Dynamic species interactions in phototrophic biofilms
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Chapter 6

Trophic interactions between benthic copepods and phototrophic biofilms: a laboratory study
Abstract

Meiofauna is potentially capable of controlling microphytobenthic biomass. It is hypothesized that intense grazing leads to succession towards less edible algal species with consequent effects on copepod survival and growth.

Monospecific biofilms of three benthic diatom species and one benthic cyanobacterial species and multi-specific biofilms with these species were offered to adult *Attheyella trispinosa* and the changing abundance of phototrophic species was observed during 17 days. The survival and development rate of nauplii of *A. trispinosa* and *Bryocamptus minutus* were measured during incubations with a similar range of biofilms supplied as food.

Feeding by *A. trispinosa* decreased the density of the diatom *Nitzschia perminuta*, while biomass of all other diatom species was generally unaffected. The biomass of the cyanobacterium *Leptolyngbya foveolarum* was either unaffected or was slightly enhanced by copepod feeding. Yet, the architecture of the biofilm changed and the cyanobacterium was ingested and partly digested by the copepod that was able to develop on this diet.

Cyanobacteria alone or combined with diatoms reduced the survival and development rate of *A. trispinosa* nauplii and diminished copepodid size. Similar observations were made for *B. minutus*. It is concluded that phototrophic species exert a strong effect on harpacticoid copepods.

Specific effects of copepod grazing on multi-species phototrophic consortia were to a large extent masked by the interaction among phototrophic species. In contradiction to our hypothesis, grazing of copepods on cyanobacteria altered the physiognomy of biofilms and promoted the persistence of (edible) diatom species otherwise overgrown by the less nutritious cyanobacteria.
Introduction

The forces structuring algal communities have received broad attention from ecologists and there is controversy on the importance and degree of influence of nutrients and other bottom-up factors (e.g. Persson et al. 1988 and references therein) versus grazing or other top-down factors (e.g. Shapiro and Wright 1984). It is accepted that grazer taxon, grazer biomass and periphyton accrual influence the magnitude of the effect exerted by grazers on periphyton (Feminella and Hawkins 1995). Macro-invertebrates clearly influence periphyton as grazers and are able to control periphyton biomass and diversity (Hillebrand 2002, 2003, Hillebrand et al. 2002).

Knowledge on the role of meiobenthic copepods in freshwater foodwebs is more limited (Gladden and Smock 1990, Sarvala 1998) although meiofauna importance in food webs is recognized (Schmid-Araya et al. 2002, Hillebrand et al. 2002). Evidence from marine environments show that the benthic microalgal biomass may be controlled by harpacticoids (Carman et al. 1997) and that the diel variation of microalgal biomass is influenced by meiofaunal grazing (Buffan-Dubau and Carman 2000).

Benthic harpacticoid copepods are often an important component of the meiofauna both in the littoral and profundal zones of lakes. The main food sources for harpacticoid copepods are assumed to be detritus, algae and bacteria (Hicks and Coull 1983, Dole-Olivier et al. 2000). Although each of the meiobenthic copepod species may exploit microalgal resources differently (Buffan-Dubau et al. 1996, Pace and Carman 1996), the spatial distribution of copepods is often directly correlated with the patchy distribution of microalgae, particularly of diatoms (Long and Ross 1999, Sandulli and Pinckney 1999). There are also indications that certain harpacticoid copepods preferentially feed on some diatom species over other diatom species (Lee et al. 1977).

In general, harpacticoids are reported to avoid the ingestion of cyanobacteria (Decho and Castenholz 1986, Buffan-Dubau and Carman 2000), although a number of harpacticoid species are able to ingest (Miliou and Moraitouapostolopoulou 1991) and assimilate (O'Neal 1998) certain cyanobacterial species. These indications imply that meiofauna represent an important link between microalgal primary production and higher trophic levels and may have the ability to drive succession from the edible to the less edible microalgal species.

