Development and lipid composition of the harpacticoid copepod *Nitocra spinipes* reared on different diets

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ABSTRACT: We reared the harpacticoid copepod *Nitocra spinipes* on diets of bacteria, a diatom, or a macroalga, evaluating survivorship and growth in short-term (≤1 generation) experiments. Lipid content of the copepods and their diets was measured and used as an index of nutrition. Although growth, survivorship and lipid content of *N. spinipes* were significantly greater when fed the diatom, which had the highest lipid content of the 3 diets, the copepod was able to develop from egg to adult when fed a lipid-poor bacterial diet. Furthermore, this species was able to go through developmental molts without the addition of food (6 individuals from a starved cohort of 25 made it to at least copepodite stage I), suggesting the uptake of dissolved organic matter for growth. This widespread estuarine benthic copepod apparently has the ability to survive on diverse and nutritionally poor diets, a quality that is useful in a variable, detritus-dominated environment.

KEY WORDS: Copepod · Feeding · Lipid · Bacteria · Nutrition

INTRODUCTION

Harpacticoid copepods comprise a large proportion of the meiofauna in many marine benthic communities (Hicks & Coull 1983). They have been described as an essential link to upper trophic levels in benthic communities and are a common food for fish larvae in aquaculture. Although it has been argued that harpacticoids are the most important component in the diets of some larval and juvenile fish (Alheit & Scheibel 1982, Hicks & Coull 1983), their overall role in nutrient and energy transfer in marine benthic food webs is not well quantified.

Studies on the trophodynamics of harpacticoids have revealed that they are often indiscriminate feeders, utilizing dissolved and particulate organic matter, microalgae, ciliate protozoa and copepods (Heinle et al. 1977, Rieper 1978, Rieper & Flotow 1981, Poulet 1983, Decho & Fleeger 1988, Lazzaretto & Salvato 1992). Much is known about the dietary components of harpacticoids, but the nutritional value of these components is not well understood.

Nitocra spinipes is a common harpacticoid species found in brackish-water environments throughout the world (Lang 1948, Muus 1967, Noodt 1970, Wulff 1972, Gopalan 1977). It is euryhaline, with a salinity tolerance well beyond the range of most natural salinity fluctuations (Wulff 1972), as is commonly found in other harpacticoids (Ranade 1957). Laboratory cultures reveal it has a wide range of suitable foods (Gopalan 1977, Weiss pers. obs.).

The ontogeny of *Nitocra spinipes* consists of 6 nonpelagic naupliar stages and 6 copepodite stages, with stage VI corresponding to the adult. Generation time

typically is 10 to 14 d at 25 to 27°C (Weiss pers. obs.). The number of eggs per brood varies between 15 and 30 (Muus 1967, Weiss pers. obs.) with a female producing 1 to 3 broods per fertilization.

We set out to answer 3 questions concerning the nutrition of this copepod. First, can *Nitocra spinipes* develop from egg to adult on a diet consisting exclusively of bacteria? Previous observations that harpacticoid copepods can be cultured on bacteria would seem to contradict the established idea that the Crustacea have strict dietary requirements, especially for lipids such as sterols and polyunsaturated fatty acids, which are usually not found in bacteria (Rieper 1978, Goad 1981, Lechevalier & Lechevalier 1988). Second, how well does a bacterial diet support growth and survivorship, compared with other common foods (a diatom and a macroalga)? Third, is dietary lipid content closely correlated with growth and lipid content of the copepods, as is the case with many other crustaceans (e.g. Provasoli et al. 1970, Lee et al. 1971, Hakanson 1984, Ahlgren et al. 1990)?

MATERIALS AND METHODS

Cultures of *Nitocra spinipes* were started from individuals obtained from the lower Patuxent River, a tributary of Chesapeake Bay, USA. They were maintained for multiple generations in the laboratory on a diet of mixed microalgae (the diatom *Thalassiosira weissflogii* and the prymnesiophyte *Isochrysis galbana*) plus any associated bacteria in the cultures. Gravid females were taken from these cultures for the feeding experiment. The experiment compared growth and survivorship on the diatom *T. weissflogii*, the macroalga *Ulva* sp., and a gram-negative bacterium isolated from the river. The 3 diets were chosen to cover a wide range in chemical composition and to reflect food types commonly found in Chesapeake Bay. Based on the results of the feeding experiment, a second experiment was done to examine more closely naupliar survivorship and development under starvation.

