Vol. 47: 99–106, 2007 | AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol | Published April 3

Bacteria associated with copepods: abundance, activity and community composition

Eva Friis Møller[1,2,*], Lasse Riemann[3], Morten Søndergaard[1]

[1]Freshwater Biological Laboratory, University of Copenhagen, Helsingørsgade 51, 3400 Hillerød, Denmark
[2]Department of Marine Ecology and Aquaculture, Danish Institute for Fisheries Research, Kavalergården 6, 2920 Charlottenlund, Denmark
[3]School of Pure and Applied Natural Sciences, Kalmar University, 391 82 Kalmar, Sweden

ABSTRACT: We measured abundance, activity and community composition of bacteria associated with *Calanus* spp. in the North Sea in late July and compared it to the free living bacteria. Both the local density and production of bacteria associated with *Calanus* spp. were magnitudes higher than the average in a comparable volume of water. As analyzed by denaturing gradient gel electrophoresis (DGGE) and 16S rRNA gene sequencing, the bacterial community associated with *Calanus* spp. consisted of members of the *Roseobacter* lineage (*Alphaproteobacteria*), whereas the free living bacteria were more diverse. Discovery of an identical bacterial phylotype on *Calanus* spp. and in the free living community at the specific depth with the highest *Calanus* spp. biomass suggested an active exchange of bacteria between copepods and the free living state.

KEY WORDS: *Calanus* spp. · Attached bacteria · Bacterial production · Community composition

INTRODUCTION

Copepods are by far the most abundant mesozooplankton in the ocean (Verity & Smetacek 1996). When they feed, they produce dissolved organic matter (DOM) by sloppy feeding, excretion or leakage from fecal pellets (Eppley et al. 1981, Roy et al. 1989, Møller et al. 2003, Steinberg et al. 2004, Møller 2007). It may therefore be advantageous for bacteria to stay in the vicinity of copepods, i.e. be situated in the same layer in the water column, or even be attached to the copepod.

In copepod populations, between 0 and 100% of the copepods are found to have bacteria attached to their surface (Nagasawa 1989). Generally, most exterior bacteria are found near the mouth, between the segments and around the anus (Carman & Dobbs 1997), i.e. where the supply of organic substrates produced by copepod feeding activity would be expected to be highest. However, reports of total abundance on copepods are scarce, probably due to methodological difficulties arisen from the uneven distribution (Hansen & Bech 1996, Tang 2005). Likewise, only few studies have measured the activity of copepod-attached bacterial communities, but it seems to be high compared with the free living bacteria (Carman 1994, Tang 2005). In this respect copepods resemble aggregates, which are often sites of elevated microbial activity (Azam & Long 2001). Compared to aggregates, which contain a finite amount of organic material, copepods will, however, be a continuous source as long as they feed (Tang 2005). It could be speculated that copepods may function as 'baby machines' where attached bacteria release most of their progeny to the surrounding environment, as has been suggested for colonized fecal pellets (Jacobsen & Azam 1984).

To our knowledge no studies have examined bacterial community composition on marine copepods. Most previous studies have focused on copepod associated bacterial communities as reservoirs of specific bacterial pathogens (e.g. Cellini et al. 2005, Huq et al. 2005) and on specific bacterial groups. For instance, Heidelberg et al. (2002) found that *Vibrio* species constituted a large proportion of the bacteria associated with copepods in Chesapeake Bay. In addition, they found a

*Email: efmoller@bi.ku.dk

correlation between zooplankton and water-column presence of single *Vibrio* species (Heidelberg et al. 2002).

In the present study we hypothesize that copepods are an advantageous surface for bacteria, and that the release of bacteria attached to copepods can affect community composition of bacteria in the surrounding water. Specifically, we compared the abundance, production and community composition of bacteria attached to copepods relative to free living bacteria and related this to the abundance and distribution of copepods. The study was carried out in the North Sea, in late July 2005, using the large dominant *Calanus* species as model organisms.

MATERIALS AND METHODS

Sampling. The study was conducted during a cruise with RV 'Dana' (Danish Institute for Fisheries Research), from 26 July to 4 August 2005. A 65 m deep station in the central North Sea (56° 39.0′ N, 03° 27.0′ E) was sampled on 27 July 2005. Bacterial abundance, production and community composition and copepod abundance were recorded every 5 m in the upper 50 m. Additionally, we examined bacterial abundance, production and community composition associated with *Calanus* spp. females and copepodite stage 5 (CV).

Temperature, salinity and fluorescence were obtained with a Seabird CTD system equipped with a fluorometer and water samples were taken with 5 l Niskin bottles. *In situ* fluorescence was converted to chlorophyll *a* (chl *a*) using a regression between *in situ* fluorescence and measurements of water column chl *a* concentrations from selected depths sampled during the whole cruise of 10 d (chl $a = 3.58 \times$ fluorescence + 0.18, $n = 88$, $R^2 = 0.80$).