Food quality has been observed to affect the growth and development of both calanoid and cyclopoid copepods (Vijverberg 1989, Twombly and Burns 1996, Koski et al. 1999) and when food is of insufficient quality, growth and development may be arrested (Hart and Santer 1994). Although the growth, development and reproduction of harpacticoid copepods has been less studied, it has been hinted that
food quality and quantity may have a similar effect in this group of copepods (Powlik et al. 1997, Pinto et al. 2001), though development may continue during some instars in the absence of food (Weiss et al. 1996).

Benthic harpacticoid copepods, intensively exploiting biofilms provide a good test system to explore the interaction of grazers with multi-specific consortia of phototrophs. In this study, we intend to investigate:
1) whether the freshwater harpacticoid copepod *Attheyella trispinosa* (Brady 1880) has the ability to drive the species composition of phototrophic consortia towards less edible cyanobacteria when nutrients are not limiting algal growth and conversely,
2) how the specific composition of biofilms influences the development and survival of the harpacticoid copepods *A. trispinosa* and *Bryocamptus minutus* (Claus 1863), especially when a cyanobacterium is present.

**Methods**

**Biofilms**

The benthic diatoms *Achnanthes lanceolata* (Brébisson Grunow 1880), *Navicula trivialis* (Lange-Bertalot 1980), and *Nitzschia perminuta* (Grunow) M. Peragallo 1903 and the benthic cyanobacterium *Leptolyngbya foveolarum* (Rabenhorst ex Gomont) Anagnostidis et Komárek 1988) were obtained from non-axenic cultures kept in the laboratory. All algal species were isolated from sediment samples collected in 1999, at the littoral zone of small lakes in the floodplains of the River Waal (a branch of the River Rhine).

*N. trivialis* (Length = 38 ± SD 3 μm) and *N. perminuta* (L = 12 ± SD 1 μm) are motile diatoms that move in close contact to the substrate while *A. lanceolata* (L = 13 ± SD 1 μm) is a sessile diatom with a size similar to that of *N. perminuta* and whose cells firmly attached to the substrate, sometimes by erect gelatinous stalks. The filamentous cyanobacterium *L. foveolarum* (Diameter = 1 ± SD 0 μm) never formed heterocysts during the experiment.

A total of eleven young biofilms of different specific composition (see Figure 6.1), were allowed to grow during 10 days on sand blasted glass discs (area = 1.5 cm²) in Petri dishes at 20 °C under a light intensity of 50 μmol m⁻² s⁻¹ and a 16:8h light:dark regime. The biovolume of the algal inocula placed in the Petri dishes was 1.7 x 10⁷ μm³ cm⁻² for the diatom biofilms and 8.5 x 10⁶ μm³ cm⁻² for the cyanobacterium monoculture biofilms.

Each of the diatom species in the multi-specific diatom films contributed to the total inoculum with an equal biovolume. For the biofilms with one diatom species plus the cyanobacterium, each inoculum consisted of 8.5 x 10⁶ μm³ cm⁻² diatom and...
4.25 \times 10^6 \mu m^3 cm^2 cyanobacterium. The inocula were allowed to settle in 30 ml Petri dishes containing the glass discs and WC medium (modified from Guillard and Lorenzen 1972). Half of the growth medium was renewed every 3 days and nutrients, in the amount present in fresh medium, were added daily from day 7 to day 10. A minimum of 10 days of biofilm incubation was necessary to obtain sufficient algal biomass for in vivo chlorophyll a fluorescence measurements.

After 10 days of incubation, the glass discs with the young biofilms were transferred to 1.5 cm diameter wells (24 well polystyrene clusters) filled with 2 ml medium. Modified WC medium was used in the first set of experiments (Grazing experiments) to test the ability of the harpacticoid copepod A. trispinosa to alter the growth, species composition and physiognomy of simple biofilms through grazing.

M4 Elendt medium (Elendt 1990) was used in the second set of experiments (Experiments on post-embryonic development) to test the effect of food quality (biofilm specific composition) on the post-embryonic development time, growth and survival of copepods. Previous experiments have shown that M4 Elendt medium is more suitable for copepod growth and development compared to modified WC medium while algae grow only at a slightly lower rate than on modified WC medium. All experiments were run in duplicate.

