Bacterivory by tropical copepod nauplii: extent and possible significance

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ABSTRACT· Copepod nauplii may be an important intermediary between the 'microbial' and 'classical' pelagic marine food webs. In studies of planktonic food webs, along a trophic gradient from eutrophic harbour through coastal to oligotrophic oceanic waters off Jamaica, West Indies, we investigated bacterivory by nauplii of 11 representative copepod taxa (n = 176 total nauplii) using fluorescently labelled bacteria (FLB) of 0.7 μm³ volume at concentrations of 1.5 to 2.5×10^6 cells ml^{-1} Seven taxa consistently ingested FLB: *Acartia liljeborgii*, *Paracalanus* spp., *Temora stylifera*, *T. turbinata*, *Oncaea* spp., *Undinula vulgaris*, *Oithona* spp.; 4 taxa consistently did not: *Centropages velificatus*, *Clausocalanus* spp., *Euchaeta marina*, and *Corycaeus* spp. These data, and the observations that naupliar moulting and growth rates were uncoupled from chlorophyll *a* concentrations in any size fraction over the range 0.09 to 4.7 mg m^{-3}, suggest that nauplii are not food limited even in oceanic waters. Calculations indicate that daily food requirements of oceanic nauplii can be met from a diet of bacteria and picoplankton, but not from a diet of nano- and net-phytoplankton. Naupliar production in oceanic waters is at least 50 to 60% of copepodite production; it appears therefore that the ecological importance of copepod nauplii in oceanic waters has been greatly underestimated.

KEY WORDS: Marine plankton · Nauplius · Bacterivory · Tropical copepods · Microbial food web

INTRODUCTION

Copepods are the most abundant metazoans on the planet (Raymont 1980) and are the primary processors of photosynthetically fixed organic matter in the oceans. The copepod life cycle comprises 4 phases: egg, nauplius, copepodite, and adult. Because most sampling for copepods has been done with larger meshes of nets (>200 μm), nauplii have historically been undersampled. However, when appropriate meshes are used, nauplii in samples can vastly outnumber older copepodite and adult stages (Turner 1982, 1993, Chisholm & Roff 1990, Webber & Roff 1995a). The lack of sufficient attention to nauplii was emphasised by Björnberg (1984) from a taxonomic perspective; we agree from an ecological perspective.

Our objective here is to present data and basic arguments in order to construct a *prima facie* case for the significance of the nauplius stage, and to evaluate its possible role as an intermediary between the microbial food web (MFW) and the 'classical' (= grazing) food web (CFW; see Turner & Roff 1993 for definitions). It is generally believed that, with a few exceptions (e.g salps, appendicularians, cladocerans), marine pelagic metazoans are not significant bacterivores (e.g. Fenchel 1984, Jørgensen 1984), however the nauplii and their feeding habits, especially in oceanic waters, have received relatively little attention.

Our observations on nauplii come primarily from ongoing studies of planktonic food webs along a trophic gradient from eutrophic harbour through coastal to oligotrophic oceanic waters off Jamaica, West Indies (see Roff et al. 1990, and references therein). With the exception of feeding studies, where animals were briefly exposed to artificial diets (i.e. flu-

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orescently labelled bacteria), nauplii were fed natural diets from water at the place of capture. Observations included: studies of bacterivory, naupliar developmental times and growth rates, and abundance and biomass of nauplii and copepodites. From the latter we calculated naupliar and copepodite production.

The emphasis in our observations was not on the rate of feeding, but on whether representative species from the naupliar community are significant feeders on bacterial sized particles. This is clearly of great potential significance, because picoplankton (<2 μm) are the dominant primary producers in oceanic waters (e.g. Stockner 1988), and bacterioplankton production is of the order of 30% of total primary production (Cole et al. 1988). If auto- and heterotrophic bacterial-sized cells are directly available to nauplii, then nauplii could act as important and direct intermediaries between the MFW and the CFW.

MATERIALS AND METHODS

Field collections. Copepods and their developmental stages were collected from 3 areas off Jamaica: off the south coast, in (1) eutrophic Kingston Harbour and (2) mesotrophic neritic Lime Cay; and (3) off the north coast in oligotrophic oceanic water approximately 1.6 km north of Discovery Bay. Water depths at these stations were approximately 15, 30, and 600 m respectively. Collections were either routine for estimation of seasonal abundances, biomass and production, or for experimental purposes. Data from Lime Cay are weekly observations over 18 mo, and from Discovery Bay are monthly observations over 2 yr. Samples for chlorophyll a were also collected at the same time (see Hopcroft & Roff 1990 for details of methods; Hopcroft et al. 1990, Webber 1993, M. K. Webber & J. C. Roff unpubl.). Zooplankton samples were collected by vertical net hauls with 0.5 m diameter, 64 μm and 200 μm mesh SCOR pattern nets. Sampling sites and techniques for preservation, enumeration, biomass determination and production calculations have been described by Chisholm & Roff (1990).

Moulting rates and growth rates. Nauplii were collected in vertical net hauls (64 μm mesh) from 200, 60, 27, or 12 m, depending on the water depth at a station. Animals were immediately transported to the laboratory after filtering through 600 μm mesh to remove gelatinous zooplankton and other major predators. Fresh seawater for incubations and size-fractionated chlorophyll a analyses (see Hopcroft & Roff 1990) was collected simultaneously with zooplankton.

