
C). The grazing impact varied significantly during the three months of the season and was highest during August, when the abundance of *P. parvus* was at its peak (see Fig 2.5E in Chapter 2). The grazing impact was relatively lower during the initial phase of the bloom, but increased several folds as the bloom progressed, later decreasing towards the declining phase of the bloom (Fig. 5.2 C).

During autumn, *T. stylifera* females grazed daily from 4.7 to 36.4% of the target diatom biomass while the total population removed from 54.1 to 227.9 % (Fig. 5.2D). The impact varied significantly over the whole season, with lowest value in October, when the standing stock of target diatoms was high, and increased in November and December, when the standing stock was low. The grazing impact was low during the initial phase of the bloom, but it increased significantly as the bloom progressed (Fig. 5.2D).

The grazing impact of the target copepod species on the total diatom standing stock at bloom and non-bloom condition is shown in Table 5.4. The diatom biomass removed daily by the four copepod species ranged between 0.4±0.9 % d$^{-1}$ to 190±1170.9 % d$^{-1}$ for the only females category and between 4.7±17.3 % d$^{-1}$ to 394.1±2017.3 % d$^{-1}$ for the total population category. The impact varied widely for the two grazer categories at both conditions. In case of the only female category, the highest impact was for *P. parvus* at both conditions, while for the total population, the impact was highest for *A. clausi* at bloom condition and *P. parvus* at non-bloom concentration. The grazing impact did not differ significantly between bloom and non-bloom conditions for all the copepod species except *P. parvus*. Overall, *P. parvus* population removed much more than the other copepod species.

On an annual scale, the grazing impact of the target copepod species on the total diatom standing stock varied widely (Fig. 5.3A). The variation can be attributed to the
changing cycle of both phytoplankton standing stock showed a steady increase from winter onward and reached the peak during March corresponding to the winter-early spring bloom and copepod abundance (Fig. 5.3). The annual cycle of total diatom standing stock showed a peak (2059.2 mg C m\(^{-2}\)) during March. The standing stock decreased from March onwards up to June, followed by another increase till October, after which it decreased till the end of the year (Fig 5.3 A).

**Table 5.4** Grazing impacts of different grazer categories of the target copepod species expressed as % of total diatom standing stock removed daily in bloom and non-bloom conditions. Values are reported as avg. ± stdev.

<table>
<thead>
<tr>
<th>Season</th>
<th>Copepod species</th>
<th>Daily grazing impact (% d(^{-1})) on total diatoms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Only females</td>
<td>Total population (f+m+j)</td>
</tr>
<tr>
<td></td>
<td>Bloom</td>
<td>Non-bloom</td>
</tr>
<tr>
<td>Spring</td>
<td>A. clausi</td>
<td>2.2±4.2</td>
</tr>
<tr>
<td>Spring</td>
<td>C. typicus</td>
<td>0.4±0.9</td>
</tr>
<tr>
<td>Summer</td>
<td>P. parvus</td>
<td>2.5±2.6</td>
</tr>
<tr>
<td>Autumn</td>
<td>T. stylifera</td>
<td>0.5±1.1</td>
</tr>
</tbody>
</table>

The total population of target copepods species also showed two peaks in their abundance, one during spring (April) and another during late spring-early summer (June-July) (Fig. 5.3 B). The highest grazing impact of target copepods on total diatom standing stock was during summer (July- August) when the copepods grazed up to 264.4 % of the diatom biomass, while the lowest was during October corresponding to 20.2 % of diatom biomass. The highest grazing impact during summer (July) was due to the combined effect of low diatom standing stock and high copepod abundance.
Figure 5.3 Annual cycle (monthly averages) of A) total diatom standing stock (mg C m\(^{-2}\)) and abundance of total population of target copepod species (ind. m\(^{-3}\)) and B) estimated grazing impact of the total population of target copepod species (corresponding to abundance shown in A) calculated by applying ingestion rates at bloom concentration for each copepod species on the total diatom standing stock for the period 1984-2009 at st. LTER-MC.
5.4 Discussion

In the present chapter, the grazing impacts of the target copepod species *A. clausi*, *C. typicus*, *P. parvus* and *T. stylifera*, which represent the key copepod grazers of phytoplankton in the Gulf of Naples, were estimated based on the ingestion rates obtained from incubation experiments with the food removal method. The selected diatom species formed a substantial part of the total diatom assemblage in the GoN during different seasons. The grazing rates which were obtained for each copepod species at different food conditions, and the vast dataset of phytoplankton and zooplankton abundance together allowed us to estimate the grazing impact for different situations in the field, which can together help to create a better understanding of the role of grazing in phytoplankton bloom dynamics in the GoN.