1. Grazing experiments

For each algal assemblage, two replicates of the young biofilms were kept without copepods (control) while a set of 15 unfertilized female copepods of A. trispinosa was placed in each of two other replicates. The copepods had been cultured in the laboratory for several generations, after collection from experimental ponds at the university campus. Prior to the experiments, female copepodids at stage 5 were isolated from the stock cultures and allowed to develop to adult stage in 4 ml wells (2 cm diameter) filled with M4 Elendt medium and a mixture of N. perminuta and N. trivialis (1:1) at a final concentration of $1 \times 10^7 \mu m^3 cm^2$.

The sets of 15 newly moulted adult females were transferred to the 2 ml wells already containing the discs with the microalgal assemblages and the modified WC medium. Concentrated nutrient stock for algal growth was added daily to every well. Chlorophyll a (CHLa) biomass and the capacity of fluorescence emission were measured by a non-destructive method, during the 17 days of the experiment.

Fluorescence measurements

The in vivo chlorophyll fluorescence of the biofilms on glass discs was measured with a PHYTO-PAM fluorimeter (Pulse Amplitude Modulation, Heinz Walz GmbH, Effeltrich, Germany) equipped with an array of light-emitting diodes featuring 4 different wavelengths: 650 nm (red), 620 (orange), 535 (green), 470 (blue) and to differentiate between the contribution of the main algal groups with different pigment composition (Bacillariophyceae and Cyanophyceae).
Algae were kept in the dark for 30 min before measurement of the fluorescence variables $F_0$, $F_m$, $F_v$ and $\Phi_0$. In accordance with Schreiber et al. (1994), $F_0$ is the fluorescence level following dark incubation (all PSII electron acceptors fully oxidized, reaction centres open), $F_m$ is the maximum fluorescence level with all PSII reaction centres closed (assuming negligible non-photochemical quenching), $F_v$ is the maximum variable fluorescence of a dark adapted sample ($F_v - F_m$) and $\Phi_0$ is the maximum quantum yield ($F_v / F_m$) of PSII photochemistry.

$F_0$ values were calibrated for CHL$a$ biomass for each monospecific algal assemblage with spectrophotometric data. As the calibration curves of the three diatom species were not significantly different ($F_{2,40} = 2.59, p > 0.05$), diatom data were pooled to calculate the curve used to calibrate the $F_0$ values for multi-specific diatom assemblages. $\Phi_0$ was calculated to obtain a measure of the maximal efficiency of electron transport of each type of microalgal assemblage (Schreiber et al. 1995), which is independent of CHL$a$ biomass.

**Figure 6.1**

Growth curves of mono-specific and multi-specific biofilms grown in the absence (Control) and in the presence (Copepoda) of copepods during the grazing experiment. Error bars indicate ± 1 SD of the mean, n = 2 for all biofilms except for N. perminuta, N. perminuta + A. lanceolata and L. foveolarum where n = 4 (data was pooled with the biofilms obtained from an inoculum of the same algal species plus N. trivialis, see methods).
Microscopic observations

To assess the relative abundance of diatom species in the multi-specific diatom assemblages two replicate discs at the beginning \((t = 0)\) and at the end of the experiment \((t = 17\) days\) were individually placed in a 10 ml vial with 1 ml of distilled water and 40 \(\mu\)l of Lugol’s iodine and stored at 4 °C for later cell counting. Before counting, the vials were sonicated for 30 sec to detach the algae from the discs (Sonifier 2210, Branson Ultrasonics). When \(A.\) lanceolata was present at \(t = 0\), the discs were previously scraped with a scalpel before sonication since this species firmly attached to the discs.

In order to check whether the copepods digested the diatoms and the cyanobacterium, a fluorescence microscope (Olympus BH-2, Japan, 40x magnification) was used to examine the presence of live algae in the faecal pellets from four sets of 15 newly moulted adult females feeding on monospecific biofilms during 12 hours.