Copepods used for experiments were collected using a WP-2 net towed vertically from 50 m depth to the surface. Samples for copepod abundance were filtered using a submersible pump (1500 l min^{-1}) equipped with a 30 µm net. Detailed copepod data will be presented elsewhere (S. H. Jónasdóttir unpubl. data) but is briefly presented here for comparison with the vertical distribution of bacterial abundance and activity.

Bacterial abundance, composition and production in water samples. Bacterial abundance in water samples was determined by flow cytometry (FACSCalibur, Becton Dickinson) after staining the fixed cells (2% glutaraldehyde) with the nucleic acid stain SYBR Green I (Molecular Probes) according to Marie et al. (1997). Briefly, 10 µl of a 100× dilution of stock SYBR Green 1 was added to 1 ml of sample, followed by 10 µl of a suspension of 2 µm fluorescent beads (Polyscience).

Bacterial production in 20 ml seawater was measured in triplicates by [^{3}H]-thymidine (10 nM final) incorporation (Fuhrman & Azam 1982) and converted to cell production assuming 2×10^{18} cells mol^{-1} thymidine incorporated (Bell 1993). Samples from 5 to 37 m depth were incubated at 16°C, while the deeper samples were incubated at 8°C. Incubations were stopped by addition of trichloroacetic acid (5% final). Samples with 5% trichloroacetic acid added prior to the addition of [^{3}H]-thymidine were used as blanks. The samples were filtered onto 0.2 µm cellulose nitrate filters.

Bacterial community composition analyses were done on 100 ml water from each depth filtered onto 0.2 µm membrane filters (Whatman Nuclepore). Filters were frozen at −80°C until nucleic acid extraction. DNA was extracted from filters using an enzyme/SDS-based protocol as described in Boström et al. (2004), but with a few modifications: no phenol/chloroform extraction and glycogen (10 µg per sample; Roche) was used as co-precipitant. DNA was re-suspended in TE (10 mM Tris, 1 mM EDTA, pH 8.0) and quantified fluorometrically (PicoGreen; Molecular Probes).

Bacterial 16S rDNA was amplified using primers GC341f (Muyzer et al. 1993) and 907r (Muyzer et al. 1998). PCR reactions (50 µl) contained 0.8 mM deoxynucleotide triphosphates, 0.5 µM of each primer, 3% DMSO (Finnzymes) and 1 unit Phusion polymerase with associated buffer (Finnzymes). For filter samples, ~2 ng template DNA was used. For copepod samples (see below), ~50 ng template DNA was required per reaction. Initial denaturation was at 98°C for 30 s followed by: denaturation for 10 s at 98°C; annealing for 30 s at an initial temperature of 63°C, decreasing 1°C every 2 cycles to a final temperature of 53°C; extension for 90 s at 72°C. Ten cycles were run at 53°C for a total of 30 cycles followed by a final 7 min incubation at 72°C. Two blank filters treated as sample filters served as negative controls. No DNA or PCR products were obtained from these.

PCR products were quantified fluorometrically and 60 ng PCR products per sample were analyzed by DGGE using the D Gene System (Bio-Rad) at 60°C for 6 h at 150 V (Riemann & Middelboe 2002). DGGE bands were excised, eluded, re-amplified, and analyzed on DGGE to confirm migration relative to the original sample. In some cases re-amplified bands yielded several faint bands. In these cases excision and re-amplification was repeated to enrich the intended band. Bands were sequenced using the DYEnamic™ ET terminator cycle sequencing kit (Amersham Biosciences) and an ABI PRISM 377 sequencer (Applied Biosystems) as described by the manufacturer. Sequences were aligned to known sequences using BLAST (Altschul et al. 1997) and analyzed by the program Chimera Check (Cole et al. 2003). DGGE band

sequences have been deposited in GenBank, using the abbreviations NS 1 to 17 (where NS refers to the North Sea), under the following accession numbers (in order): DQ839246 to DQ839262.

Bacterial abundance, composition and production associated to copepods. Samples for copepod abundance were preserved in 4% buffered formalin. Copepodites were identified to species or genera, and the length of 10 individuals from each group was measured in each sample. The copepod biomass was calculated on the basis of length-carbon regressions from the literature (Berggreen et al. 1988, Hay et al. 1991, Hirche & Mumm 1992, Sabatini & Kiørboe 1994, Satapoomin 1999, Uye et al. 2002).

The abundance and composition of bacteria attached to *Calanus* spp. were measured on females and CV incubated in 0.2 µm filtered seawater for 4 h to allow them to empty their guts. After this incubation they were rinsed 3 times in 0.2 µm filtered seawater, blot dried on pre-combusted GF/F filters, and transferred to sterile Eppendorf tubes using a pair of tweezers. Two batches of 20 copepods were frozen (–80°C) until DNA extraction with the Aquapure Genomic DNA Isolation Kit (Bio-Rad). Conventional DNA extraction, as used for filters, conferred complete lysis of copepods and inhibition of PCR amplification. A more gentle extraction method with a commercial DNA isolation kit, where copepods were not visibly lysed, and the use of a relatively high DNA template amount allowed for PCR amplification.