The estimated grazing impact varied widely depending upon the abundance of the grazer community and the co-occurring diatom standing stock. The estimated grazing impact of all the four copepod species on the ensemble of target diatom species was considerable and at time matched or exceeded their standing stock. The copepods removed between 5 % and 1239 % of biomass of bloom-forming species during autumn and spring respectively. This large temporal variation in the grazing impact reflects the differences in copepod abundance, their feeding rates and diatom standing stock in the respective season. The highest rate of grazing impact of *P. parvus* is not surprising considering its high abundance compared to the other three copepods.

When considering the total diatom standing stock, the daily grazing impact ranged between 0.4 % to 394 %. The magnitude of the impacts appeared to be primarily related to the abundance of grazers and was highest during summer when the grazer abundance was high and decreased in autumn with a decrease in the grazer abundance.
Overall, the grazing impact estimated in the current study falls within the range reported for mesozooplankton grazing in the different marine environments of the world (Table 5.5). The present values are also comparable to those obtained during other studies with variable results for the Mediterranean Sea (reviewed by Siokou-Frangou et al., 2010). In the Gulf of Lion (NW Mediterranean), zooplankton grazing contributed significantly to the regulation of primary production and removed between 5.3 to 83.1 % of primary productivity depending upon the season (Gaudy et al., 2003). Copepods grazing in the South Aegean Sea removed between 14-35% of phytoplankton production, which mainly consisted of >3μm cells (Siokou-Frangou et al., 2002). In the North-East Aegean Sea, small copepods including A. clausi, C. typicus, P. parvus, Oithona similis and Oncaea spp. removed up to 32% of the primary production (Zervoudaki et al., 2007). The lower grazing impact on diatoms observed in the bloom condition in the present study at st. LTER-MC ranged between 0.4 to 12.2 % d⁻¹. Considering the total phytoplankton standing stock, the values of grazing impact would be much lower. In that case, it would be similar to the highest impact reported by Saiz et al. (2007) and Broglio et al. (2004) who estimated a grazing impact of <1% d⁻¹ grazer community comprising of copepods and cladocerans on phytoplankton standing stock in the Catalan Sea.

During spring, A. clausi and C. typicus are the most abundant copepods at st. LTER-MC and are characterized by a peak period extending from April to July (Mazzocchi et al., 2011, 2012). Due to their numerical abundance they can together exert a considerable control on the diatom standing stock. The investigation of the grazing impact of these species showed a very high, but, different daily removal of 454.0 and 28.6 % of the target diatom standing stock. The spring bloom populations in the GoN are dominated by large colonial diatoms including several species of...
Chapter 5. Grazing Impact

Chaetoceros, and Pseudo-nitzschia (Ribera d’ Alcalà et al., 2004). The high grazing impact observed for the target diatoms species used in the present study can therefore indicate a high grazing pressure on these spring blooming species as well. However, when considering total diatoms, the grazing impact of *A. clausi* and *C. typicus* were quite low (0.4 and 16.9 % d⁻¹). One of the reasons for this low impact can be the highly diverse plankton community during the spring. In fact, the target bloom-forming species formed only 30% of the total diatom population, indicating dominance of other diatoms. Along with diatoms, the spring season is also characterised by a parallel increase in ciliates and dinoflagellates (Modigh, 2001; Ribera d’Alcalà et al., 2004), which increase the phytoplankton diversity even more. A highly diverse food environment would mean increased prey choice for a selectively-feeding copepod. Both *A. clausi* and *C. typicus* have displayed a selective feeding in grazing of the bloom-forming diatoms as well as on different size categories in the grazing experiments (Chapter 3). These two species are known to switch between suspension and ambush feeding modes depending upon the prey type and show a preference for larger motile prey when available (Calbet et al., 2007; Gismervik and Andersen, 1997). It is therefore possible that *A. clausi* and *C. typicus* also feed on prey other than diatoms, which would dilute their grazing impact on the diatoms. During periods of high food availability like during a bloom, food quality may be the deciding factor for food selection. In this context, both ciliates and dinoflagellates have been suggested to be qualitatively important as food source for copepods because of their low carbon to nitrogen ratios compared to algae, making them a more efficient source of proteins and amino acids (Stoecker and Capuzzo, 1990a). This behaviour has also been reported for copepods when feeding on natural food assemblage (e.g. Atkinson, 1996; Broglio et al., 2004; Stoecker and Capuzzo, 1990). Although sent grazing experiments were based only on diatoms, this
consideration can be valid taking into account the selectivity and high ingestion of the most abundant diatom in the mixed diet by both the copepod species, a strategy to optimize feeding to meet the body carbon requirements.