Calculations and statistics

The variation in the CHLa biomass of mono and multi-specific biofilms growing in the presence of copepods was compared to the variation in the CHLa biomass observed for control biofilms using a repeated measures test (Zar 1999). Because \(N.\) trivialis did not grow in the multi-specific biofilms containing \(N.\) perminuta (no visual observations at \(t = 0\)) and was detected together with the cyanobacterium on only one date, the analyzed data set contained 4 replicates for the mono-specific biofilms of \(N.\) perminuta and \(L.\) foveolarum and the bi-specific assemblage of \(N.\) perminuta + \(A.\) lanceolata.

All other analyses were applied on 2 replicates for each biofilm. Repeated measures analysis was used to test the effect of the cyanobacterium on \(N.\) perminuta and \(A.\) lanceolata. As \(A.\) lanceolata biomass data violated the assumption of sphericity (Huynh and Feldt 1970) required to perform repeated measures analysis, ANCOVA was applied on LN transformed biomass data, using time as covariate.

Figure 6.2
Algal biomass increments of a diatom biofilm growing in the absence (filled squares) and presence (open squares) of copepods.
The growth rate ($\mu$) of algae growing in the absence of copepods was calculated from CHL$a$ biomass variation in time. The CHL$a$ biomass was calibrated to carbon biomass for each algal assemblage to enable comparisons among algal assemblages. The carbon content of a set of biofilms growing under the experimental conditions in the absence of copepods was measured using a T.O.C. Analyzer (Model 700, O.I. Co., USA) after measuring chlorophyll fluorescence and significant regressions were derived from these data.

In order to account for biomass changes in the biofilms ascribed to autogenic factors and not to herbivory (Peterson and Renaud 1989) the algal biomass increment between adjacent dates of fluorescence measurement ascribed to copepod herbivory was calculated by subtracting the observed increment from the expected biomass increment in the absence of herbivory. The expected increment for any given algal biomass was derived from statistically significant regressions obtained from control data.

ANOVA's were applied to compare the observed biomass increment of microalgae (2 replicates for each biofilm type) to the expected increment in the absence of copepods at each date of fluorescence measurement. ANOVA was applied to carbon increment data from day 3 to day 6, using the type of biofilm as factor to test if copepods removed different amounts of carbon from different biofilms. Multicomparison of the means (Tukey HSD, 5% error probability) was performed to establish differences between pairs of biofilm types.

ANOVA was applied to test the effect of copepod presence on the relative abundance of $N.\ perminuta$ in the multi-specific diatom biofilms on day 17. The same test was applied to $N.\ trivialis$ growing together with $A.\ lanceolata$. It was observed that $N.\ trivialis$ was absent from multi-specific biofilms containing $N.\ perminuta$ at $t = 0$. Thus, the results from the biofilm $N.\ perminuta + A.\ lanceolata + N.\ trivialis$ were pooled with those from $N.\ perminuta + A.\ lanceolata$ biofilms (4 replicates).

![Graph showing estimated daily amount of cyanobacterial (a) and diatom (b) carbon removed per copepod, between day 3 and 6, from biofilms containing the cyanobacterium $L.\ foveolarum$ (Cyano) and the diatoms $N.\ perminuta$ (Np), $N.\ trivialis$ (Nt) and $A.\ lanceolata$ (Al).]
2. Experiments on copepod post-embryonic development

In order to obtain nauplii for development experiments, 24 ovigerous females or mating pairs of *A. trispinosa* and *B. minutus* were transferred from the stock cultures and individually placed in 4 ml wells (2 cm diameter, 12 well polystyrene clusters) filled with M4 Elendt medium and a mixture of *N. perminuta* and *N. trivialis* (1:1) at a final concentration of $1 \times 10^7 \mu m^3 cm^{-2}$. After hatching, ten 24h - 48h old nauplii were transferred to each 2 ml well already containing the discs with the several microalgal assemblages and 2 ml of M4 Elendt medium. Previous observations have revealed that 24 - 48h nauplii endure handling better than nauplii younger than 24h.

