ISSN 0240-8759

Vie et Milieu

Life and Environment

Vol. 45 nº 1 – Mars 1995

Périodique d'écologie - Publication trimestrielle

VIE ET MILIEU

Life and Environment

PÉRIODIQUE D'ÉCOLOGIE GÉNÉRALE
JOURNAL OF GENERAL ECOLOGY

LABORATOIRE ARAGO — UNIVERSITÉ P. et M. CURIE

Vie et Milieu – Life and Environment publie des contributions concernant les divers aspects de l'écologie, la biologie et la systématique dans les milieux marins, lagunaires et terrestres :

- biologie et écologie des organismes, des populations et des écosystèmes
- réseaux trophiques, flux énergétiques
- écologie benthique
- pollution
- écologie numérique, modélisation
- évolution et phylogénie

Un numéro à thème paraît chaque année.

La revue est une publication trimestrielle; chaque volume annuel comprend 4 fascicules groupant 400 pages et présente des articles originaux, des communications de synthèse et des notes brèves, en Français ou en Anglais.

Les manuscrits sont reçus par le Professeur A. GUILLE, Directeur de la Publication, ou par M^me^ N. COINEAU, Rédaction (Laboratoire Arago, 66650 Banyuls-sur-Mer, France). Ils ne seront acceptés qu'après avoir été soumis à l'approbation scientifique de deux lecteurs dont les commentaires restent anonymes.

Vie et Milieu – Life and Environment publishes contributions in the fields of Ecology, Biology and Systematics with emphasis on marine, lagoonar and terrestrial milieus :

- Organisms, communities and ecosystems biology and ecology
- Trophic webs, energetics
- Benthic ecology
- Pollution
- Numerical ecology, modelling
- Evolutionary biology and phylogeny

Each year, a single or double issue is devoted to a special topic.

The journal is quaterly. Each volume (four fascicles) of ca 400 pages presents original articles, reviews and short papers, in french or english.

Manuscripts are received by Professor Dr. A. GUILLE, Editor in Chief, or by Dr. N. COINEAU, Executive Editor (Laboratoire Arago, F. 66650 Banyuls-sur-Mer). Papers are only accepted after review by two anonymous referees.

Publié avec le concours financier de l'Association Naturalia et Biologia et du Conseil Général des Pyrénées-Orientales

---

ABONNEMENTS/SUBSCRIPTIONS 1995

	FRANCE	AUTRES PAYS
Un an (4 numéros) / *Annual subscription (4 issues)*.....	685 F	890 F

Adresser le paiement à l'ordre de / *Address payment to the order of :*

Vie et Milieu, Laboratoire Arago, 66650 BANYULS-SUR-MER. FRANCE

Les abonnements partent du premier numéro de l'année / *subscriptions begin with the first calendar year*

Numéros séparés de l'année, volumes antérieurs, suppléments et faunes des Pyrénées-Orientales, collections complètes, disponibles / *Back Issues and volumes, supplements and Faunas of Pyrénées-Orientales, complete collections, available :*

VIE ET MILIEU — Laboratoire Arago — F. 66650 BANYULS-SUR-MER

VIE MILIEU, 1995, 45 (1) : 1-9

RATES OF METAZOAN MEIOFAUNAL MICROBIVORY : A REVIEW

PAUL A. MONTAGNA

The University of Texas at Austin, Marine Science Institute, P.O. Box 1267, Port Aransas, Texas 78373 U.S.A.