When considering the two food conditions of bloom and non-bloom, the grazing impact of *A. clausi* and *C. typicus* on the total diatoms did not show any statistical difference despite the difference in the total diatom standing stock. The highest grazing impact of 16.9 % d\(^{-1}\) by the population of *A. clausi* even at the non-bloom condition is moderate compared to the higher values reported in literature (see table 5.4). Such paradox appears widespread in temperate regions and can be due to the difference in the growth rates of the phytoplankton and mesozooplankton. The grazing impact of *C. typicus* was even lower than *A. clausi*, ranging between 0.4 to 6.2 % d\(^{-1}\). These low values are in agreement to those reported for *C. typicus* on natural communities (reviewed by Calbet *et al.*, 2007). These results are also consistent with the feeding response of these copepods observed in the grazing experiments in the present study, when both copepod species did not show any significant difference in the ingestion rates for the mixed diet at the two food concentrations. Also, for the monospecific diet experiments, the growth of diatoms in the test bottles exceeded losses due to grazing pressure. The absence of a significant grazing impact at high food concentration implies a lack of grazing control during a bloom. It seems therefore that *A. clausi* and *C. typicus* do not have a significant impact on the total diatom standing stock during spring. However, the copepods can have a significant impact on the target bloom-forming species, especially in the initial stage of the bloom. The impact decreased when the standing stock reached its peak and continued to remain low due to the very high standing stock. It is possible that the presence of alternative prey and overall high growth rates of the phytoplankton due to favourable environmental condition dilutes the
effect of copepod grazing on these selected blooming species over the course of the bloom period.

During summer, *P. parvus* could have a significant impact on the standing stock of the selected bloom–forming diatoms. The grazing impact in this case was extremely high, and showed a steady increase keeping pace with the increasing standing stock, which may have lead to the subsequent decrease in the standing stock. It is therefore possible that *P. parvus* grazing was an important loss factor during the summer diatom blooms observed at st. LTER-MC, especially when the target bloom-forming species dominate the total diatom standing stock. The grazing impact showed a significant increase from the bloom to non-bloom condition. Calbet (2001) found a significant inverse relationship between the productivity of the ecosystem and the grazing impact of mesozooplankton and highlighted a general trend in decrease of the mesozooplankton grazing impact with increasing productivity on a global scale. The relative proportion of primary production removed by mesozooplankton grazing ranged from 40.4% for unproductive to 10.1% for highly productive communities. Calbet *et al.* (2009) also found that the mesozooplankton grazing impact was three times higher in the oligotrophic regions as compared to the most productive regions in the Atlantic. The different grazing impacts of *P. parvus* observed in the present study appeared to be primarily caused by differences in the standing stock of diatoms for the two conditions. The summer bloom at st. LTER-MC is dominated by small-sized, mostly solitary diatoms and the phytoplankton peaks alternate with periods of low values, reflecting the fluctuation in the nutrient availability (Ribera d’Alcalà *et al.*, 2004). *Paracalanus parvus* is known to find 20-40 μm particles as an optimum size for ingestion, but it can collect < 5 μm cells passively (Bergreen *et al.*, 1988; Price *et al.*, 1983). In addition to this, *P. parvus* is known to be extremely well adapted to conditions in which food
availability is limited (Paffenhofer and Stearns, 1988; Sautour et al., 1996; Schnack-Schiel, 1982). These traits, suitable to exploit the summer phytoplankton in addition to its high abundance in that season (24.0±12.9 % of total copepods during the period 1984-2009, Mazzocchi and Di Capua, unpublished) can together lead to a high grazing impact of *P. parvus* as estimated in this study. The impact can be significant especially during the non-bloom condition or the periods of low standing stock observed during the summer.