Half of the medium was replaced every 2 days and concentrated nutrient stock for algal growth was added on the intermediate days. Naupliar development time was established by the time taken for half of the naupliar population to moult into the first copepodid stage and copepodid development time was the time required for half of the population to moult into the adult stage. Copepod development stage was checked daily with a binocular microscope. After the last moult, adult length was measured from the anterior end of the cephalothorax to the posterior end of the abdomen (without the furcal rami).

Naupliar and copepodid development times and survival data were subjected to ANOVA followed by *a posteriori* test (Tukey's B, $\alpha = 0.05$) to identify groups of biofilms with similar influence on these parameters. ANOVA were also applied to data of female and male length of both copepod species to assess the influence of biofilm type on body growth. Unplanned comparisons Hochberg’s GT2 tests (because of unequal sample size) were used to identify homogeneous sub-sets.

![Figure 6.4](image)

Fraction of each diatom species in multi-specific diatom biofilms at the beginning ($t = 0$ days) and at the end ($t = 17$ days) of the experiment.
Results

1. Grazing experiments

The copepods generally had a negligible effect on the biomass of the tested diatom biofilms with a clear exception for *N. perminuta* whose biomass was significantly reduced in the presence of copepods ($F_{1,6} = 20.43$, $p < 0.01$, Figure 6.1). The biomass of the cyanobacterium *L. foveolarum* was not negatively affected by the presence of copepods (maximum $F_{1,6} = 0.38$, $p > 0.05$). From day 10 onwards, *L. foveolarum* attained even higher biomass in the presence of copepods.

The presence of the cyanobacterium significantly decreased diatom biomass in bi-specific biofilms during biofilm formation (see values at $t = 1$, Figure 6.1) and that trend continued throughout the experiment: *N. trivialis* was only detected on day 13; the biomass of *N. perminuta* was lower than in mono-specific biofilms ($F_{1,2} = 18.65$, $p < 0.05$), decreasing below the limit of detection level by day 10 in the absence of copepods and *A. lanceolata* registered an overall reduced biomass relative to when growing alone (ANCOVA $F_{1,21} = 216.90$, $p < 0.001$).

*A. lanceolata* exhibited a particular feature when growing with the cyanobacterium i.e. on day 6 of the experiment, a thin film of *A. lanceolata* was visible near the surface of the medium. On day 10, *A. lanceolata* was no longer detected in the biofilm attached to the glass disc, but was forming a distinct, brownish film floating near the surface of the medium.

Except for *N. trivialis*, all control biofilms have shown a decrease in maximum quantum yield of PSII photochemistry throughout the experimental period. After day 10, 5 - 20% higher photosynthetic capacity was observed in the presence of the grazers for all biofilm types, relative to the controls.

![Figure 6.5](image)

Mean survival rate of the copepod species grown on the several biofilms (see legend of Figure 6.3). Error bars indicate ± 1 SD of the mean, $n = 2$. Means that are not significantly different (Tukey comparisons, $\alpha = 0.05$) are indicated by the superscript letters.
During most of the time intervals and for all algal assemblages, the removal of algae by the copepods (i.e. decrease in algal increment relative to the control) was not statistically significant. After day 10, the negative trend on daily increments was reversed to a positive trend (Figure 6.2) as carrying capacity was being approached in the controls. The copepods removed generally more cyanobacterial carbon from the biofilms containing the cyanobacterium relative to diatom carbon from the biofilms containing only diatoms ($F_{9,10} = 3.15, p < 0.05$, Figure 6.3). However, multicomparison tests failed to find any significant paired differences. The removal of cyanobacterium carbon biomass was not dependent on the specific composition of the biofilm ($F_{3,4} = 1.54, p > 0.05$, Figure 6.3a).

When *A. lanceolata* was present however, the copepods removed smaller amounts of the cyanobacterium. The copepods showed a clear preference for staying on/under the floating *A. lanceolata* film instead of staying in/on the cyanobacterium layer. In the absence of copepods, the cyanobacterium grew in vertical tufts. In the presence of copepods, the cyanobacterium formed a compact three-dimensional network of threads that grew in all directions forming a cohesive film.