Enumeration of attached bacteria was done on rinsed copepods added to 1.5 ml of 0.2 µm filtered seawater containing glutaraldehyde (2%) and stored at 4°C. Before counting, individual copepods were blot dried on a pre-combusted GF/F filter, transferred to 10 ml of 0.2 µm filtered seawater and sonicated at 50 W, 2 × 30 s (Velji & Albright 1993), which was enough to disintegrate the copepod, although parts of the antennas were sometimes still recognizable. Since the copepod used for counting had empty guts, bacteria in fecal pellets were excluded from the counts. However, the disintegration meant that no differentiation could be made between bacteria on the copepod's exterior and bacteria inside the copepod. The samples were then stained with DAPI (Porter & Feig 1980) and bacteria were counted using epifluorescence microscopy. In total, 10 copepod samples were counted. Three samples containing only 10 ml of 0.2 µm filtered water and processed the same way as the copepod samples served as blanks.

Bacterial production on *Calanus* spp. was measured on live copepods, which had been starved for 4 h and subsequently rinsed 3 times in 0.2 µm filtered seawater. Five replicates of 2 copepods (BP_{cop}) were incubated with [^{3}H]-thymidine (10 nM) at 16°C in 18 ml water in 20 ml vials containing 0.2 µm filtered water from 10, 37 (fluorescence maximum) and 45 m, respectively. After the incubation it was verified that the copepods were still active. Three controls ($BP_{control}$) were performed from each depth. These contained 0.2 µm filtered seawater plus the same amount of water from the rinsed copepod culture as added to the copepod bottles but without copepods. Blinds, both with and without copepods, were prepared by addition of trichloroacetic acid before [^{3}H]-thymidine. The bacterial production associated with the copepods was calculated as $(BP_{cop} - blind_{cop}) - (BP_{control} - blind_{control})$.

The bacterial growth rate (µ) was calculated for the free living and copepod associated bacteria assuming exponential growth: $N_t = N_0 \times e^{t \times \mu}$, where N_0 is the measured abundance, N_t is the N_0 plus the bacterial production during the time (t).

RESULTS

During our study the water column was stratified with well-mixed warm water (>15°C) overlying a cold (<7°C) bottom layer. The thermocline was located at ~30 m and was ~15 m thick. A chl *a* maximum with concentrations up to 3 times higher than at the surface was found at the base of the thermocline (Fig. 1A). Copepods were dominated by *Calanus* spp., which constituted between 10 and 92% of the total biomass (Fig. 1B). *C. helgolandicus* were most important in the upper 30 m while *C. finmarchicus* dominated closer to the bottom. Adults and CV comprised more than 90% of the *Calanus* spp. biomass. Other copepods consisted of *Oithona similis* mainly in the upper 25 m, *Metridia lucens* and *Microsetella norvegica* in and below the thermocline, while *Temora longicornis* was important in the thermocline.

Bacterial abundance and activity

The abundance of free living bacteria in the water column was 1 to 5×10^5 cells ml^{-1}, with a small peak in the chl *a* maximum (Fig. 1C). The abundance of bacteria associated with *Calanus* spp. was about 1.9×10^5 cells copepod^{-1} (Table 1). Given a body volume of ~0.001 ml (assuming 0.13 pg C µm^{-3}, Berggreen et al. 1988) for *Calanus* spp. the local bacterial population densities associated with the copepod would be 1.9×10^8 ml^{-1}. Thus, the local density of bacteria associated with *Calanus* spp. was 1000 times higher than in the surrounding water.

The bacterial production in the water column showed distinct peaks at 15 m (7.8×10^3 cells ml^{-1} h^{-1}) and at 35 to 37 m associated with the chl *a* maximum