During autumn, *T. stylifera* had a significant grazing impact on the standing stock of the target diatoms. Over the course of the bloom, the impact increased steadily as the standing stock of the target diatoms decreased. It is possible that the decrease in the standing stock of the target diatoms was due to the high grazing control of *T. stylifera*. The annual phase of phytoplankton growth in the GoN during the autumn is represented by a peak of colonial diatom species including *Pseudo-nitzschia* spp. and *Leptocylindrus* spp. The target bloom-forming diatoms form nearly 60% of the total diatoms population. So, considering that the phytoplankton community is dominated by these diatoms, grazing by *T. stylifera* can be an important loss factor to the autumn blooms. However, the estimated values for the grazing impact on the total diatom standing stock were too low to affect the bloom. This implies that the impact of *T. stylifera* may be significant only when the total diatoms community is less diverse and is dominated by a few selected diatoms.

5.5 Conclusions

The magnitude of the impact of grazing on the diatoms varied depending on the copepod species and abundance, diatom standing stock, and stage of the bloom with different seasons over an annual scale.
The estimated grazing impact of the four copepods showed a significant removal of the target bloom-forming diatom species.

Though, all the copepods showed a high removal of target diatoms, *P. parvus* and *T. stylifera* seem to be able to control the diatom abundance even when the standing stock increased several fold.

The highest grazing impact on the total diatom standing stock was observed for *P. parvus* during summer as compared to the other copepod species.

During spring, impact of the *A. clausi* was more important than *C. typicus*; however, their grazing impacts, even when considered together, were modest to control the bloom.

During autumn, the grazing impact of *T. stylifera* was high enough to cause the decline of the target diatom abundance, but not sufficient to affect the removal of the total diatom standing stock.

Overall, it can be concluded that the grazing impact of the four key copepod species was low on the total diatoms standing stock, and may not contribute to the loss of the bloom. However, the copepods can significantly reduce the standing stock of the bloom-forming diatoms, and the impact would be much higher at low cell concentrations or during the initial stage of the blooms. The fate of the bloom may therefore depend on the composition of the phytoplankton assemblage and the ability of the copepods to efficiently remove the standing stock during the initial phase of the bloom.
Table 5.4. Grazing impact of copepods on the total phytoplankton production in different ecosystems.

<table>
<thead>
<tr>
<th>Region</th>
<th>Copepod species</th>
<th>Grazing impact</th>
<th>Method used</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plymouth (SW England),</td>
<td>Medium-sized copepods</td>
<td>30-40% (&lt;10μm)</td>
<td>Gut fluorescence</td>
<td>Bautista, 1992</td>
</tr>
<tr>
<td>Oyashio region, northwestern</td>
<td><em>Eucalanus, Metridia</em> and <em>Neocalanus</em> species</td>
<td>28%</td>
<td>Gut fluorescence</td>
<td>Kobari, 2010</td>
</tr>
<tr>
<td>Pacific Ocean.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equatorial and North African</td>
<td>Size-fractionated copepod</td>
<td>&gt;100%</td>
<td>Gut fluorescence</td>
<td>Huskin et al., 2001</td>
</tr>
<tr>
<td>upwellings,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE Atlantic</td>
<td><em>Calanus spp., Metridia</em> <em>spp., Oithona spp.</em></td>
<td>&lt;2%</td>
<td>Gut content</td>
<td>Morales et al., 1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bay of Biscay</td>
<td><em>Temora longicornis</em> and <em>Paracalanus parvus</em></td>
<td>35-68%</td>
<td>Gut content</td>
<td>Soutour, 1996</td>
</tr>
<tr>
<td>Location</td>
<td>Prey Species</td>
<td>Grazing Impact</td>
<td>Method</td>
<td>Reference</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-------------------------------------------------</td>
<td>----------------</td>
<td>------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Pearl river estuary, China</td>
<td><em>Acartia spinicauda</em>, <em>Paracalanus</em> spp. and <em>Oithona</em> spp.</td>
<td>20-100% (winter-summer)</td>
<td>Gut fluorescence</td>
<td>Tan <em>et al.</em>, 2004</td>
</tr>
<tr>
<td>NW Spain, Atlantic</td>
<td><em>Oithona</em> spp., <em>Paracalanus parvus</em> and <em>Clausocalanus</em> spp.</td>
<td>&lt;3%</td>
<td>Gut content</td>
<td>Barquero <em>et al.</em>, 1998</td>
</tr>
<tr>
<td>Subtropical convergence (spring)</td>
<td><em>Pleuromamma abdominalis</em> and <em>Metridia lucens</em></td>
<td>90%</td>
<td>Gut fluorescence</td>
<td>Pakhomov and Perissinotto, 1997</td>
</tr>
<tr>
<td>Skagerrak, North sea</td>
<td>Small cyclopoid and harpacticoid spp.</td>
<td>(57%)</td>
<td></td>
<td>Maar <em>et al.</em>, 2004</td>
</tr>
</tbody>
</table>
CHAPTER 6.