BACTÉRIES
MICROALGUES
NUTRITION
RÉPONSE FONCTIONNELLE

RÉSUMÉ – La méiofaune consomme des Bactéries, des microalgues, des Protozoaires, du matériel détritique et d'autres méiobenthontes. Elle peut également absorber de la matière organique dissoute. L'objet de cet article de synthèse concerne la méiofaune microphage, et en particulier la prédation sur les Bactéries et les microalgues par les Nématodes et les Harpacticoïdes. La plupart des études utilisent les techniques radioisotopiques. Elles se divisent en deux catégories : celles qui envisagent d'élucider la biologie de la nutrition de la méiofaune, et celles qui cherchent à évaluer l'impact de l'importance de la méiofaune dans le recyclage du carbone. La méiofaune broute (sélectionne) les petites particules de la biomasse microbienne. Le taux de broutage de la méiofaune augmente lorsque l'abondance de la nourriture bactérienne disponible s'accroit. C'est une réponse fonctionnelle que les modèles d'optimisation prédisent. La méiofaune présente également des taux de consommation différents selon les sources de nourriture. Il existe des différences ontogénétiques du taux de broutage et de sélection. Ces différences intragénériques sont faibles. Les modes de nutrition varient avec les diverses phases du cycle de vie. En dépis d'une forte variabilité d'un point à l'autre du globe, le taux moyen de broutage de la méiofaune est de 0,01 · h^{-1}, ce qui représente 1 % par heure de la biomasse des hétérotrophes et des autotrophes. Ainsi, la méiofaune a un impact global significatif sur tous les processus faisant intervenir des microbionthes.

BACTERIA
MICROALGAE
FEEDING
FUNCTIONAL RESPONSES

ABSTRACT – Metazoan meiofauna eat bacteria, microalgae, protozoans, detritus, and other meiofauna. They also have the ability to absorb dissolved organic matter. The focus of this review is on meiofaunal microbivory, particularly the use of bacteria and microalgae by nematodes and harpacticoids. Most of the studies utilize radioisotope techniques. The studies fall into two categories : those done to elucidate meiofaunal feeding biology, and those done to assess the impact and importance of meiofauna in carbon cycling. Meiofauna graze on (i.e., select) small particles of microbial biomass. Meiofaunal grazing rates increase when offered increased abundances of microbial food. This is a functional response predicted by optimization models. Meiofauna also have different grazing rates on different species of food. Ontogenetic grazing rate and selection differences exist. The evidence for intrageneric differences in grazing rates is weak. Differences of feeding modes exist with different life history stages. There is much variability from site to site worldwide, but on average meiofauna graze at a rate of 0.01 · h^{-1}, or 1 % of the standing stock of both heterotrophs and autotrophs per hour. Therefore, meiofauna have a significant global impact on microbially mediated processes.

INTRODUCTION

During the 1970's, little was known about how, what, and how much meiofauna consumed (Fenchel, 1978; Coull and Bell, 1979). A lot has changed. The kinematics of meiofaunal feeding has been defined. There are four harpacticoid feeding groups : point feeders that are selective epistrate pickers, line feeders that scrape edges of particles, plane sweepers that sweep food into their mouths from two-dimensional surfaces, and solid feeders that either eat or clean whole particles (Marcotte, B.M. 1977, Ph.D. Thesis, Dalhousie University, 212 pp., described *in* Hicks and Coull, 1983). There are also four nematode feeding groups : deposit feeders, epistrate feeders, scavengers and predators (Jensen, 1987). Although some nematodes (as well as meiofaunal sized annelids) are deposit feeders, most metazoan meiofauna behavior is adapted to pick specific microbial food items, e.g., microalgae, bacteria, and protozoans. Some workers have referred to this feeding habit as « grazing, » and have measured

how much microbial biomass is consumed by meiofauna (Montagna, 1984; Decho, 1988; Blanchard, 1991). Whereas macrofaunal (and presumably meiofaunal) deposit feeders have adaptations to acquire food by ingesting large volumes of sediment (Lopez and Levinton, 1987), meiofaunal grazers have adaptations to pick out specific microbial particles (Marcotte, 1977 *in* Hicks and Coull, 1983; Jensen, 1987).

One of the most successful areas of study during the 1980's was to measure how much meiofauna eat in the field. It was found that meiofauna can vary their ingestion rates of microbes in response to changes in food quality or quantity (Deco, 1988; Montagna and Yoon, 1991). These results suggest that meiofaunal grazing rates are a functional response to changes in the environment. I use this term in the same way that Taghon and Green (1990) defined functional response : « how any animal changes its feeding rate in response to changes in abundance of its food. » Meiofauna, particularly harpacticoids, can exponentially increase feeding rates as a function of increased microphytobenthos (Montagna, *et al*, 1995). More information is needed on how to apply models to meiofauna feeding, but we need a reliable and easy way to obtain feeding rate measurements. The current radioisotope techniques are neither well understood, nor easy to use (Montagna, 1993).