Preparation of diets. Bacteria were isolated from the lower Patuxent River on enriched seawater agar using a streak plate technique described in Benson (1990). A yeast-tryptone seawater medium was inoculated with single colonies collected from the agar plates and incubated at 27°C on a countertop shaker. After 24 h the bacteria were centrifuged (20 min at 6000 rpm, 3800 × g), washed with deionized water to remove salt and medium, and centrifuged again. The pellets were transferred to 20 ml scintillation vials and freeze-dried. The isolate used in the feeding experiment was identified as a gram-negative rod, but was not characterized further The centric diatom *Thalassiosira weissflogii*,

obtained from non-axenic, unialgal cultures maintained at Chesapeake Biological Laboratory, was centrifuged, washed and freeze-dried as described above. *Ulva* sp. was collected from local waters, freeze-dried, ground, and sieved into <240 µm diameter particles.

Bacteria, diatom, macroalga feeding experiment. Treatments in this experiment (copepods fed either diatoms, macroalgae, or bacteria, plus a starved control) were replicated 4 times. The starved controls consisted of only filtered, autoclaved seawater. Five gravid females per replicate were placed in 200 ml plastic containers filled with 50 ml of 0.45 µm filtered and autoclaved seawater (salinity = 15 psu). The containers were maintained at 25 to 27°C, a temperature range commonly used in previous studies (e.g. Gopalan 1977). Feeding of the diet treatments initially consisted of 50 µg (dry weight) of food per container per day. After hatching of nauplii, 500 µg of food per container was given daily. Copepods were collected every other day on a 64 µm mesh, then gently washed off the mesh into clean water supplied with food. On days when water was not changed, 10 ml water samples were taken from each container, stained with acridine orange, and examined under an epifluorescent microscope to assess bacterial or protozoan contamination (Hobbie et al. 1977). At the conclusion of the 14 d experiment, the copepods were narcotized with MS-222 (Durbin et al. 1990), separated into 3 developmental stages (nauplii, copepodite, and adult) and counted. We did not measure clutch size or number of clutches per female separately; thus changes in abundance were due to combined differences in fecundity, development and survival on the different diets. One-way ANOVA and Fisher's multiple comparison test (α = 0.05) were used to quantify differences in copepod abundance between treatments.

Starvation experiment. Because some copepods survived and even continued development in the starved controls during the first experiment, we conducted a second experiment to evaluate the effects of starvation when cannibalism was not possible. We started with 25 nauplii hatched from the same clutch. They were separated, rinsed, and placed in individual wells filled with 1 ml of 0.45 µm filtered, autoclaved seawater. Survivors were identified to stage and transferred to clean filtered seawater every second or third day until the last copepod died on Day 23.

Lipid extraction and analysis. Individuals within each treatment in the first experiment were pooled and collected by filtration through combusted glass-fiber filters (Whatman GF/C) for lipid analysis. Copepods were washed with deionized water and transferred 3 times before filtering to remove organic debris. Samples of each freeze-dried diet were also prepared for lipid analysis.

Extraction of lipids was carried out using procedures described in Harvey et al. (1987). Total neutral lipid content and class composition (copepod samples only) were measured using a thin layer chromatography-flame ionization detection system (TLC-FID; Volkman et al. 1986, Parrish 1987). An aliquot of the lipid extract was examined further to determine individual sterols present in adult copepods by gas chromatography, using methods given in Harvey & McManus (1991). For all adult copepods, cholesterol (5-cholest-5-en-3-ol) was the only sterol detected.

RESULTS

Bacteria, diatom, macroalga feeding experiment

There were significant differences in the yield (p < 0.0001; all developmental stages combined) of copepods raised on the different diets (Fig. 1). The abundance of copepods that had been fed the diatom *Thalassiosira weissflogii* was significantly greater (p < 0.0001) than that of individuals in the other treatments (Fig. 1). The total number of surviving offspring in the diatom treatment was 35% greater than in the bacterial and macroalgal treatments, and 80% greater than in the starved control. Treatment error (coefficient of vari-
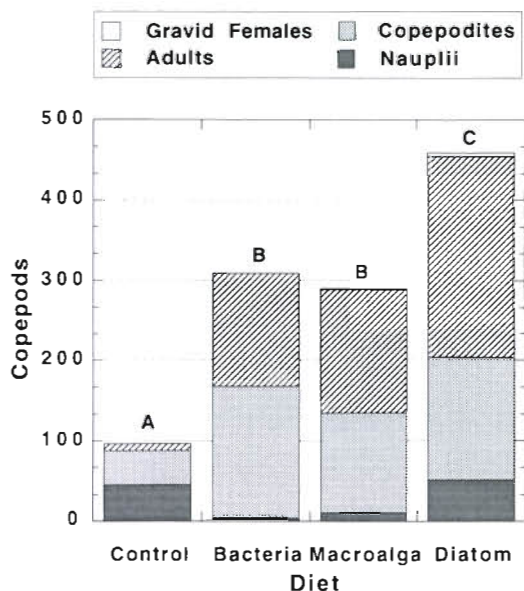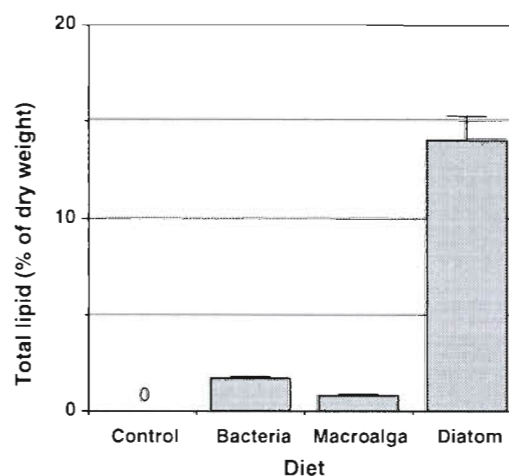