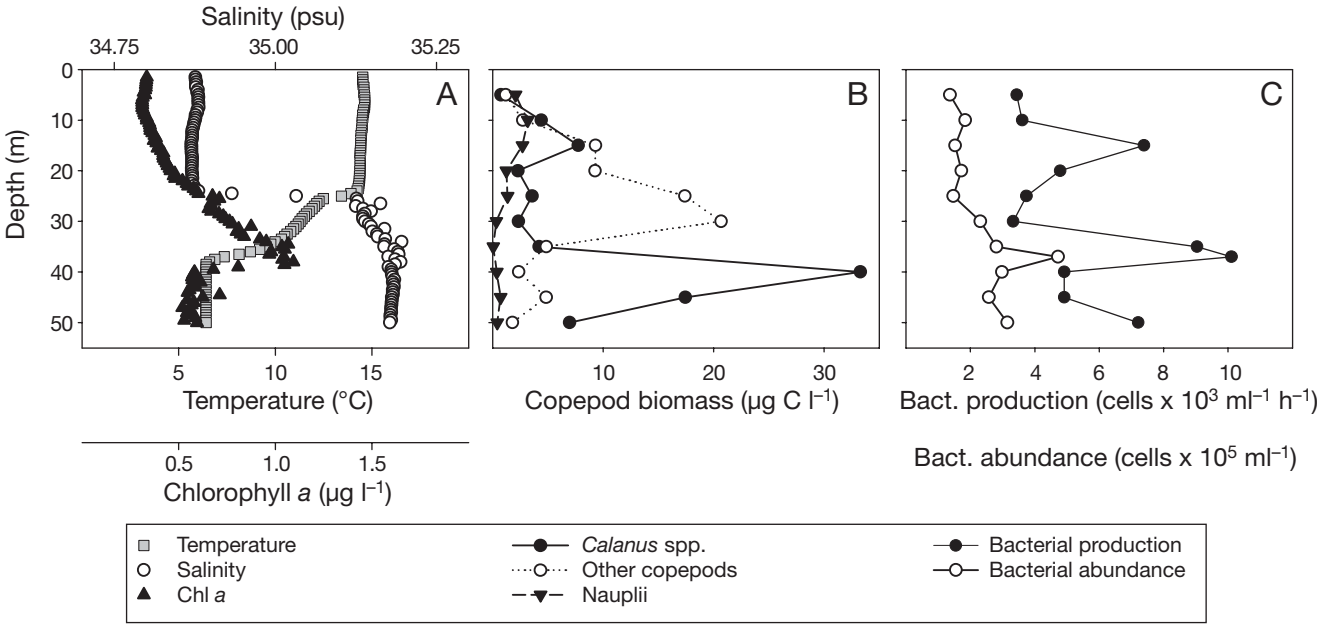

Fig. 1. Vertical depth profiles of (A) temperature, salinity and chlorophyll *a*; (B) biomass of *Calanus* spp., nauplii and other copepods (S. H. Jónasdóttir unpubl.); (C) bacterial abundance and production

(1.0×10^4 cells $ml^{-1} h^{-1}$, Fig. 1C). The bacterial production associated with *Calanus* spp. was between 5.3×10^3 and 9.3×10^3 cells $copepod^{-1} h^{-1}$, corresponding to $5–9 \times 10^5$ cells $ml^{-1} h^{-1}$. Thus, like for abundance, the local copepod associated production was magnitudes higher than the production of the free living bacteria. The growth rate of the copepod associated bacteria was 0.7 to 1.2 d^{-1} while the free living bacteria were growing at rates of 0.4 to 0.5 d^{-1} (Table 1).

Bacterial community composition

We used PCR-DGGE analysis of 16S rDNA to compare the community composition of bacteria attached to copepods with free living bacteria (Fig. 2). Ideally, this analysis provides a graphical 'fingerprint' of the bacterial community composition. Potential biases inherent in the DNA extraction and PCR-DGGE protocols are described elsewhere (Wintzingerode et al. 1997, Muyzer et al. 1998). The copepod samples consisted of less than 5 discernible bands. Identical bands were observed in the 2 copepod samples; though the lower-most bands were hardly visible in sample A, likely due to inefficient PCR amplification. In contrast, the free living bacterial communities were represented by 14 to 23 bands with an increase in the number of bands from the surface to deeper samples (Fig. 2, Table 2).

Twenty DGGE bands were excised, cloned and sequenced to obtain an impression of bacterial community composition and to compare the identity of bac-

Table 1. *Calanus helgolandicus* and *C. finmarchicus*. Bacteria associated with females and copepodite stage 5. Bacterial abundance and production on the copepod corresponds to 1.9×10^8 cells ml^{-1} and 5 to 9×10^6 cells $ml^{-1} h^{-1}$, respectively, assuming copepod volume to be 0.001 ml

Depth (m)	Copepod			Water column		
	Bacterial abundance[a] (cells $copepod^{-1}$ ± SE)	Bacterial production (cells $copepod^{-1} h^{-1}$ ± SE)	Growth rate (d^{-1})	Bacterial abundance (cells ml^{-1})	Bacterial production (cells $ml^{-1} h^{-1}$ ± SE)	Growth rate (d^{-1})
5	$1.9 \times 10^5 \pm 2.1 \times 10^4$	$7.6 \times 10^3 \pm 2.3 \times 10^3$	1.0	1.4×10^5	$3.4 \times 10^3 \pm 5.2 \times 10^1$	0.4
37	$1.9 \times 10^5 \pm 2.1 \times 10^4$	$5.3 \times 10^3 \pm 2.0 \times 10^3$	0.7	5.0×10^5	$1.0 \times 10^4 \pm 4.3 \times 10^2$	0.5
45	$1.9 \times 10^5 \pm 2.1 \times 10^4$	$9.3 \times 10^3 \pm 4.7 \times 10^3$	1.2	2.7×10^5	$4.9 \times 10^3 \pm 1.7 \times 10^3$ [b]	0.4

[a]Not separated between depths
[b]This incubation was at 8°C, all others at 16°C