Together nematodes and harpacticoids usually make up 90-98 % of the meiofaunal community (McIntyre, 1969; Coull and Bell, 1979; Dye and Furstenburg, 1981; Platt, 1981). This paper focuses on nematodes and harpacticoids because they dominate the community. So, when I refer to metazoan meiofauna, I am referring to mainly nematodes and harpacticoids and exclude benthic microfauna (flagellates, ciliates and foraminifera). There is no doubt that microfauna are important, but they may have different behavior and metabolism than metazoan meiofauna (Rivkin and De Laca, 1990). Meiofauna can utilize organic matter in diverse forms, but this review is focusing on the utilization of the microflora : bacteria and microphytobenthos. The main purpose of this paper is to review the current literature on meiofaunal feeding rates, so that the role of meiofauna in transferring microbial carbon into food webs can be better appreciated.

TECHNIQUES

Most meiofaunal grazing rate studies have employed radioisotopic tracers. There are two notable exceptions. One study used chlorophyll-pigment gut-content analysis to measure grazing on microalgae (Deco, 1988), and one study used fluorescence-labeled bacteria to measure grazing on bacteria (Epstein and Shiaris, 1992). Both of these fluorescence techniques are very powerful and have advantages for studies specific to bacteria or microalgae. The radioisotope approach has problems, but it is currently the only way to measure grazing on both bacteria and microalgae. Therefore, this review will mostly cover radioisotope tracer studies. There are two techniques for employing radioactive tracers to measure the invertebrate feeding via grazing. Microbial food is either pre-labeled (Haney, 1971) or labeled while it is being grazed (Daro, 1978). Both techniques have advantages and disadvantages that limit their use to either laboratory or field studies. The pre-labeling technique requires growing microbes with a radioactive tracer, introducing the labeled microbe to the existing microbial community and knowing the specific activity of the food source. Such conditions are best achieved in laboratory studies. The synoptic labeling technique is more amenable to in situ studies, because only the radioactive tracer is introduced and microbial uptake of the tracer and meiofaunal grazing can be measured at the same time.

It is difficult to review the literature and determine the comparative quantitative differences in meiofaunal microbivory, because of differences in approach to measuring feeding rates and differences in reporting the rates measured. In large part, the differences exist because different endpoints are desired. Studies on carbon flow might report rates on a biomass specific basis (e.g., $\mu g\ C \cdot ind \cdot h^{-1}$), whereas studies on the impact of meiofauna grazing on microbial populations might report the flow rate (e.g., h^{-1}). The focus of this review is on assessing the latter issue, therefore I have reported flow rates. This requires that the amount of food (e.g., carbon, chlorophyll, or number of cells) offered or available for eating is known and reported. In this case it is simple to calculate the flow rate, i.e., the percent grazed per unit time. An advantage to reporting the flow rate is that the inverse is the turnover time required for the microbial population to maintain itself under the grazing pressure of meiofauna. Turnover time is an interesting number, because if the grazing rate is near the prey population growth rate then there is a tight linkage between predator and prey.

MEIOFAUNAL GRAZING RATE STUDIES IN THE LABORATORY

The earliest study on nematodes was performed by Duncan *et al.* (1974). They pre-labelled bacteria, *Acinetobacter* sp., with ^{14}C-glucose and fed

them to a freshwater nematode, *Plectus palustris*, in hanging drops on slides. Although the grazing rate was low (Table I), the amount of bacteria consumed was 650 % of the nematode body weight d^{-1}. Another early study on the marine nematode, *Adoncholaimus thalassophygas*, demonstrated that this predator could take up dissolved organic matter (DOM) in the form of ^{14}C-glucose, but did not eat pre-labelled bacteria (Lopez *et al.*, 1979).

Admiraal *et al.* (1983) fed the nematode *Eudiplogaster pararmatus* ^{14}C-labelled algae, *Navicula pygmaea*. They used an interesting technique. The diatoms were solidified in agar and nematodes introduced to the agar drops. This is one of the most complete studies in terms of measuring functional responses, since they used three concentrations of food, two ages of nematodes, and two species of food. Feeding rates increased with increasing food concentration. In two of the three experiments, there appears to be a saturation of the feeding rate. Feeding rates doubled with doubled nematode age, but the weight specific grazing rate decreased nearly an order of magnitude. One experiment was performed with the diatom *N. salinarum*, and the feeding rate was four times higher on this diatom, indicating that selection of food species exists.