Fig. 2. Total lipid content of the 4 diet treatments. Lipid is expressed as a percentage of the dry weight of each diet

ation) was 11% for the control and <8% for the other treatments. A second generation of gravid females was apparent only among copepods fed the diatom diet. No significant difference in abundance (p = 0.2197) was detected between copepods reared on *Ulva* sp. and the bacterial diet. The number of individuals in the starved control was significantly lower (p < 0.0001) than that of the other 3 treatments. There was no bacterial or protozoan contamination apparent in the treatments, although these diets were not initially axenic.

The diatom diet had a much higher proportion of lipid per milligram dry weight than the bacteria or macroalga diet (Fig. 2). Similarly, copepods fed the diatom contained over 5 times more total neutral lipid per individual than that recorded for the other 3 treatments (Fig. 3a). Triacylglycerol, which made up 72% of the total neutral lipid in copepods fed the diatom, was not detectable in copepods fed the other diets (Fig. 3b). Copepods fed the diatom also had approximately 3 times as much cholesterol as those fed the bacterium or *Ulva* sp. Sterol comprised 32 and 43% of the neutral lipid in copepods fed *Ulva* sp. and bacteria, respectively. All of the neutral lipid detected in the starved copepods was present as cholesterol.

Starvation experiment

When nauplii from a single clutch were starved in individual wells separate from other nauplii, most perished within the first week (50% survival at ca 5 d; Fig. 4). The last survivor lasted 22 d. Despite complete lack of particulate food, however, some individuals passed through successive molts of the naupliar stages and became copepodites. Of the original 25 nauplii, 24% made it to the copepodite stage.

Fig. 1. *Nitocra spinipes*. Abundance of copepods for each diet treatment after the 14 d feeding experiment. Bars represent treatment means (n = 4) and are divided into the 4 developmental stages: nauplii, copepodites, adults, gravid females. Error within treatments is given in the text. Bars with same letters were not significantly different (α = 0.05; Fisher's multiple comparison test)
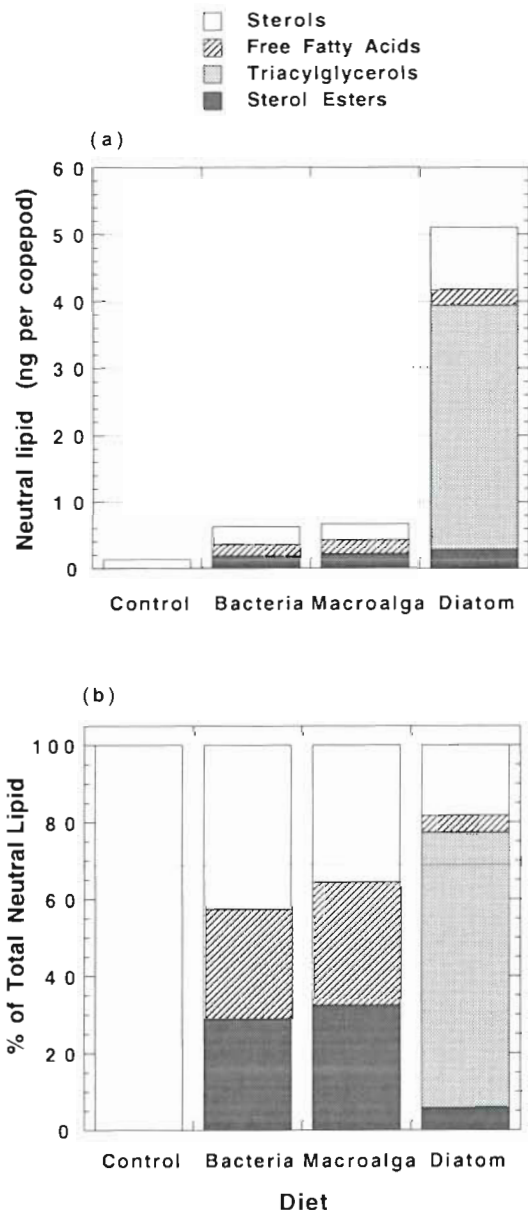
Fig. 3. *Nitocra spinipes*. Abundance of neutral lipids in copepods after 14 d feeding experiment as (a) absolute amount (ng lipid per copepod, all stages combined) and (b) as a percent of total lipid. The control treatment was copepods reared in filtered, autoclaved seawater only
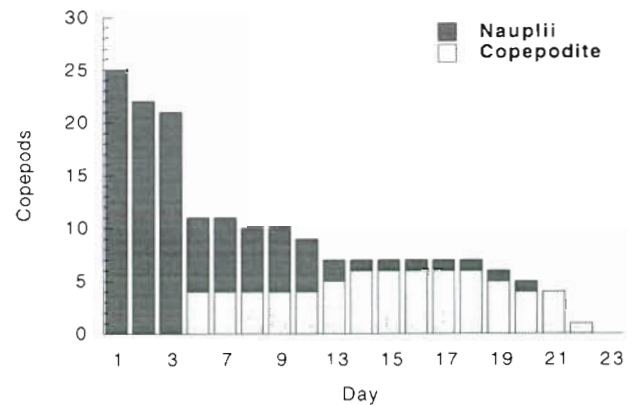