Conclusions
The aim of this thesis was to evaluate the role of copepod grazing in the dynamics of phytoplankton blooms. Towards this aim, the important predator-prey interactions between key grazers and bloom-forming diatoms were investigated following a species-based approach. The study was applied to the pelagic system at st. LTER-MC in the inner Gulf of Naples (GoN) site of a long-term time-series characterized by recurrent seasonal patterns in the phyto- and zooplankton communities. The focus was on the small calanoids *Acartia clausi*, *Centropages typicus*, *Paracalanus parvus* and *Temora stylifera*, which comprise a major fraction of the copepod abundance in the GoN during different seasons. Their grazing rates and behavior were analyzed while feeding on the diatoms *Chaetoceros socialis*, *Leptocylindrus danicus*, *Leptocylindrus aporus* and *Pseudo-nitzschia calliantha* which represent the most widespread and abundant species during the blooms in GoN (Chapter 2 and Ribera d’Alcalà *et al.*, 2004). Finally, the impact of copepod grazing on diatom removal was evaluated during the different phases of their time course.

During the development of a phytoplankton bloom, the algal biomass shows several stages starting from initiation to senescence, and, the fate of the bloom is likely to change depending on various factors, including the stage-specific control of grazers. The effect of grazing on the bloom progression is suggested to be more relevant during the early initial or pre-bloom stage, when the cell number is low (Uye, 1986). The non-bloom concentrations used in the present study correspond to pre-bloom or post-bloom phases as recorded in coastal waters of the GoN, with much lower cell concentrations as compared to those observed during blooms. The different diatoms were offered as monospecific and mixed diets to reproduce two different conditions, i.e. when a single diatom dominates the phytoplankton assemblage, a situation which may commonly occur over the temporal scale of the blooms, and, when multiple diatoms are responsible
of the bloom assemblage. Both these conditions are realistic when considering the available information on the bloom dynamics. In the coastal GoN, intense phytoplankton blooms are generally composed of diversified diatom assemblages, but monospecific patches may occur at the small scales typical of individual copepod surroundings.

6.1 Response of target copepod species to different food environments

The results obtained from the present study on the feeding responses of the target copepod species from incubation experiments with the food removal method have highlighted significant differences in the copepod feeding rates and selectivity for the different bloom-forming diatoms as well as when feeding at two extremely different food concentrations. All the copepods grazed efficiently on all the different diatom species offered. However, the quantitative and qualitative response of each copepod species to each diatom species and the general food environment varied, indicating their different roles in the copepod-diatom interactions. The four target copepod species chosen for this study differ in their feeding habits and dominate the zooplankton community during different periods of the year in the GoN, and are therefore exposed to different food environments. The observed inter-specific differences in the feeding response to different diatom diets are therefore not surprising. The most interesting findings of the present study are rather how each of these copepod species adapts to contrasting food environments by changing its feeding rates and selectivity in the short time scale. Such adaptations are relevant to a pre bloom or bloom condition of phytoplankton community which can be crucial in the overall dynamics and fate of the bloom.

Controlling a bloom at its onset requires that grazers effectively feed on the pre-bloom phytoplankton species when they are at lower concentration. However, it is
likely that the copepods greatly reduce or even stop their feeding at a lower food concentration (Frost, 1993; Jonsson and Tiselius, 1990; Paffenhofer and Stearns, 1988; Price and Paffenhofer, 1986). The decrease in the rates at lower food concentration can be considered as an adaptation to conserve energy by reducing the appendage movement or the time spent in creating feeding currents (Paffenhofer and Stearns, 1988; Price and Paffenhofer, 1986). It is also possible that at lower concentrations of specific phytoplankton, zooplankton may switch to more abundant prey species (Landry, 1981). In the present study, all the copepods fed at the lower food concentrations and the ingestion was generally higher on the most abundant species in the mixed diet experiments. The different diatom species in the mixed diets were available at similar carbon concentrations, but they varied significantly in their shape and size. The difference in the feeding rates therefore may be due to their different encounter rates.