Herman and Vranken (1988) fed the nematode *Monohystera disjuncta* with the bacterium *Alteromonas haloplanktis* in bacto-agar. Feeding rates increased with age, but females had greater feeding rates than males (Table I). They also measured defecation rates and used a model to calculate assimilation efficiency. The assimilation efficiency was low : 18 % for juveniles, 27 % for adult males, and 26 % for adult females.

Diatom ingestion rates increase with age and size for the nematode *Chromadorita tenuis* (Jensen, 1984). The nematode body weight increases about 20 times during its life cycle, and the ingestion rate increases from about 1 to 3 µg algae d^{-1}.

Table I. – Meiofaunal grazing rates using prelabelled food. The grazing rate was calculated as the fraction of radioactivity incorporated per individual. Units are in $\times 10^{-4}$ h^{-1}, which is equivalent to % $\times$ 100 h^{-1}.

Taxa	Bacteria	Microalgae	Reference
Nematodes			
Plectus palustris	3.2	-	Duncan *et al.* (1974)
Adonchholaimus thalassophygas	0.016	-	Lopez *et al.* (1979)
Eudiplogaster pararmatus	-	0.02	Admiraal *et al.* (1983)
Monohystera disjuncta (Juvenile)	17.8	-	Herman and Vranken (1988)
Monohystera disjuncta (Male)	15.3	-	Herman and Vranken (1988)
Monohystera disjuncta (Female)	53.1	-	Herman and Vranken (1988)
Harpacticoids			
Tisbe californicus	-	1.6	Lear & Oppenheimer (1962)
Tisbe holothuriae	-	1.2	Vanden Berghe & Bergmans, (1981)
Tisbe holothuriae (sand)	19.7	-	Rieper (1978)
Tisbe holothuriae (suspension)	0.059	-	Rieper (1978)
Tisbe battagliai	-	1.2	Vanden Berghe & Bergmans, (1981)
Tisbe furcata	-	0.51	Vanden Berghe & Bergmans, (1981)
Paramphiacella vararensis (sand)	0.059	-	Rieper (1978)
Paramphiacella vararensis (suspension)	0.0049	-	Rieper (1978)

It appears that all aspects of nematode biology is strongly influenced by the amount of food available. Schiemer (1982) measured respiration, growth, and reproduction of *Caenorhabditis briggsae* as a function of different concentrations of the bacterium, *Escherichia coli*. Nematode body length and weight increased concordantly when grown with increasing concentrations of bacteria. Nematode respiration increased with increasing food. Growth rates increased with increasing food to a threshold value, and maturation was earlier at higher food densities. Fecundity, egg size, and egg production also increased with increasing food. The nematode *Plectus palustris* showed a similar response (Shiemer *et al.*, 1980). Both species have increased assimilation, growth, and population rate with an increase in bacterial food density (Schiemer, 1983). These studies demonstrate the tight linkage between nematodes and the amount of bacterial food available.

The first study on harpacticoids was performed by labelling the green alga, *Platymonas subcordiformis*, with ^{90}Sr and ^{90}Y (Lear and Oppenheimer, 1962). However, they used *Tigriopus californicus*, a harpacticoid that is not benthic. *Tigriopus* is a littoral tide pool species. Syvitski and Lewis (1980) studied sediment ingestion and mineral transformation by *T. californicus*. They found that ingestion rate was dependent on suspension concentration.

Rieper (1978) performed one of the first studies feeding bacteria to harpacticoids. She used a planktonic species, *Tisbe holothuriae*, and a benthic species, *Paramphiascella vararensis*. She prelabelled five different strains of bacteria with ^{3}H-glucose or ^{3}H-leucine, and offered the harpacticoids a sand-paste consisting of pelleted bacteria in sterilized beach sand and bacteria in suspension. We don't know in which experiments glucose or leucine were used, and this could have affected the results. Both species had higher grazing rates when offered bacteria in a sand paste over bacteria in suspension. Unexpectedly, the planktonic species grazing rate was three orders of magnitude greater on sand than suspension, but the difference was only one order of magnitude for the benthic species (Table I). This suggests that even planktonic harpacticoids are episubstrate feeders. Rieper obtained three other results : the planktonic species ate more than the benthic species, both species had different rates on different species of bacteria, and both species had higher grazing rates when the cell concentration were increased.