**Figure 6.6**

Naupliar and copepodid mean development times (in days) of the copepods *A. trispinosa* and *B. minutus* growing on the several biofilms (see legend of Figure 6.3). Error bars indicate ± 1 SD of the mean, $n = 2$, except (*) where $n = 1$. Copepodid means that are not significantly different (Tukey comparisons, $\alpha = 0.05$) are indicated by the superscript letters.


*N. perminuta* exhibited the highest maximum growth rate ($\mu = 0.58$ day$^{-1}$) relative to *A. lanceolata* and *N. trivialis* that had similar maximum growth rates ($\mu = 0.33$ and $0.35$, respectively). *N. trivialis* failed to establish a population in the presence of *N. perminuta* (0.47% of total abundance at $t = 0$; 0% at $t = 17$) and was poorly represented in the presence of *A. lanceolata* (Figure 6.4). *N. perminuta* was removed from the bi-specific biofilms by the copepods (Figure 6.4) enabling *A. lanceolata* to be represented at a higher percentage than in the absence of copepods ($F_{1,8} = 64.3$, $p < 0.01$). However, because *N. perminuta* was the most abundant diatom in the biofilm, the copepods seem to have been using the food in the same proportion as it was available.

The abundance of *N. trivialis* growing with *A. lanceolata* decreased throughout the experiment regardless of the presence of copepods ($F_{1,4} = 0.99$, $p > 0.05$). *A. lanceolata* growing with other diatoms was always very closely attached to the glass discs, especially in the presence of copepods (visual observation).

Microscopic examination of faecal pellets indicated that copepods digest diatoms well but have some difficulties digesting the cyanobacterium. Diatoms were represented by empty frustules or single diatom valves, while more than half of the cells in the centre of the filaments of the cyanobacterium (up to 20 cells in length) survived gut passage.

### 2. Copepod post-embryonic development

The specific composition of the biofilm influenced significantly the survival of both *A. trispinosa* ($F_{9,10} = 7.17$, $p < 0.01$) and *B. minutus* ($F_{910} = 7.89$, $p < 0.01$) (Figure 6.5). The survival of both copepod species was lower when growing on biofilms containing the cyanobacterium or the diatom *N. trivialis* and higher when growing on biofilms containing *A. lanceolata* or *N. perminuta* (Figure 6.5).

![Figure 6.7](image)

Female (filled circles) and male (open circles) total body length of copepods grown on the different biofilms (see legend of Figure 6.3). For *A. trispinosa*, females $F_{3,50} = 14.69$, $p < 0.001$ and males $F_{3,13} = 13.47$, $p < 0.001$. For *B. minutus* females $F_{3,60} = 0.19$, $p > 0.05$ and males $F_{3,20} = 2.05$, $p < 0.10$. Error bars indicate ± 1 SD of the mean.
The naupliar development time of both *A. trispinosa* and *B. minutus* was significantly longer when growing on the cyanobacterium monoculture relative to any other microphytobenthic assemblage ($F_{9,10} = 14.43$, $p < 0.001$, Tukey’s B, $\alpha = 0.05$ and $F_{9,10} = 24.70$, $p < 0.001$, Tukey’s B, $\alpha = 0.05$, respectively, Figure 6.6).

The copepodids developed significantly faster when feeding on any diatom diet containing *N. perminuta* and slower when feeding on any algal assemblage containing the cyanobacterium or *N. trivialis* (Figure 6.6). Both males and females of *A. trispinosa* have shown significantly larger total body length when feeding on a mixture with *N. perminuta* and *A. lanceolata* compared to when feeding on other biofilm types (Figure 6.7).

Although the smallest individuals were the copepods grown on the cyanobacterium, a posteriori multicomparison test revealed that the length of these copepods was not significantly different from the length of copepods growing on *N. perminuta* alone. Both females and males of *B. minutus* tended to be consistently smaller when feeding on biofilms containing the cyanobacterium although the differences were too small as to be significant (Figure 6.7).