In the laboratory nauplii were identified with the aid of Björnberg (1972) or by rearing eggs from adults of known species. Field-collected nauplii were identified to species or genus, except at Discovery Bay where individual animals were identified only in feeding studies. Live nauplii were individually sorted with pipettes into 60 ml tissue culture flasks, and incubated for up to 48 h in either 64 or 20 μm screened natural seawater from the collection site. These individually reared nauplii were observed every 3 h for moulting. In other incubations, nauplii were reared in batches of 10 in 200 ml tissue culture flasks, also in either 64 or 20 μm screened natural seawater from the collection site, and checked for moulting between 12 and 20 h after incubation. For individual nauplii, time to moult was noted; for batch-reared nauplii number of exuviae were counted at the end of the experiment, and moulting rates were calculated.

The stage at which exogenous feeding first begins is not known for all species. It is to be expected that nauplii which are not exogenously feeding (i.e. which are lecithotrophic) would develop at rates regulated by temperature rather than food concentration. To the best of our knowledge, and as verified by feeding studies, the nauplii for which we report development times here were exogenously feeding, with the exception of *Euchaeta marina*.

Growth increments for the commonest species in the harbour and off Discovery Bay were calculated by 2 methods. In the harbour, change in naupliar size and volume over time was measured directly from enclosure studies (R. R. Hopcroft & J. C. Roff unpubl.) using the digitising system of Roff & Hopcroft (1986). Dry mass was calculated from relationships of Lawrence et al. (1987). For oceanic waters, consecutive naupliar stages of 12 common species (Webber & Roff unpubl.) off Discovery Bay were digitised from scaled drawings and volumes calculated. These data were then used with moulting rates to yield growth rates from:

$$g = 1/D \ln(V_{S+1}/V_S) \quad \text{or:} \quad g = 1/D \ln(M_{S+1}/M_S)$$

where D = moulting rate in days; M = dry mass of nauplius; V = volume of nauplius; S = development stage. A daily specific growth rate (G) was then derived from:

$$G = e^g - 1$$

Feeding experiments. A total of 176 copepod nauplii of 11 taxa (species or genera) were examined for ingestion of fluorescently labelled bacteria (FLB) in 6 sets of experiments. In addition, for comparative purposes, we offered FLB to adults and/or copepodites of selected species and the marine cladoceran *Penilia avirostris*. The taxa examined are representative of those in tropical coastal and oceanic waters, and comprise the dominant species found around Jamaica (Chisholm & Roff 1990, Webber & Roff unpubl.). We used freshly caught specimens in all feeding studies and kept them at low densities for brief

periods, so as to avoid possible biases from specimens kept under culture conditions.

FLB were obtained from E. & B. Sherr (Oregon State University). These were *Escherichia coli* (~0.7 µm³ cell volume) prepared according to Sherr et al. (1987) and mailed to us in freeze-dried form. Stock and working suspensions of FLB were prepared following procedures of Turner & Tester (1992) to produce experimental concentrations of 1.5 to 2.5×10^6 cells ml^{-1}, which is within the reported range of natural bacterial concentrations (e.g. Ducklow 1983). Stock and experimental suspensions were produced fresh for each set of experiments, and were microscopically checked for clumping before use. Any suspensions which revealed that FLB were not dispersed solely as single cells were discarded; on rare occasions (<0.1% of cell counts) cells were observed in couplets or triplets.

In typical experiments, 400 µl of stock FLB suspension in Milli-Q deionized water was added to 22 ml of GFF glass fiber filtered seawater from the site of collection of experimental animals. Experimental incubations were of short duration, generally 30 to 60 min, in an effort to avoid long-term inefficient accumulation of FLB, for example in incidental feeding, or through time-mediated clumping of cells. Concentrations of nauplii were kept low (5 to 10 per 25 ml), because Bergreen et al. (1988) showed that clearance rates declined when copepods were held at concentrations >1 per ml. Preliminary incubations on *Temora* nauplii showed that turnover time of gut contents (to production of fecal pellet) was 20 to 40 min. Following incubation, each nauplius was separately examined for the presence of ingested FLB under 40× or 100× oil-immersion using a Leitz Laborlux epifluorescence microscope. Incubation temperatures were the same as ambient seawater temperature, 29 ± 0.5°C.

RESULTS AND DISCUSSION

Moulting rates and growth rates

Mean naupliar moulting rates for representative species of calanoid copepods were not significantly different between Kingston Harbour, Lime Cay and Discovery Bay stations (Table 1), although there was a considerable range in values and the lowest development times (= fastest moulting rates) were observed in oceanic waters. We could not make direct comparisons of any species across the entire trophic gradient, since none of the species occurred in all 3 locations at observation times. The corresponding data for chlorophyll a concentrations over an annual cycle are shown in Table 2. The chlorophyll a measurements for Kingston Harbour (taken at the east harbour mouth) should be viewed as conservative, because concentrations increase by an order of magnitude into the harbour proper (Webber 1990, unpubl.).