Fig. 4. *Nitocra spinipes*. Survival and development of 25 nauplii from a single clutch, reared separately in well-plates in filtered, autoclaved seawater. Plates were not examined on Days 4, 5, 11, and 12

DISCUSSION

Our experiments with *Nitocra spinipes* were motivated initially by reports that some harpacticoids have been cultured successfully on diets consisting exclusively of bacteria (e.g. *Tisbe holothuriae* and *Paramphiascella vararensis*; Rieper 1978). This is puzzling because with few exceptions bacteria lack sterols (Lechevalier & Lechevalier 1988), a class of lipid that is

an absolute dietary requirement for the Crustacea (e.g. Goad 1981). We thus first set out to confirm this observation. In our experiment with the bacterial diet, we checked carefully to eliminate the possibility of contamination by protozoa. Since bacterial lipid content is so low (<2% of dry weight), small numbers of protozoa could have contributed significantly to the lipid content of the diet even though they comprised a small fraction of the total caloric intake. Our results confirm that *N. spinipes* can indeed reproduce and grow on a strictly bacterial diet. Cannibalism and maternally derived energy stores could account for some, but not all, of the survivorship and growth. For example, the yield from our original 20 females in the bacteria-fed treatment was over 300 copepods, including over 100 that made it to the adult stage. Clearly, *N. spinipes* can convert bacterial food into more *N. spinipes*. How it obtains sterols under such conditions is not presently known. Possibly, it harbors gut symbionts that produce sterols, as do some insects (e.g. Douglas 1988). We also do not know how long this animal can continue to survive on a sterol-free diet. Further work rearing them through multiple generations is needed.

The results of our control in the feeding experiment, and of the starvation experiment, show that nauplii can develop to at least the early copepodite stages in filtered seawater. Since they are increasing in mass as they develop, they may be using dissolved organic matter (DOM). Direct uptake of DOM by copepods has been observed in several studies (Hicks & Coull 1983, Poulet 1983), however, the mechanism and nutritional importance of this type of feeding behavior remain unclear. Again, the possible role of symbionts, either epibiotic or within the gut, is unknown.

Growth, development, and neutral lipid content were greatest in *Nitocra spinipes* when fed the relatively lipid-rich diatom *Thalassiosira weissflogii*. Our

results suggest that diets rich in lipid maximize growth and lipid content in this species, as observed in a calanoid copepod (Hakanson 1984) and in several cladoceran species (Ahlgren et al. 1990). Lipid analysis of *N. spinipes* showed that 72% of the total neutral lipid in copepods fed the diatom was in the form of triacylglycerol, a storage lipid. This suggests that the lipid content of the diatom exceeded the daily metabolic demands of *N. spinipes*, permitting lipid storage to take place. No triacylglycerols were found in copepods raised on the other diets. For this copepod, living in a bacteria- and detritus-rich environment, where lipid content of the available food may often be low, the capacity to store lipids in the form of triacylglycerols may complement its apparently low lipid requirement.

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