**Discussion**

The copepods were found to exert a generally low grazing pressure that allowed the algal populations to grow and equilibrate at a high biomass as previously reported for other meiofaunal grazers (e.g. Admiraal *et al.* 1983). Indeed, a low grazing impact has been demonstrated to result from a small number or a small biomass of herbivores relative to the available algal biomass (Bott and Borchardt 1999) or from the presence of high nutrient levels that enabled the algal populations to outgrow their losses (Hillebrand *et al.* 2000). The impact of grazing on the biofilm may have been further reduced by an enhancement of algal biomass through removal of decaying algal cells and accompanying bacteria, thus allowing for stimulated algal growth.

Our observation on copepods grazing on cyanobacteria confirm the coupling of consumption and stimulation. As in Schäffner *et al.* 1994 copepods were found to graze on filaments by biting off portions in addition to consuming entire filaments and forage unsystematically on the clipped trichomes. On the one hand, there is release of nutrients by this ‘sloppy’ feeding through the mechanical breakage of cells and by copepod excretion while grazing (O’Neill *et al.* 1996). On the other hand, non-ingested clipped trichomes may continue to grow, enabling the growth of the cyanobacterium in various directions thus altering the architecture of the cyanobacterial film and enabling filaments to be fully exposed to light. A higher maximum quantum yield (i.e. healthier cells) registered after day 13 for all biofilms with
copepods relative to controls, is consistent with a positive effect of grazing on biofilm algae.

Different harpacticoid species have been shown to specialize on the use of the available microbial sources (Carman and Thistle 1985) and to exhibit particular feeding techniques that determine the ability to use different diets (Marcotte 1984). A similar role has been suggested for amphipods that may favor an increase of the number of epiphyte taxa (Jernakoff and Nielsen 1997). Hence, copepods, through removal of more edible food species, such as *N. perminuta*, may be responsible for an impact on species composition of microphytobenthos rather than on biomass.

However, the potential effects on the phototrophic species that differed widely in substrate adherence and digestibility were apparently overruled by the direct interaction of algal species. In fact, the biofilms after incubation (at $t = 0$) have shown that *N. trivialis* was unable to establish a large population in the presence of any other diatom species and the cyanobacterium prevailed over any diatom species.

During biofilm incubation and during the experiment, *N. perminuta* showed the highest growth rate of all diatom species and was thus the most successful diatom in multi-specific assemblages. Cyanobacteria grow well at and above 20 °C when nutrients are available and this trend has been indicated for both marine (e.g. Watermann et al. 1999) and freshwater species (Chapter 3).

The better performance of *A. lanceolata* relative to the other diatom species growing in the presence of *L. foveolarum* may result from a modified growth strategy switching from firm adherence to floating of cells, an adaptive response of a diatom species that is, to our knowledge, new to the literature. The floating diatom film may have profited from mitigated inhibitory effects by the cyanobacterium (Abarzua et al. 1999) and decreased shading by its filaments. It is remarkable that the copepods feeding on the mixed *A. lanceolata-L. foveolarum* biofilms enhanced the survival of the diatom relative to control biofilms thus contributing to the survival of the most edible food species.

Although our study did not have the purpose of estimating carbon consumption rates, our attempt to estimate biomass removal by the copepods suggested that a higher biomass of *L. foveolarum* was ingested relative to that of diatoms. Grazing rates for meiofauna organisms in general and harpacticoid copepods in particular are scarce in the literature. Perlmutter and Meyer (1991) have estimated a grazing rate of 0.03 - 0.47 µg C ind$^{-1}$ day$^{-1}$ for *Attheyella* spp. on bacteria.