The only prior study systematically comparing development times of copepod nauplii across a range of productivity appears to be that of Hart (1990), which emphasises freshwater and marine neritic species. Björnberg (1972) indicated that several species moult about once per day, ranging from 16 h to 2.5 d per moult at temperatures between 16 and 28°C. Some smaller species may moult faster than this (see e.g. Hart 1990), and other larger species which have inherited yolk reserves (lecithotrophic) may also moult at faster rates prior to exogenous feeding. The species investigated here, predominantly in the size range 150 to 400 µm length, were all feeding exogenously (see below), except probably *Euchaeta marina*. Our data on moulting rates of nauplii in oceanic waters may be biased low because of the inclusion of *Euchaeta*

Table 1. Development times of nauplii of representative species, from locations off Jamaica, West Indies, at 29.3 ± 0.8°C

Species	Location	Stage of development	No. of observations	Mean development time (h)	Standard deviation
Temora turbinata	Kingston Harbour	Various (N4–C1)	35	27.5	12.5
Temora turbinata	Lime Cay	Various (N4–C1)	5	32.0	16.0
Centropages velificatus	Kingston Harbour	Various (N4–C1)	28	38.9	24.7
Centropages velificatus	Lime Cay	Various (N4–C1)	34	49.4	25.3
Undinula vulgaris, Euchaeta marina, Clausocalanus furcatus, Paracalanus spp.	Discovery Bay[a]	Various (N4–C1)	103	21.0	12.5
Euchaeta marina	Discovery Bay	N1–N2	21	25.2	10.7

[a]Individual nauplii not distinguished to species in these experiments

Table 2. Chlorophyll *a* concentrations (total and size-fractionated), at 3 locations off Jamaica, West Indies. Values cover the entire annual cycle at each station, and are either depth-weighted for means (DW), or from mid-euphotic zone for maxima and minima (to avoid automatic increase of variation with depth). Net: >20 µm; nano: 2–20 µm; pico: <2 µm

| Location | | | Chlorophyll *a* (mg m^{-3}) | | | | No. of |
			Pico	Nano	Net	Total	observations
Kingston	Max	(5 m)	0.450	1.52	1.04	3.01	
Harbour	Mean	(DW)	0.243	0.517	0.151	0.911	504
	Min	(5 m)	0.045	0.091	0.012	0.148	
Lime	Max	(10 m)	0.174	0.148	0.166	0.488	
Cay	Mean	(DW)	0.105	0.079	0.074	0.258	720
	Min	(10 m)	0.048	0.018	0.004	0.070	
Discovery	Max	(60 m)	0.190	0.083	0.190	0.463	
Bay	Mean	(DW)	0.063	0.03.7	0.011	0.111	828
	Min	(60 m)	0.032	0.012	0.004	0.048	

marina in our observations (see Table 1). However, this bias may not be great because separate observations on *Euchaeta marina* N1 and N2, reared from eggs,

Table 3. Means and ranges of daily specific growth rates (*G*, d^{-1}) for nauplii and copepodites (all stages, excluding moult from copepodite stage 5 to adult. Data from Chisholm & Roff (1990), Webber (1993), Hopcroft & Roff (unpubl.)

Location	Order/Species	Mean (range)
	Nauplii	
Kingston Harbour	Cyclopoida *Oithona* spp.	0.64 (0.34–0.99)
	Calanoida *Centropages velificatus* *Paracalanus crassirostris* *Acartia* spp. *Temora turbinata*	0.61 (0.38–1.33)
Discovery Bay	Cyclopoida *Oithona* spp.	0.36 (0.23–0.38)
	Calanoida *Clausocalanus* spp. *Paracalanus crassirostris* *Temora turbinata*	0.57 (0.38–0.98)
	Copepodites	
Lime Cay and Kingston Harbour	Calanoida *Temora turbinata* *Centropages velificatus* *Paracalanus aculeatus*	0.61 (0.50–0.71)
Discovery Bay	Calanoida *Paracalanus/ Clausocalanus* spp. *Euchaeta marina* *Undinula vulgaris*	0.48 (0.12–1.49)
	Cyclopoida *Oithona plumifera*	0.21 (0.09–0.37)

showed a mean moult time of 25.2 h (n = 21).

There were no significant relationships (ANOVAs p ≫ 0.1) between naupliar development rates and total chlorophyll *a* concentration or its concentration in any size fraction, over the range of 0.048 µg l^{-1} (total chlorophyll *a* in oceanic water) to 3.01 µg l^{-1} (total chlorophyll *a* in harbor water); data are given in Tables 1 & 2. There were no significant differences in moulting rates of nauplii reared in 64 µm or 20 µm filtered seawater (*t*-test, p > 0.1).

Mean daily specific growth rates of nauplii in harbour waters were similar for calanoids and cyclopoids (Table 3). In oceanic waters, calanoid growth rates were essentially identical to those in harbour waters, while cyclopoids were somewhat lower (Table 3). Thus the average '*G*' for harbour calanoid nauplii appears indistinguishable from oceanic species, despite a 10-fold difference in mean chlorophyll *a* concentration and a 100-fold difference in extremes of the ranges (see Table 2). In contrast, calanoid copepodite growth rates were distinctly lower in oceanic waters, with cyclopoid copepodites lower still. Because of confounding body size effects in this data (J. C. Roff, M. K. Webber & R. R. Hopcroft unpubl.), we will not present statistical analyses of the data here.

These moulting and growth rates were derived either from laboratory observations or from 'artificial' cohorts in field manipulations, where mortality is effectively zero (Hopcroft & Roff unpubl.); the cautions of Lopez (1991) on biases in cohort development times do not therefore apply. Overall then, naupliar moulting rates and growth rates are largely 'uncoupled' from chlorophyll *a* concentrations. Hart (1990) arrived at essentially the same conclusion, following a survey of naupliar and copepodite development times in fresh and coastal marine waters.