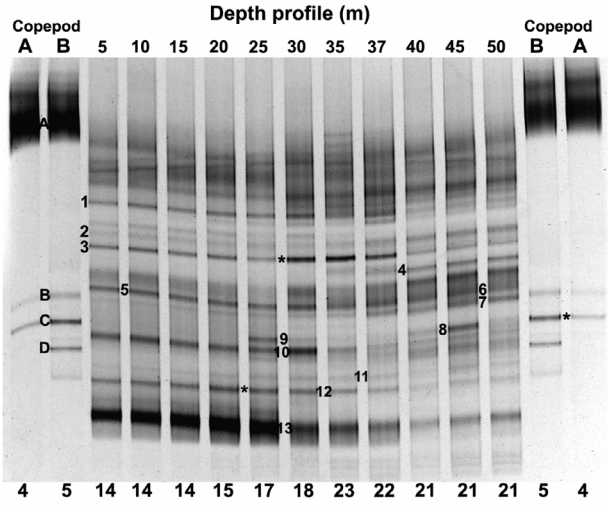

Fig. 2. Bacterial community composition profiles of samples from various depths (5 to 50 m) and from copepod samples (A and B). A denaturing gradient top to bottom of 29 to 55% was applied. The total number of discernible bands in each sample is shown below the gel. Excised and sequenced bands are labelled to the left of the lane. *: excised band with the same vertical position as Bands CopC, Free3, and Free12, respectively. The relationships of excised band sequences to other sequences in the GenBank database are shown in Table 2

teria on copepods with the total free living community. Bands from different samples with identical vertical positions in the gel had identical sequences (see Bands Free3, Free12, and CopC; Fig. 2). The 13 bands successfully sequenced from the free living bacterial community consisted of *Alphaproteobacteria* (6 bands), *Bacteroidetes* (3 bands), *Gammaproteobacteria* (2 bands), *Actinobacteria* (1 band) and *Cyanobacteria* (1 band) (Fig. 2, Table 2). The 3 bands successfully sequenced from the bacterial community attached to the copepods were exclusively related to *Alphaproteobacteria*. Except for Bands Free2 and Free13, the amplicons were closely related to marine sequences available in GenBank (95.1 to 100% similarity). No heteroduplexes or chimeras were found among the excised bands. All 9 *Alphaproteobacteria* bands were affiliated with the marine *Roseobacter* lineage and showed between-sequences similarity with on average 93% (range 89 to 100%). Interestingly, an identical *Roseobacter* sequence was found on copepods (CopC) and in the free living phase (Free8). These 2 bands showed a 100% nucleotide match (503 bp) but differed slightly in total length; explaining the 2 different nearest relatives indicated in Table 2.

DISCUSSION

The local density and production of bacteria living on the copepods *Calanus* spp. were magnitudes higher than those of bacteria living in the water column. These data are among the first for bacteria living on copepods *in situ*. They are, however, in the same range as found in mixed zooplankton populations (Heidelberg et al. 2002) and in laboratory cultures of the smaller copepod *Acartia tonsa* (Hansen & Beck 1996, Tang 2005). In comparison, bacteria colonizing marine particles also

Table 2. Closest relatives of the sequenced DGGE bands

Band	% similarity	Alignment[a]	Closest relative	Taxonomic affiliation as reported in GenBank	Accession no.[b]
Free1	100.0	552/560	Clone PI_RT311, Massachusetts coast	*Bacteroidetes*	AY580639
Free2	92.0	552/554	Clone SPOTSAPR01_5m189, California coast	*Bacteroidetes*	DQ009093
Free3	99.8	500/500	Clone OCS155, Pacific Ocean	*Actinobacteria*	AF001652
Free4	96.7	513/534	Clone SBI04_55, Western Arctic Ocean	*Bacteroidetes*	DQ186969
Free5	99.6	539/539	Clone NAC11-3, North Atlantic	*Alphaproteobacteria*	AY145625
Free6	95.1	508/508	Clone EB080-L11F12, Monterey Bay	*Alphaproteobacteria*	AY627365
Free7	97.1	413/431	*Roseobacter pelophilus*, North Sea sediment	*Alphaproteobacteria*	AJ968651
Free8	99.3	536/543	Clone NH10_29, Oregon coast	*Alphaproteobacteria*	DQ372849
Free9	99.6	497/502	Clone PI_RT264, Massachusetts coast	*Alphaproteobacteria*	AY580469
Free10	99.8	527/527	Clone EF100-65C12, Monterey Bay	*Alphaproteobacteria*	AY627371
Free11	99.3	551/565	Clone KTc1119, North Sea	*Gammaproteobacteria*	AF235120
Free12	100.0	554/560	Clone PI_RT273, Massachusetts coast	*Gammaproteobacteria*	AY580747
Free13	91.4	533/549	*Synechococcus* sp. Almo3, Red Sea	*Cyanobacteria*	AY172800
CopA	99.2	706/712	*Calanus pacificus*, 18S sequence	*Eukaryota*	L81939
CopB	99.8	528/535	Clone NAC11-6, North Atlantic	*Alphaproteobacteria*	AY664344
CopC	99.4	502/503	Clone NH10_05, Oregon coast	*Alphaproteobacteria*	AF466885
CopD	98.2	495/514	Clone H25, North Sea	*Alphaproteobacteria*	AY277267

[a]Part of the total sequence used in alignment
[b]Nucleotide sequences can be accessed via www.ncbi.nlm.nih.gov/Entrez/

often have elevated growth rates relative to the free living bacteria, yet these rates may sometimes be much higher than the 0.7 to 1.2 d^{-1} found in the present study (Smith et al. 1995, Riemann et al. 2000, Grossart et al. 2003, Kiørboe et al. 2003). It should be noted that in our study copepods were starved 3 to 4 h before and during the bacterial production measurements. Hence, in this period there was no production of substrates due to copepod feeding. Although DNA synthesis does not respond immediately to changes in environmental conditions (Bell 1993), it seems likely that our estimates are conservative relative to the bacterial production on copepods *in situ*.