*In situ* grazing rate measurements of meiofauna (copepods, nematodes and ostracodes) of 0.042 µg bacterial C and 0.53 µg algal C ind$^{-1}$ day$^{-1}$ during the summer were reported by Montagna (1984). Admiraal et al. (1983) measured an average nematode grazing rate of 0.165 µg diatom C ind$^{-1}$ day$^{-1}$. Our estimated values for cyanobacterium carbon removal were close to the low bacterial carbon requirement (1 µg C copepod$^{-1}$ day$^{-1}$) estimated for the harpacticoid *Heteropsyllus pseudonunni* by
Grazing

Rieper (1978). Cyanobacteria are generally considered to be nutritionally inadequate (Gulati and DeMott 1997) and the present observations confirm earlier indications on copepods ingesting large quantities of cyanobacteria to compensate for their poor nutritional value (Chen and Folt 1993, Koski et al. 1999).

Although being able to grow and develop on an exclusive cyanobacterial diet, both *B. minutus* and *A. trispinosa* took longer to develop and experienced a higher mortality rate relative to the copepods feeding on diatom diets (this study). Harpacticoid copepods are highly flexible in their uptake of microalgae, bacteria or detritus, which makes them an important link in aquatic food webs representing alternative pathways to larger predators (Schmid-Araya et al. 2002). The ability to survive on poor diets may be an important asset for harpacticoid copepods that thrive in variable environments dominated by detritus as previously pointed by Weiss et al. (1996).

When feeding on both *A. lanceolata* and *N. perminuta*, the copepods experienced the shortest development times and reached larger body length than when feeding exclusively on either *N. perminuta* or *A. lanceolata*. The availability of an extra algal species may further enrich a nutritious diet since dietary diversity may contribute to obtain a nutritionally complete diet (Kleppel and Burkart 1995, Schmidt and Jonasdottir 1997).

The copepods had difficulty in digesting the cyanobacterium (half of the cells in the ingested trichomes were not digested), which may have led to nutrition deficiencies. When *N. perminuta* or *A. lanceolata* were present in small quantities in the biofilms with the cyanobacterium, the copepods developed at a rate similar to that of copepods feeding on the diatom assemblages thus suggesting again that the cyanobacterium is nutritionally deficient as sole food resource.

The poor quality of the cyanobacterium as food was especially severe on *B. minutus* that failed to develop beyond copepodid stage four in one of the replicates. Food of low quality causes reduced moulting frequencies of copepods (Vijverberg 1989, Pinto et al. 2001) and when food lacks essential nutrients, growth and development may be arrested (Hart and Santer 1994).

*A. trispinosa* and *B. minutus* copepodids had a longer development time than the naupliar stages, in accordance with data from Sarvala (1979) for *Attheyella crassa* and *Bryocamptus echinatus*, respectively. In the present study, *A. trispinosa* feeding on diatoms had a development time similar to that of the marine harpacticoids *Tigriopus californicus* growing at 18 - 20 °C (Powlik et al. 1997) and *Amphiascoides atopus* growing at 23 °C (Sun and Fleeger 1995). *B. minutus* developed faster than the related species *B. zschokkei* at 18 °C (O'Doherty 1985) and it seems to be surpassed only by fast developing *Tisbe* species (Battaglia 1957, Gaudi and Guerin 1977).

In general, naupliar development time seems to be less affected by the quality of food than later development stages. Twombly and Burns (1996) have found a
similar trend for the calanoid copepod *Boeckella triarticulata*. In the present study, the
nauplii may have benefited from diatoms still in the biofilms also containing the
cyanobacterium in the first days of the experiment. Additionally, the earlier naupliar
stages carry stored nutrients obtained from maternal source that may enable them to
overcome food deficiencies in the earlier development stages.

The present study shows that
1) harpacticoid copepods feed on widely different algal species, albeit the ingestion
rate of algal species may differ. However, a clear top-down control of overall
microphytobenthic biomass was not evident in the absence of nutrient limitation
for algal growth, and
2) the various algal species have distinct effects on the survival, growth and
development of juvenile stages of the copepods and a combination of diatom
species was shown to provide the best food for the two copepod species.
Our hypothesis that intense copepod grazing leads to non-edible microphytobenthos
was not confirmed. On the contrary, feeding of copepods on multi-species consortia
of cyanobacteria and diatoms may affect the architecture of biofilms and break up
dominant cyanobacterial mats, thereby allowing diatom species of a high nutritional
value to persist.

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