The simplest explanation of these unexpected observations is that exogenously feeding nauplii of tropical copepods may not be food-limited even in oligotrophic oceanic waters. Part of the explanation for this may be that nauplii have low metabolic rates, and correspondingly high energy efficiency (Paffenhöfer 1976, Epp & Lewis 1980). However, the question remains as to how naupliar stages obtain sufficient food to maintain their growth rates. One possibility is that these nauplii feed predominantly on smaller-sized particles, e.g. bacterioplankton and picoplankton. The numbers of these organisms per unit volume are known to be considerably less variable across a gradient of trophic status

than the larger sized nano- and netplankton (Hopcroft & Roff 1990, Rath et al. 1993), therefore providing both a more constant and a more homogeneous food resource. Data summarised in Table 2 show that the average picoplankton concentration varied by less than a factor of 4 from harbour to oceanic waters, while nano- and net-plankton varied by a factor of 14.

Naupliar abundances, biomass and production

The relative ecological significance of any group of organisms has traditionally been evaluated in terms of its abundance. However, biomass or preferably production are more appropriate units for such comparisons. Production, i.e. the sum of growth increments of all members of a population (Downing & Rigler 1984), represents the amount of growing tissue; this must be directly related to the amount of energy processed by a group of organisms. For purposes of comparison between coastal and oceanic waters, we present all 3 measures of significance for nauplii and copepodites (Table 4).

There are few reliable estimates of naupliar production in marine waters. Chisholm & Roff (1990) estimated that total naupliar production was 11.9% of total copepodite production at inshore Lime Cay (Table 4). Landry (1978) estimated this was 15% in a coastal population of *Acartia clausi*. In offshore waters (Discovery Bay), however, it appears that naupliar production is far more important (Table 4). Here, for the upper 60 m, naupliar production was 50.4% of copepodite production and for the upper 200 m it was 59.5%. These higher ratios are a function of larger mean size and greater relative abundances of nauplii in offshore waters, and slower average growth rates of oceanic copepodites (Webber & Roff 1995a, b). Offshore data are taken from Webber (1993) and methods and results will be more fully presented and defended elsewhere. All the data in Table 4 were collected and processed by precisely the same techniques and thus are directly comparable. Corresponding data for Kingston Harbour have been collected, but are not yet available.

These data clearly show that nauplii rival the ecological significance of copepodite stages in oceanic waters. We believe that this is the first documentation that nauplii constitute such an important component of metazoan production in oceanic waters. Even the data in Table 4 are conservative estimates of the relative importance of nauplii, because abundances of the smallest and most numerous calanoids and cyclopoids are underestimated by passage through 64 µm mesh nets (Böttger 1987, Hopcroft & Roff unpubl.). Calibrations between meshes show that up to 90% of the nauplii of *Paracalanus crassirostris*, and 33% of the total biomass of the naupliar community passes through a 64 µm mesh (Hopcroft & Roff unpubl.). Converting these revised biomass estimates to production reveals that naupliar production may exceed 80% of copepodite production (because mean growth rates of nauplii are higher than mean growth rates of copepodites) in oceanic waters.

The higher ratio of nauplii to copepodites in oceanic waters could be explained by 3 possibilities: (1) the food climate there is more conducive to nauplii than to copepodites, relative to coastal waters, (2) predation pressure is lower on nauplii than on copepodites, again relative to coastal waters, (3) fecundity of copepods is higher in oceanic than in coastal waters. Our data (J. C. Roff, C. Clarke, R. R. Hopcroft, G. Persad & M. K. Webber unpubl.) show that predator groups (e.g. chaetognaths, cnidaria, ctenophores, decapod larvae etc.) all decline in abundance into oceanic waters. However, at present there is no reason to suppose that a selective predatory pressure is exerted on copepodites rather than nauplii in oceanic waters. If anything the reverse is expected, simply as a function of size and relative abundance. Thus although possibility (2) cannot be excluded, it seems ecologically unlikely. Possibility (3) can be discounted because of our observations (Webber & Roff 1995b) which show that average fecundity in oceanic copepods is significantly lower than in neritic species, and from many reports (e.g. Checkley 1980) that rates of egg production decline with decreasing chlorophyll *a* concentration.

This reasoning therefore left us with possibility (1), and led us to suspect that the disproportionately higher numbers and production of nauplii in oceanic waters was most probably the result of relatively greater availability of food for nauplii, and that they might, in fact, directly exploit food of microbial size. This reasoning was also supported by the observations of

Table 4. Mean abundances and biomass, and annual production of nauplii (Naup) and copepodites (Copep) of the entire copepod community from Lime Cay and offshore Discovery Bay stations

	Lime Cay 0–30 m		Discovery Bay 0–60 m		Discovery Bay 0–200 m	
	Naup	Copep	Naup	Copep	Naup	Copep
Abundance ($\times 10^4$ m^{-2})	25.50	14.40	11.14	2.61	20.08	7.86
Biomass (g AFDW m^{-2})	0.01	0.15	0.012	0.036	0.021	0.079
Production (kJ m^{-2} yr^{-1})	50	419	40.7	80.8	90.2	151.5
Mean weight (µg AFDW)	0.04	1.042	0.106	1.38	0.106	1.005

Turner & Tester (1992) that copepod nauplii are bacterivorous, and the knowledge that nauplii, in general, ingest smaller particles than adults (see Turner 1984 and references therein).