When measuring bacterial production we included a control where the copepod was killed, thereby accounting for thymidine adsorbed to the copepod. This control does not take into account the thymidine that potentially may be absorbed by live copepods. In a study with *Acartia tonsa* that had recently moulted, i.e. with a potentially less dense copepod associated bacterial community (Carman & Dobbs 1997), neither bacterial uptake nor adsorption or absorption of thymidine were found (Møller 2007). Hence, we do not expect that absorbance of thymidine by the copepods has lead to a significant overestimation of copepod associated bacterial production in our study.

While the present study excluded the bacteria in fecal pellets because all copepod had empty guts, it did not differentiate between bacteria on the copepod's exterior and bacteria inside the copepod. In another study based on direct counts, approximately the same number of bacteria was found in the gut as on the surface of *Acartia tonsa* (Hansen & Beck 1996). Using leucine incorporation and autoradiography, which allow visual inspection of the location of activity, Carman (1994) found that bacterial activity was concentrated on the exterior surfaces of copepods, particularly where bacteria were most abundant, e.g. around mouth parts. However, both that study and ours may underestimate the bacterial production in the gut if labelled compounds are not equally distributed outside and inside the copepod. On the contrary, Tang (2005) suggested that copepod ingestion primarily supports the growth of gut bacteria and that copepods can be important hot spots in the ocean because defecation releases a significant amount of bacteria into the water column when the copepod is feeding.

Bacteria associated with the *Calanus* spp. had elevated local abundance and growth rates compared to the free living bacteria. However, their total abundance and production never constituted more than 0.3 and 0.1% of the total water column bacterial abundance and production, respectively (Fig. 3). Even if it is assumed that the bacterial density and activity per copepod are the same for the other copepods present

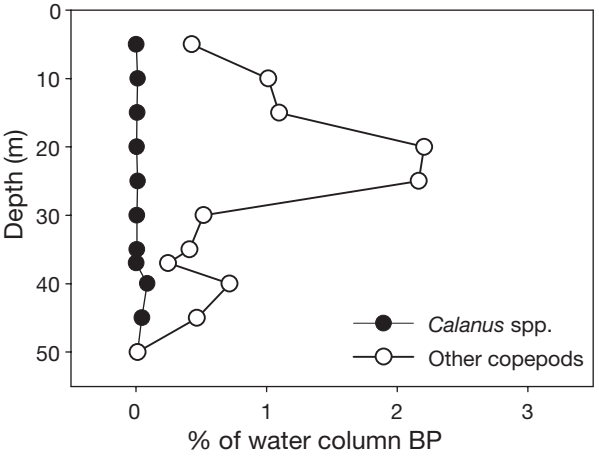

Fig. 3. Potential production of bacteria associated with *Calanus* spp. and the total copepod community compared to water column bacterial production (BP)

(Hansen & Bech 1996, Tang 2005) as for *Calanus* spp., the copepod associated bacterial abundance and production would not constitute more than 1.5 and 2%, respectively, of that of the free living bacteria (Fig. 3). Similarly, in Chesapeake Bay, Heidelberg et al. (2002) found that the proportion of total bacteria being associated with zooplankton larger than 202 µm was generally insignificant (down to 0.01%), though occasionally these bacteria constituted up to 40% of the total bacterial community. In addition, Carman (1994) found that the bacterial activity associated with copepods in the northern Gulf of Mexico could sometimes account for up to 20% of total bacterial activity.

During the present study the food available to the copepods *in situ* were dominated by large phytoplankton species (M. Koski unpubl. data). This suggests that the DOC production by sloppy feeding may be significant (Møller 2007). The DOC production by *Calanus* spp. would be 4 µg C $copepod^{-1}$ d^{-1} if, as a rough estimate, it is assumed that *Calanus* spp. weigh 75 µg C, graze 50% their own bodyweight per day, and loose 10% of what they clear by sloppy feeding. Compared with the bacterial production associated with *Calanus* spp. of 0.07 to 0.012 µg C $copepod^{-1}$ d^{-1} (recalculated from values in Table 1 assuming 53 fg C $bacterium^{-1}$; average for marine snow attached bacteria in Simon et al. 1990) it is obvious that the carbon demand of the attached bacteria will be insignificant relative to the DOC amount produced by the copepod. Thus, 'excess' DOC will be available to the bacteria in the surrounding water, and a local high copepod concentration in the water column could, presumably, cause an increased bacterial production. Such a relationship may, however, be difficult to notice because of other equally important sources of DOM in the water column, e.g. phytoplankton cell leakage, protozooplankton graz-