For *A. clausi* and *C. typicus*, whose populations grow and peak during the spring-early summer, the feeding rates did not change from non-bloom to bloom concentration despite the large difference in the food availability (100-800 µg L⁻¹). However, both copepods showed diatom-specific differences in feeding and selectivity. *A. clausi* was more selective at the non-bloom concentration and showed preference for *C. socialis* in addition to *P. calliantha*, which was the most abundant diatom available. This behaviour of *A. clausi* can be referred to as "peak tracking" or "opportunistic feeding" when the copepods tend to feed on the most abundant food item that represents the biomass peak, and can be a strategy to compensate the energy requirements at lower food conditions (Poulet, 1978; Richman *et al.*, 1977). The theory suggests that the removal rates are highest on the most abundant particle size peak regardless of the size of the particles comprising that peak. In case the grazers exhibit such feeding behavior, the diversity of the phytoplankton in that grazing pressure will be reduced on those
species lying outside the dominant sized peak. Such active selection of the dominant and/or most actively growing peak will tend to enhance the ability of the copepod to control the phytoplankton bloom by concentrating grazing pressure on the most rapidly growing segment of the phytoplankton assemblage (Donaghay, 1988). *C. typicus* also exhibited selective feeding and selected different diatoms at the two concentrations, showing the tendency to feed on the more abundant food item available. Also, at the two contrasting food concentrations used in the grazing experiments, the daily ration obtained at the bloom concentration was significantly higher for all the copepods except for *P. parvus*. This indicates that the copepods were food limited at the non-bloom concentrations. The higher selectivity at lower food concentration by the copepods can thus be explained by the need to meet their basic energy requirements. These copepods can therefore affect the composition of the phytoplankton assemblage during the early stage of the bloom through selective feeding when the concentration of cells is lower.

*P. parvus* showed a significant difference when feeding on the same diatom at the two concentrations, but the feeding rates for the mixed diet were similar at both concentrations. Also, the daily ration of the copepod did not vary for the different food concentrations, indicating that the copepod is very well adapted to feed even at lower food concentrations and therefore an increase in food availability does not have a major effect on this species. Instead, *T. stylifera*, which is an important autumn copepod, showed significant difference when feeding at the two food concentrations. The carbon ingestion rates and the daily ration was higher at the bloom concentration suggesting that this copepod was food limited at lower food quantity, and, may react quickly to increase in food availability. Also, this species showed selectivity for the larger cells at lower food concentration, which also contributed a major portion to its dietary carbon. Based on these results it can be concluded that *T. stylifera* may have a significant effect
on the phytoplankton community under lower food availability due to higher ingestion, and its size selective feeding on larger cells may favor the occurrence of smaller cells

### 6.2 Effect of food environment on feeding behaviour

The variable feeding response of the copepods to the different diets is reflected by their different feeding behaviours as observed at small scale. For all planktonic copepods, motion and feeding are closely linked, as it appeared in the species analyzed during the present study, i.e., *A. clausi*, *C. typicus*, and *T. stylifera*. All the three copepods studied showed an ability to modify different parameters of their feeding and motion with respect to the different diets offered. Perception of food environment in copepods occurs through chemo-and mechanoreception and they can modify their feeding modes and the intensity of their movements according to this information to maximize the probability of encountering food. A copepod can influence the probability of encountering the prey by varying the flow field of its feeding current, changing its swimming speed or varying the time spent in swimming by modifying its appendage movement, or move in a complex swimming path to stay in a patch with higher food concentration (e.g., Bundy *et al.*, 1993; Buskey, 1984; Gerritsen and Strickler, 1977). The three copepod species analysed in the present study showed variations in the motion pattern when feeding on the different diets. The most significant effect was observed for *T. stylifera*, which showed a species-specific response to the different diets by changing the beat frequency of its feeding appendages, with higher rates for the colonial *C. socialis* and *L. danicus*. The difference in the beating frequency may explain the different feeding rates observed for *T. stylifera* from the grazing experiments. In case of *A. clausi* and *C. typicus*, the overall feeding behaviour did not change significantly for the different diets at the high food concentration, except for the jump frequency. Both copepods showed a higher jump frequency for the more abundant prey.
This behaviour is consistent with an area-restricted-search foraging strategy which allows the copepods to find and remain within patches of food. On detection of a patch of high food concentration, a copepod may have to increase the jumps in order to relocate itself back to retain its original position, which may change due to sinking due to gravity. This may imply that the denser the patch, the more frequent will be the jumps. However, it is difficult to say if change in mere jump frequency should be enough to affect the feeding rates. Considering that there was no change in other feeding and motion parameters, like time allotted to hovering or slow swimming for *A. clausi* and *C. typicus* when feeding on the different diatom diets, it can be concluded that feeding responses for the different diets were similar. Although, it was clear from the video observations, that both *A. clausi* and *C. typicus* did feed on the different diatoms, the low time spend in feeding might explain the low values of feeding rates, as observed in the grazing experiments.