Feeding experiments

Of the 11 species examined, 7 showed clear evidence of feeding on FLB (Table 5), with 92% of their nauplii ingesting FLB. FLB were clearly visible in the guts of many nauplii (Fig. 1), and usually comprised a discrete feeding (foregut) or 'fecal' (hindgut) bolus of cells (Fig. 2). After only 30 min, fecal pellets were being produced, already filled with FLB. Presence of FLB in guts cannot be interpreted as incidental feeding or 'drinking' (Turner & Tester 1992), because 4 species consistently failed to ingest them. Numbers of FLB in
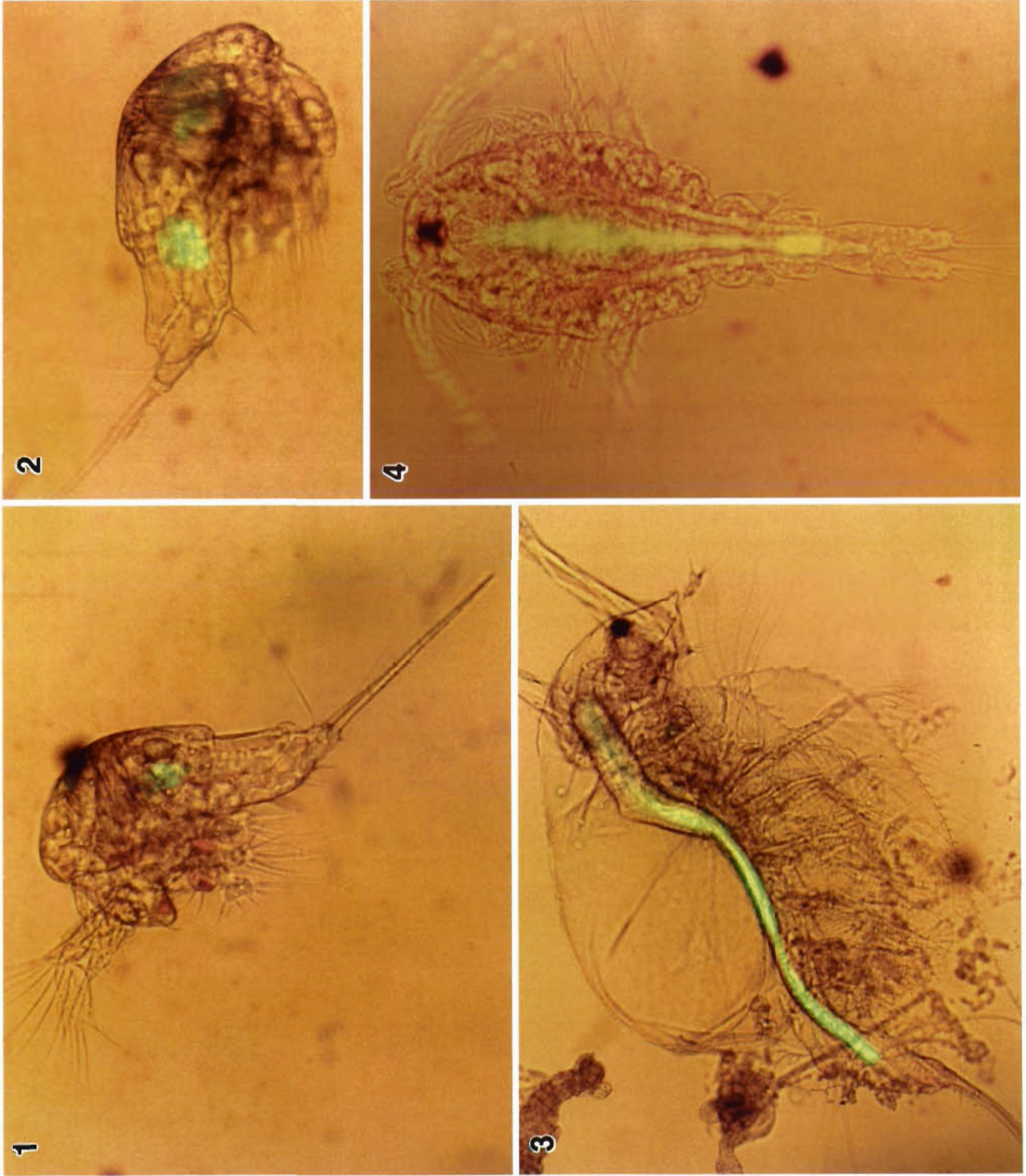
guts and fecal pellets could not be counted with confidence, but as judged from intensity of fluorescence (not measured) many thousands must have been ingested. The reduction in numbers of FLB during experiments lay within the limits of counting error, and could not be distinguished from background. Thus, because of the preferred experimental design we have opted to survey the extent of bacterivory by nauplii in these observations, rather than attempting to document feeding rates or efficiencies. Nauplii must be maintained at high food densities for long periods in order to observe decreases in food concentrations and avoid high counting errors; this we believe is one reason for the wide range in feeding rates of nauplii on FLB observed by Turner & Tester (1992).