ing, and viral lysis. Accordingly, we found that one of the 2 peaks in bacterial production was associated with the fluorescence maximum, while the other was situated at 15 m where also a peak in *Calanus* spp. was found. In contrast, the peak of other copepods at 25 to 30 m did not coincide with increased bacterial production (Fig. 1). A remarkable difference in diversity, as judged from DGGE band number, was observed between the copepod associated (5 bands) and the free living (14 to 23 bands) bacterial communities. Thirteen DGGE bands were sequenced from the free living bacteria community; of these, 8 were from the marine *Roseobacter* lineage. From the bacterial community attached to the copepods only 3 bands were sequenced, all belonging to the *Roseobacter*. Members of *Roseobacter* are among the most predominant bacterial phylotypes recovered in marine plankton clone libraries (Buchan et al. 2005) and have been found to be dominant in the North Sea, especially in summer time (Eilers et al. 2001, Zubkov et al. 2001). The finding that copepod-attached bacterial phylotypes were related to the *Roseobacter* lineage is not too surprising, considering that this group is known to successfully colonize marine particles (e.g. DeLong et al. 1993, Rath et al. 1998, Riemann et al. 2000); however, it complements existing studies of copepod associated bacteria, which have primarily reported on the importance of *Vibrio* species (*Gammaproteobacteria*; Kaneko & Colwell 1978, Heidelberg et al. 2002).

Interestingly, the 2 bands Free8 and CopC aligned vertically in the DGGE analysis and showed 100% sequence similarity (503 bp). This *Roseobacter* phylotype was almost identical (>99%) to a clone obtained during a coastal diatom bloom in Oregon, USA (Morris et al. GenBank, see http://www.nncbi.nlm.nih.gov) and was seen as a dense bacterial band in both copepod samples and as a distinct band appearing at 40 to 50 m depth. At this specific depth, a pronounced peak in *Calanus* spp. biomass was observed (Fig. 1).

The bacterial production associated with the *Calanus* spp. was less than 1% of the free living bacteria (Fig. 3). Hence, intuitively a potential export of bacterial progeny from the copepods to the water column would be anticipated to have only a limited effect on the community composition of the free living bacteria. Nevertheless, our finding of an identical bacterial phylotype on copepods and in the free living community, only at the specific depth with the highest copepod biomass, indicates that this phylotype was indeed exchanged between copepods and the free living state.

Acknowledgements. We thank P. Griekspoor for technical assistance in PCR – DGGE – 16S rDNA sequencing analyses. E.F.M. was financially supported by a grant from the Carlsberg Foundation (04-0383/20). L.R. was supported by Kalmar University.

LITERATURE CITED

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Azam F, Long RA (2001) Oceanography — sea snow microcosms. Nature 414:495–496

Bell R (1993) Estimating production of heterotrophic bacterioplankton via incorporation of tritiated thymidine. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, FL, p 495–503

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

Boström KH, Simu K, Hagström Å, Riemann L (2004) Optimization of DNA extraction for quantitative marine bacterioplankton community analysis. Limnol Oceanogr Methods 2:365–373

Buchan A, González JM, Moran MA (2005) Overview of the marine *Roseobacter* lineage. Appl Environ Microbiol 71: 5665–5677

Carman KR (1994) Stimulation of marine free-living and epibiotic bacterial activity by copepod excretions. FEMS Microbiol Ecol 14:255–262

Carman KR, Dobbs FC (1997) Epibiotic microorganisms, on copepods and other marine crustaceans. Microsc Res Tech 37:116–135

Cellini L, Di Campli E, Grande R, Di Bartolomeo S, Prenna M, Pasquantonio MS, Pane L (2005) Detection of *Helicobacter pylori* associated with zooplankton. Aquat Microb Ecol 40: 115–120

Cole JR, Chai B, Marsh TL, Farris RJ and 7 others (2003) The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. Nucleic Acids Res 31:442–443

DeLong EF, Franks DG, Alldredge AL (1993) Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. Limnol Oceanogr 38:924–934

Eilers H, Pernthaler J, Peplies J, Glöckner FO, Gerdts G, Amann R (2001) Isolation of novel pelagic bacteria from the German Bight and their seasonal contributions to surface picoplankton. Appl Environ Microbiol 67: 5134–5142

Eppley RW, Horrigan SG, Fuhrman JA, Brooks ER, Price CC, Sellner K (1981) Origins of dissolved organic matter in Southern California coastal waters: experiment on the role of zooplankton. Mar Ecol Prog Ser 6:149–159

Fuhrman JA, Azam F (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar Biol 66:109–120

Grossart HP, Hietanen S, Ploug H (2003) Microbial dynamics on diatom aggregates in Oresund, Denmark. Mar Ecol Prog Ser 249:69–78

Hansen B, Bech G (1996) Bacteria associated with a marine planktonic copepod in culture. I. Bacterial genera in seawater, body surface, intestines and fecal pellets and succession during fecal pellet degradation. J Plankton Res 18: 257–273