Harpacticoids also may have intrageneric differences in feeding rates and preferences. Three species of the planktonic genus *Tisbe* were fed pre-labelled algae (*Dunaliella tertiolecta*) and unlabelled bacteria (Vanden Berghe and Bergmans, 1981). One species had a low feeding rate on algae and preferred the bacteria, and two had the same rate and preferred the algae (Table I). However, I think that Vanden Berghe and Bergmans may have misinterpreted the results. They performed seven experiments, but the experiments usually contained different amounts of added algae and bacteria for different replicates. Within the experiments, there are trends of increasing grazing rates with increasing added algae (Fig. 1). Although, *T. furcata* had an average grazing rate four times lower than the other two species, the experiments also contained an average of 40% less food in each replicate. Rather than an intrageneric effect, they may have simply observed a functional response to the amount of added food (Fig. 1).

Ontongenetic feeding shifts also occur within the harpacticoids (Decho and Fleeger, 1988). *Nitocra lacustris* feed on diatoms only from the second or third copepodite stage. Nauplii and early copepodite stages ingest bacteria by scraping the outer surface of diatoms. Adults ingest bacteria coincidently with ingested diatoms.

Food quality, as measured by C:N ratios, does not affect ingestion rates of *Tisbe cucumariae*. Guidi (1984) fed *T. cucumariae* seven pre-labelled foods with varying C:N ratios and protein content. Whereas nitrogen, protein level, and C:N ratios significantly correlated with survival and development times, ingestion rates were not greatly affected.

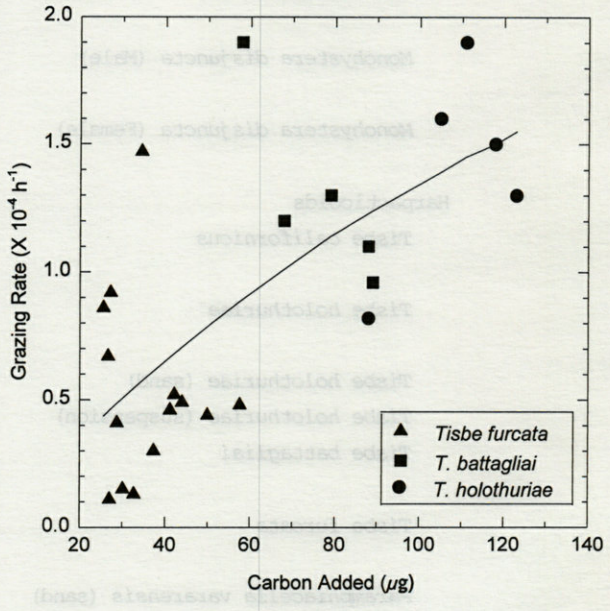

Fig. 1. – Functional response of *Tisbe* spp. to added food. Data from Vanden Berghe and Bergmans (1981) is fit to exponential model, $G=G_{max}\ (1-e^{(-k\ C)})$, suggested by Montagna *et al.* (1995).

One measure of food quality is egg production. The harpacticoid copepod, *Heteropsyllus pseudonunni*, produced more eggs when fed detritus from *Juncus roemerianus* than when fed the microalga, *Dunaliella* sp. (Ustach, 1982). The copepod also produced more eggs when fed *Juncus* detritus and bacteria than when fed the microalga *Nitzschia* sp. (Ustach, 1982). These results suggest that detritus and the associated bacteria are important to *H. pseudonunni*. However, the copepod *Scottolana canadenensis* produced more eggs on a mixed diet of algae and bacteria than either diet alone (Heinle *et al.*, 1977).