In several species, notably *Acartia liljeborgii*, *Temora turbinata* and *Undinula vulgaris*, there was clear evidence of digestion of FLB, with fluorescent

Table 5. Summary of observations on naupliar bacterivory on FLB in Kingston Harbour (KH), Lime Cay (LC) and Discovery Bay (DB). Fluorescence levels as follows: 0: no material for observation; −: no fluorescence observed; +: low fluorescence due to few FLB observed; •highly fluorescent due to many FLB

Location	Date (1992)	Taxon	Naupliar stage	FLB (10^6 cells ml^{-1})	No. fluorescent/ no. observed	Fluorescence level Foregut	Hindgut	Fecal pellet	Time (min)
KH	2 Aug	*Centropages velificatus*	I/II	1.8	0/2 (0%)	−	−	0	60
KH	2 Aug	*C. velificatus*	III/IV	1.8	0/9 (0%)	−	−	0	60
KH	2 Aug	*C. velificatus*	V/VI	1.8	0/8 (0%)	−	−	0	60
KH	2 Aug	*Acartia liljeborgii*	III/IV	1.8	16/16 (100%)	•	•	0	60
KH	2 Aug	*A. liljeborgii*	V/VI	1.8	1/3 (33%)	−	+	0	60
KH	3 Aug	*Temora turbinata*[a]	I	1.8	0/2 (0%)	−	−	0	60
KH	3 Aug	*T. turbinata*	II/III	1.8	18/19 (95%)	•	•	0	60
KH	3 Aug	*Oithona* spp.	II/III	1.8	18/18 (100%)	+	+	0	60
KH	9 Aug	*A. liljeborgii*	II/III	2.5	4/6 (67%)	+	+	0	60
KH	9 Aug	*Paracalanus aculeatus*	III	2.5	4/5 (80%)	•	+	+	60
KH	9 Aug	*P. aculeatus*	IV/V	2.5	5/5 (100%)	•	+	+	60
LC	1 Aug	*C. velificatus*	I/II	1.5	0/2 (0%)	−	−	0	30
LC	1 Aug	*C. velificatus*	III/IV	1.5	0/4 (0%)	−	−	0	30
LC	1 Aug	*C. velificatus*	V/VI	1.5	0/2 (0%)	−	−	0	30
LC	1 Aug	*A. liljeborgii*	III/IV	1.5	2/2 (100%)	+	•	0	30
LC	1 Aug	*Oncaea* spp.	V/VI	1.5	4/4 (100%)	•	•	0	30
LC	1 Aug	*Corycaeus* spp.	V/VI	1.5	0/2 (0%)	−	−	0	30
LC	1 Aug	*Temora stylifera*	V/VI	1.5	3/3 (100%)	•	•	0	30
KH	1 Aug	*C. velificatus*	III/IV	1.8	0/2 (0%)	−	−	0	35
KH	1 Aug	*T. turbinata*	V/VI	1.8	14/15 (93%)	•	•	•	35
KH	1 Aug	*T. turbinata*	III/IV	1.8	9/9 (100%)	+	•	0	35
KH	1 Aug	*A. liljeborgii*	I/II	1.8	2/2 (100%)	+	+	0	35
KH	1 Aug	*A. liljeborgii*	III/IV	1.8	1/2 (50%)	−	+	0	35
KH	1 Aug	*A. liljeborgii*	V/VI	1.8	1/1 (100%)	+	+	0	35
DB	8 Aug	*Undinula vulgaris*	V/VI	1.8	10/10 (100%)	•	•	+	65
DB	7 Aug	*U. vulgaris*	IV/V	1.8	4/4 (100%)	•	•	0	45
DB	7 Aug	*Euchaeta marina*	II/III	1.8	0/3 (0%)	−	−	0	80
DB	7 Aug	*E. marina*	III/IV	1.8	0/3 (0%)	−	−	0	80
DB	7 Aug	*E. marina*	IV–VI	1.8	0/5 (0%)	−	−	0	80
DB	7 Aug	*Clausocalanus* sp.	IV–VI	1.8	0/6 (0%)	−	−	0	110

[a]Hatched in the laboratory from eggs produced by adults from Kingston Harbour

Figs. 1 to 4. Examples of results of feeding experiments. Photomicrographs of tropical marine crustacean zooplankton (approx. 250×) fed fluorescently labelled bacteria (FLB) at concentrations of 1.5 to 2.5 × 10⁶ cells ml⁻¹. Fig. 1. *Temora turbinata* stage 4 nauplius showing concentrations of FLB in foregut (live, partly squashed preparation). Fig. 2. *T. turbinata* stage 5 nauplius showing concentrations of FLB in hindgut (live preparation). Fig. 3. *Penilia avirostris*, showing entire gut full of FLB (live preparation). Fig. 4. *Oithona* spp. early copepodite showing entire gut full of FLB (live preparation)

label released from the cells and forming a 'paint-like' emulsion when the nauplius was gently compressed under a cover slip. In other species, notably earlier stages of *Paracalanus aculeatus*, *Temora stylifera* and *Oncaea* sp., there was less evidence of digestion and individual FLB cells could be recognised in the gut and even in fecal pellets of some individuals which had ingested fewer cells.

The only species that consistently failed to ingest FLB were *Euchaeta marina*, *Centropages velificatus*, *Clausocalanus* sp. and *Corycaeus* sp. The very earliest stages of some species may not ingest bacteria or other particles because they may have yolk reserves. For instance, *Temora turbinata* NI nauplii did not ingest FLB, but later stage nauplii of this species did.

Our observations that a variety of nauplii appear to feed extensively on bacteria-sized particles is supported by observations of Turner & Tester (1992) with nauplii of *Acartia tonsa*, but is apparently at odds with results of Berggreen et al. (1988) for that species which indicate low feeding efficiency at the smaller end of the food particle size spectrum (< 5 to 10 μm).