**6.3 Implications of copepod feeding behaviour on grazing impact**

Concentration and composition of food are important environmental variables to be considered for determining to what extent the grazing activity impacts the phytoplankton assemblage. However, the capability of copepods to gather food through behavioural adaptations should also be taken into account. The effect of grazing on plankton communities can be both quantitative, in terms of removal of the phytoplankton standing stock by the grazing impact, as well as qualitative, by affecting the composition of the bloom through selective feeding. As evident from the results of the incubation experiments and the direct video observations, the different feeding response of the copepods to the variable food environments should reflect into a different grazing impact during the different stages of the bloom.
The estimated grazing impact based on individual feeding rates acquired in incubation experiments and standing stock data recorded in the field at st. LTER-MC indicate that the target copepods have a potential to impact severely the standing stock of the target bloom-forming diatom species. When extended to the total diatom assemblage, the estimated impact decreases by several order of magnitud. For all the copepods, the estimated impact is highest during the pre-bloom stage, when the food concentration is lower and when the diatom assemblage is dominated by a single species. During the spring, *A. clausi* and *C. typicus* have a significant impact on the total diatom standing stock in the initial stage of the bloom. However, the higher growth rates of the diatoms favored by environmental conditions, exceed the loss due to grazing, leading to the increase in the standing stock and to the development of the spring bloom. The high grazing impact of *P. parvus* during summer seems to keep in pace with the increasing diatoms. It seems therefore that the high grazing control exerted by the high abundance of *P. parvus* on summer bloom prevents the development of an important bloom. In the autumn, the grazing impact of *T. stylifera* exceeded the diatom standing stock during autumn and seems responsible of the demise of the bloom.

In conclusion, the results of the present study highlight the importance of species-specific and small-scale differences related to the variable feeding responses of copepods that can eventually affect the predator-prey interactions on a larger scale. The feeding performances of the target copepod species resulted into a grazing impact on diatoms that is variable and differentiated according to the species or community level, and to the bloom or non-bloom concentrations. Based on the further analysis of the different components of copepod feeding performances and behavior, it can be affirmed that the abundant calanoids *A. clausi, C. typicus, P. parvus* and *T. stylifera* have a
considerable effect on the bloom dynamics of the diatom species bloom-forming diatoms occurring in the GoN. Moreover, the high degree of behavioral plasticity and the ability to adapt to the different food environments may explain why these copepods are successful in dynamic coastal waters.

This is the first comprehensive study on copepod grazing for the Gulf of Naples and the findings of the species-based study highlight the importance of the key species of the plankton community at the study site. The estimated grazing impact further suggests the significance of the small copepod grazers in affecting the plankton dynamics not only through removal of standing stock, but also due to their ability to change the composition of the phytoplankton assemblage. The role of copepod grazing may explain part of the temporal variance observed for the phytoplankton in the time series LTER-MC. As evident from the present study, copepods can remove a significant amount of standing stock of particular diatom species, which increases at lower cell concentrations. The change in abundance and timing of occurrence of some species might therefore result from higher grazing pressure of copepods. The behavioral adaption of copepods to the different food environments and the diatom-specific selectivity implies the ability of these copepods to successfully exploit the changing food supply and respond quickly to such changes. These adaptations in turn can be related to the evolution of the different copepod species according to the different food regimes.