Hay SJ, Kiørboe T, Matthews A (1991) Zooplankton biomass and production in the North-Sea during the autumn circulation experiment, October 1987 – March 1988. Cont Shelf Res 11:1453–1476

Heidelberg JF, Heidelberg KB, Colwell RR (2002) Bacteria of

the γ-subclass *Proteobacteria* associated with zooplankton in Chesapeake Bay. Appl Environ Microbiol 68: 5498–5507

Hirche HJ, Mumm N (1992) Distribution of dominant copepods in the Nansen Basin, Arctic Ocean, in summer. Deep-Sea Res 39:485–505

Huq A, Sack RB, Nizam A, Longini IM and 9 others (2005) Critical factors influencing the occurrence of *Vibrio cholerae* in the environment of Bangladesh. Appl Environ Microbiol 71:4645–4654

Jacobsen TR, Azam F (1984) Role of bacteria in copepod fecal pellet decomposition — colonization, growth-rates and mineralization. Bull Mar Sci 35:495–502

Kaneko T, Colwell RR (1978) The annual cycle of *Vibrio parahaemolyticus* in Chesapeake Bay. Microb Ecol 4:135–155

Kiørboe T, Tang K, Grossart HP, Ploug H (2003) Dynamics of microbial communities on marine snow aggregates: colonization, growth, detachment, and grazing mortality of attached bacteria. Appl Environ Microbiol 69:3036–3047

Marie D, Partensky F, Jacquet S, Vaulot D (1997) Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. Appl Environ Microbiol 63: 186–193

Møller EF (2007) Production of dissolved organic carbon by sloppy feeding in the copepods *Acartia tonsa*, *Centropages typicus*, and *Temora longicornis*. Limnol Oceanogr 52:79–84

Møller EF, Thor P, Nielsen TG (2003) Production of DOC by *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* through sloppy feeding and leakage from fecal pellets. Mar Ecol Prog Ser 262:185–191

Muyzer G, De Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695–700

Muyzer G, Brinkhoff T, Nübel U (1998) Denaturing gradient gel electrophoresis (DGGE) in microbial ecology. In: Akkermans ADL, van Elsas JD, De Bruin FJ (eds) Molecular microbial ecology manual, p 1–27, Kluwer Academic, The Netherlands

Nagasawa S (1989) Bacterial epibionts of copepods. Science Prog 73:169–176

Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943–948

Rath J, Wu KY, Herndl GJ, DeLong EF (1998) High phylogenetic diversity in a marine-snow-associated bacterial assemblage. Aquat Microb Ecol 14:261–269

Riemann L, Middelboe M (2002) Stability of bacterial and viral community compositions in Danish coastal waters as depicted by DNA fingerprinting techniques. Aquat Microb Ecol 27:219–232

Riemann L, Steward GF, Azam F (2000) Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. Appl Environ Microbiol 66:578–587

Roy S, Harris RP, Poulet SA (1989) Inefficient feeding by *Calanus helgolandicus* and *Temora longicornis* on *Coscinodiscus wailesii* — quantitative estimation using chlorophyll-type pigments and effects on dissolved free amino-acids. Mar Ecol Prog Ser 52:145–153

Sabatini M, Kiørboe T (1994) Egg production, growth and development of cyclopoid copepod *Oithona similis*. J Plankton Res 16:1329–1351

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

Simon M, Alldredge AL, Azam F (1990) Bacterial carbon dynamics on marine snow. Mar Ecol Prog Ser 65:205–211

Smith DC, Steward GF, Long RA, Azam F (1995) Bacterial mediation of carbon fluxes during a diatom bloom in a mesocosm. Deep-Sea Res II 42:75–97

Steinberg DK, Nelson NB, Carlson CA, Prusak AC (2004) Production of chromophoric dissolved organic matter (CDOM) in the open ocean by zooplankton and the colonial cyanobacterium *Trichodesmium* spp. Mar Ecol Prog Ser 267:45–56

Tang KW (2005) Copepods as microbial hotspots in the ocean: effects of host feeding activities on attached bacteria. Aquat Microb Ecol 38:31–40

Uye S, Aoto I, Onbé T (2002) Seasonal population dynamics and production of *Microsetella norvegica*, a widely distributed but little-studied marine planktonic harpacticoid copepod. J Plankton Res 24:143–153

Velji MI, Albright LJ (1993) Improved sample preparation of aggregated aquatic substrate bacteria. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, FL, p 139–142

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

Wintzingerode FV, Göbel UB, Stackebrandt E (1997) Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS Microbiol Rev 21:213–229

Zubkov MV, Fuchs BM, Archer SD, Kiene RP, Amann R, Burkill PH (2001) Linking the composition of bacterioplankton to rapid turnover of dissolved dimethylsulphoniopropionate in an algal bloom in the North Sea. Environ Microbiol 3:304–311

Editorial responsibility: Klaus Jürgens, Rostock, Germany

Submitted: September 28, 2006; Accepted: January 30, 2007
Proofs received from author(s): March 7, 2007