Clearance rates per day for our 'average' nauplius are in fact comparable between the 2 sets of authors (see below), but in Bergreen et al. (1988) a clearance rate of 3.6 ml d^{-1} was not reached until a particle size of 7.2 μm (on *Dunaliela* spp.). Although we have considered several alternatives, we cannot yet advance a convincing explanation for this discrepancy between studies. The generation of highly fluorescent guts full of FLB in our feeding experiments is, however, compelling visual evidence. At this point we can only suggest, as did Bergreen et al. (1988), that prior food his-

tory, possibly variable yolk reserves passed into eggs by females, and thus perhaps variable onset of first feeding, may affect particle size selectivity.

Discrepancies in feeding studies are not uncommon among (and even within) authors. This was one reason for including *Penilia avirostris* in our observations. After only 20 min exposure to FLB, *Penilia* guts (Fig. 3) shone like neon lights. It was the most heavily bacterivorous of the species we examined, yet Turner et al. (1988) failed to demonstrate bacterivory in this species by conventional clearance studies, using natural bacterioplankton.

Among other species examined (Table 6), adult females of *Temora turbinata*, *Paracalanus aculeatus* and early copepodites of *Oithona* spp. (Fig. 4) showed clear evidence of bacterivory.

Relationship between the naupliar community and the MFW

Relationships between the MFW and the CFW must be viewed as a 2-way exchange (see Turner & Roff 1993), i.e. the contribution of energy *from* the MFW to the CFW, and the effect of the CFW *on* the MFW. An effect which is important in one direction may however be only incidental in the other. Thus, whether we consider a linkage between the MFW and the CFW as 'efficient', or not, may depend on the direction in which we view the exchange, as we shall now argue.

Having established that nauplii of many of the dominant copepod species are capable of bacterivory, we first ask: What is the possible significance of bacteria in

Table 6. Summary of observations on bacterivory on FLB by copepod adults and copepodites and marine cladocerans in Kingston Harbour (KH) and Discovery Bay (DB). Fluorescence levels as follows: 0: no material for observation; –: no fluorescence observed; +: low fluorescence due to few FLB observed; •highly fluorescent due to many FLB

Location	Date (1992)	Taxon	Stage or sex	FLB (10^6 cells ml^{-1})	No. fluorescent/ no. observed	Foregut	Hindgut	Fecal pellet	Time (min)
						Fluorescence level			
KH	2 Aug	*Penilia avirostris*	Adult	1.8	18/18 (100%)	•	•	•	45
KH	2 Aug	*Oithona oculata*	Female	1.8	0/4 (0%)	–	–	0	60
KH	2 Aug	*Evadne* sp.	Adult	1.8	0/7 (0%)	–	–	0	60
KH	2 Aug	*Acartia liljeborgii*	Female	1.8	1/7 (14%)	–	–	+	60
KH	2 Aug	*Temora turbinata*	Female	1.8	6/6 (100%)	+	•	0	40
KH	9 Aug	*Paracalanus aculeatus*	Female	2.5	7/10 (70%)	•	•	0	60
KH	9 Aug	*P. aculeatus*	CI	2.5	1/3 (33%)	+	+	0	60
KH	9 Aug	*P. aculeatus*	CII/CIII	2.5	0/4 (0%)	–	–	+	60
KH	9 Aug	*P. aculeatus*	CIV	2.5	0/1 (0%)	–	–	0	60
DB	8 Aug	*Oithona* sp.	CII–CIV	1.8	7/10 (70%)	•	•	0	60
DB	8 Aug	*Oithona* sp.	CV/female	1.8	0/9 (0%)	–	–	0	60
DB	8 Aug	*Microsetella* sp.	Female	1.8	0/4 (0%)	–	–	0	60
DB	8 Aug	*Saphirella* sp.	CV/female	1.8	0/5 (0%)	–	–	0	60
DB	8 Aug	*Oncaea* spp.	CV/female	1.8	0/5 (0%)	–	–	0	70
DB	8 Aug	*Corycaeus* spp.	Female	1.8	0/7 (0%)	–	–	0	65

their diet and to their production? This is presently a difficult question because, in a mixed food climate, we do not know the relative proportions of bacterial vs other foods actually taken. However, assuming that nauplii eat only bacterial sized particles (i.e. bacteria and picoplankton <2 µm), and knowing the daily clearance rates of nauplii, we can establish whether the daily food reqirements of nauplii could be met from this food source.

We took an average clearance rate per nauplius (*Acartia tonsa*) from Turner & Tester (1992) of 3.6 ml ind.$^{-1}$ d^{-1} (range 1.2 to 7.7 ml; see also Bergreen et al. 1988). Nauplii of this species are comparable in size to those in oceanic waters which average 0.106 µg AFDW (Table 4). A nauplius of this size (comparable to N3 *Acartia clausi* (Landry 1978) contains approximately 0.053 µg C. Calanoid nauplii in the offshore copepod community have an average G of 0.57. Growth efficiencies of nauplii are not known with any degree of certainty, but we took values of 30 to 50 %, given that they are believed to be relatively efficient feeders at low food concentrations (Paffenhöfer 1976, Epp & Lewis 1980; but see Bergreen et al. 1988). Such an average nauplius would need to ingest between 115 and 190 % of its own body mass per day to sustain its growth and other metabolic requirements, i.e. a minimum of 0.060 µg C d^{-1}. Nauplii larger or smaller than our average would clear a greater or smaller volume per day, and their required ration would also scale according to body size.