6.4 Future perspectives

The present study represents a further advance in the long-term ecological research conducted in the inner Gulf of Naples and contributes to a better understanding of trophic interactions within the plankton communities and their potential role in
affecting the temporal patterns of prey populations. The species- based approach followed to address the role of copepod feeding in relation to bloom-forming species has emphasized the consideration for both the grazer and prey characteristics at species level which can have implication on the feeding response during blooms. The grazing rates from this study can be used directly for modeling studies which can incorporate species-specific selectivity and functional response in the design and can provide a more realistic scenario of functioning of the ecosystem (Frangoulis et al., 2010). Such parameters of zooplankton are known to have a large influence on the models sensitivity (Carlotti and Poggiale, 2010). Biogeochemical models, where mesozooplankton is often the highest trophic level of the food web can benefit vastly from the inclusion of prey preference by copepods (Buitenhuis et al., 2006). However, more field studies on grazing at different time points on a diverse food assemblage and different grazer communities are necessary to provide a more clear picture of the plankton dynamics over an annual scale.

Recently, modern molecular techniques involving q-PCR have shown promising application in the study of copepod grazing on the natural plankton assemblage. These techniques allow highly sensitive estimates of predator-prey relationship towards a precise definition of the pelagic food webs (e.g., Nejstgaard et al., 2003; Simonelli et al., 2009; Troedsson et al., 2009). Under natural conditions, these techniques provided highly sensitive estimates of prey cell numbers (Durbin et al., 2011). Moreover, it allows acquisition of copepod feeding rates on specific prey species almost simultaneously with the sampling process without incubation. Because the assay targets prey DNA as an universal marker, direct quantification of in situ copepod feeding rates on non-phytoplankton taxa as protozoa and metazoan is possible (Durbin et al., 2008).
Various extensions of the present research can be foreseen. For example, this study was restricted to only the adult females of the target copepod species. However, a large portion of the copepod biomass at sea is comprised of the numerous and abundant developmental stages, from the juvenile copepodites to the larval nauplii. Due to their higher numerical abundance and the supposed stage-specific feeding habits, the grazing impact of this category can be expected to differ from that of adults.

During the present study, the focus was on the three periods of phytoplankton peak corresponding to diatom blooms, i.e. spring, summer and autumn. In addition to these seasonal blooms, the Gulf of Naples also experiences a modest and sporadic winter biomass increase, mainly caused by small flagellates and small non-colonial diatoms (Zingone et al., 2010). The hypothesis that the low abundance of copepod grazers may be one of the factors responsible of this increase can be explored in the future, to better understand the role of grazing on the whole annual cycle.

The aspect of selective grazing investigated in the present study is certainly a step forward in our understanding of planktonic interactions in the GoN, but, it was limited to few dominant diatom species. As evident from results of the grazing experiments, the copepods exhibited a wide selectivity not only on different diatom species, but also on different size categories of cells which could have important implication on the phytoplankton composition in the field. The phytoplankton community in the GoN is highly diverse with several groups which are represented by many less numerous species, or species which appear only briefly. Including such species in future studies will undoubtedly contribute to a greater understanding of the functioning of the ecosystem. Finally, following a multidisciplinary approach, integrating the laboratory studies involving the classical and advances molecular techniques with modelling studies is crucial to address the complex processes which drive the food webs.
Figure 6.1 Schematic of grazing studies carried out with the target copepod species and bloom-forming diatoms and its possible implications on the phytoplankton blooms in the Gulf of Naples.

**Copepod grazing and its implications on seasonal phytoplankton blooms in Gulf of Naples**

- **Copepods**: Variable feeding rates, selectivity, behavioural plasticity
  - *Acartia clausi*
  - *Centropages typicus*
  - *Paracalanus parvus*
  - *Temora stylifera*

- **Grazing impact**
  - Grazing impact only selected diatom species; it does not control bloom comprised of diverse phytoplankton
  - Grazing impact is relevant only in initial stage of blooms
  - Selective grazing can affect the composition of the bloom

- **Diatoms**: Diverse in morphology (size, chains, single cells)
  - *Chaetoceros socialis*
  - *Leptocylindrus appanus*
  - *Leptocylindrus danicus*
  - *Pseudo-nitzschia calliantha*

- **Grazing**
  - Small-scale interactions of key species

- **Seasonal phytoplankton blooms**
  - Spring
  - Summer
  - Autumn

- Grazing does not control blooming diatoms
- Significant grazing impact on blooming diatoms in initial stages of bloom
- High grazing control on blooming diatoms

- Grazing not affected by cell conc. Selectivity on larger cells
- Feeding not affected by cell conc. Selectivity on different cell size.


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