Data on bacterial biomass in the oligotrophic Caribbean Sea indicate an average of from 4 to 7.2 µg C l^{-1} (Ducklow 1983, Rath et al. 1993). To this we can add a further average of 0.063 µg chlorophyll a l^{-1} as picoplankton (Table 2). Allowing a conversion of chlorophyll a : C of 60:1 (Chavez et al. 1991), 3.6 ml of oceanic water offers an average food resource of bacteria + picoplankton combined of 0.028 to 0.039 µg C (~10 µg C l^{-1}) A volume of 7.7 ml offers an average food resource of 0.060 to 0.084 µg C, sufficient to feed our average nauplius. If nauplii were to restrict their feeding to the nano- plus net-phytoplankton size fractions (>2 µm), which offer 0.048 µg chlorophyll a combined (Table 2), or 0.023 µg C per 7.7 ml (~2.5 µg C l^{-1}), nauplii would fall far short of meeting their daily food requirements. By restricting their intake to particles >5 µm, nauplii would fare even worse. Nauplii in oceanic waters in fact probably exploit the whole size spectrum of particles available to them, including heterotrophic flagellates and ciliates and detritus. However, our feeding experiments and these calculations strongly suggest that nauplii could sustain 50 to 100 % of their food requirements from bacterioplankton and picoplankton alone. If the major source of food for nauplii is *indirectly* from bacteria and picoplankton, via

flagellates and ciliates, as is suggested for metazooplankton in general (see e.g. Sherr & Sherr 1988), then the available food supply per unit volume will be diminished in proportion to the number of trophic steps (see e.g. Pomeroy & Wiebe 1988), and must again be too low on average to support naupliar growth.

Food levels <300 µg C l^{-1} are generally stated as limiting to overall copepod growth, although Hart (1990) suggested the limiting concentration may be as low as 100 µg C l^{-1} for marine copepods. Even this lower figure is in sharp contrast to the average ~10 µg C l^{-1} offered by bacterio- and pico-plankton (<2 µm), or the 12.7 µg C l^{-1} offered by bacteria plus *all* phytoplankton in oceanic waters off Discovery Bay. Nauplii may also exploit detritus, although the extent to which they do so and its assimilability are largely unknown. POC off Discovery Bay averaged 166 µg l^{-1} with approximately 50 % of this as protein (Webber & Roff 1995a). Because our station here was close to shore and affected by coastal-derived detrital material, these values are considerably higher than the range of 5.2 to 19.1 µg POC l^{-1} reported for surface waters of the central Sargasso Sea by Wangersky (1974). The average concentration of POC in open oceanic waters could therefore also support naupliar growth, but the majority of it must be in the form of phytoplankton anyway, and this solution also begs the question of its origin and rate of supply.

Inshore, nauplii could feed on any size range of particles without being food limited, but in oceanic waters the most abundant source of live available food is in the bacterial size range (Rath et al. 1993). Nauplii in offshore waters do not appear to be food limited with respect to their counterparts in coastal waters. In contrast, copepodites in oceanic water exhibit decreasing growth rates as they increase in size (Webber & Roff 1995b), and appear to become increasingly food limited, presumably as they exploit progressively larger, but more dilute, food particles. This confirms the theoretical analysis and arguments of Huntley & Boyd (1984). It is logical to infer that such progressive food limitation would result in a pattern of differential size and stage mortality (see e.g. Lopez 1991), leading to proportionately greater survivorship and production of nauplii in oceanic than in coastal waters. This is in fact exactly the result (Table 4).

Having made the case that the MFW is potentially of great significance in supporting at least the nauplii of the CFW, we can now ask: What is the impact of naupliar grazing on the biomass or production of bacteria? In oceanic waters off Jamaica there is an average of 2 nauplii l^{-1} (see Table 4). Assuming an average clearance of 3.6 ml d^{-1}, the total grazing pressure of the naupliar community on bacteria and picoplankton is clearly negligible, i.e. 0.7 % of the biomass per day. This is in agreement with the results of several studies

(see e.g. Sherr & Sherr 1988), which also showed that grazing by the metazoan community on the MFW was insignificant in oceanic waters, and that the major grazers were phagotrophic flagellates and ciliates. Even in coastal waters, where mean naupliar abundance was 8.5 l^{-1} (Table 4) the grazing pressure on microbial particles will be low (~3 % of the biomass per day), even if other larger particles of the size-spectrum are not exploited.

We caution however that our interpretations are based on field data of naupliar abundances collected with 64 μm nets. We have shown that this mesh underestimates naupliar abundances by up to 90 %, and biomass by about 33 % (see above). However, even these biases are not sufficient to affect the argument that the grazing impact of nauplii on bacteria and picoplankton is negligible.

What we have documented here is the fact that several species of nauplii are capable of bacterivory. This is especially important in oceanic waters where bacterial sized particles constitute the dominant food resource. The rates of utilisation of particles of such size, and their contribution to nutritional requirements, now require careful investigation, as well as the feeding habits of those nauplii which are not bacterivorous.

In summary, in oceanic waters the MFW (based on autotrophic picoplankton and heterotrophic bacterioplankton; Sherr & Sherr 1988) appears to be significant in 'supporting' at least the juvenile phases of the CFW. In coastal waters, because of the usual existence of abundant other food resources, this is unlikely to be the case as argued by Bergreen et al. (1988). In the opposite direction however, even in oceanic waters the nauplii and copepods of the CFW appear to play a very minor role in the overall dynamics of the organisms *at the base* of the MFW.

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