IN SITU GRAZING RATE STUDIES

Microbial prey can also be labeled while they are being grazed (Daro, 1978). I have adapted this technique for use in sediments to measure meiofaunal grazing rates (Montagna, 1984; Montagna and Bauer, 1988; Montagna, 1993). This allows for in situ studies to be performed. Meiofaunal grazing rates on bacteria and microalgae are calculated using a model proposed by Daro (1978). The meiofaunal grazing rate (G) is the proportion of material flowing from the donor (or prey) compartment to the recipient (or grazer) compartment per unit time. The model assumes that label is in excess and that during the incubation period label does not become limiting, prey uptake is linear, and grazer label recycling is zero. In this case, G is expressed in units of h^{-1} and is calculated as follows :

$$G = 2F/t \quad (1)$$

$$F = M/B \quad (2)$$

where F is the fraction of label uptake in the grazer, M (in this case meiofauna), relative to the prey, B (bacteria or microalgae), at time, t in hours. M and B are in units of disintegrations per minute (DPM). Since $2/t$ is a constant, variability in G is due to variability in F, i.e., M and B. In situ grazing studies require controls, because meiofauna may absorb DOM (Montagna and Bauer, 1988), or adsorb dissolved inorganic carbon (Montagna, 1983). A properly designed in situ meiofaunal grazing experiment must consider and correct for isotopic uptake by microbes, meiofauna, and macrofauna via non-grazing processes. This requires parallel incubations to obtain experimental and control values.

Carman and Thistle (1985) used this technique to study species interactions and feeding strategies. They injected ^{14}C-bicarbonate or ^{14}C-acetate into undisturbed sediment cores and incubated for 4 and 8 hours. They found that three co-occurring species had three different feeding strategies. *Thompsonula hyaenae* preferred microalgae, *Halicyclops coulli* preferred bacteria, and *Zausodes arenicolus* did not exhibit preferences (Table II). In general, the grazing rates from field studies (Table II) are much lower than the grazing rates obtained from laboratory studies (Table I). This could be explained in two ways. There may be methodological problems with the in situ technique, or the lower rates may be a response to lower amounts of food. It is more likely that food is added in excess in laboratory culture experiments than is available in the natural sediments during field studies. Therefore, it is possible (and perhaps likely) that the lower grazing rates are reflecting lower amounts of available food.

Recently, Carman (1990) has suggested that these differences in grazing rates among the three species were due to label uptake by epicuticular bacteria, and not grazing on labeled bacteria. Male copepods do not feed while clasping females, but the females continue to feed. No bacteria associated label was seen in copepod guts by autoradiography, and non-feeding males still incorporated label on their cuticle. Carman (1990) concluded that differences in feeding rates on bacteria among the harpacticoids listed in Table II is due to differences in uptake by epicuticular bacteria, not grazing on labeled bacteria. This study illustrates the importance of using live controls to subtract label uptake by non-grazing processes.

Most authors have used the in situ technique to measure carbon flow, and not individual species responses to environmental factors. The goal in these types of studies is to determine the impact

Table II. – Copepoda grazing rates from studies that label food while grazing occurs. Grazing rate was calculated as two times the fraction of radioactivity incorporated per unit time (Daro, 1978). Units are in $\times 10^{-4}$ h^{-1}, which is equivalent to % $\times$ 100 h^{-1}.

Taxa	Bacteria	Microalgae	Reference
Thompsonula hyaenae	0.002	0.056	Carman & Thistle (1985)
Halicyclops coulli	0.016	0.002	Carman & Thistle (1985)
Zausodes arenicolus	0.008	0.007	Carman & Thistle (1985)

of meiofauna on benthic microbial productivity, and the flow of carbon from microbes to meiofauna. I have performed five such studies (Table III).

The community grazing rates on bacteria range only within one order of magnitude, from 0.003 to 0.03 h^{-1}. The range on microalgae is fives times greater, i.e. a 50 fold difference, from 0.0008 to 0.04. The range on both grazing rates reflects the range of environmental conditions, e.g., temperature, season, and food quality. In my studies, the differences can be explained by temperature and organic carbon content of the sediment (Montagna and Yoon, 1991). In each study, different taxa dominate the grazing process, reflecting differences in community structure in each locale and experiment. The average grazing rate over all studies is the same for both bacteria and microalgae : 0.01 h^{-1}. This suggests, that on average the meiofaunal community is removing 1 % of the (heterotrophic and autotrophic) microbial standing stock per hour worldwide (in shallow ecosystems).

The mesocosm study by Nilsson *et al.* (1991) was the only experimental study. They were investigating the effect of increased nitrogen (N) and phosphorous (P) loading on the lower trophic levels of a sand system. Additions of N and P increased primary production within 2-3 weeks, and this lead to increases in meiofaunal grazing rates. During the four week experiment, bacterial production was not stimulated, and grazing rates on bacteria did not change. This study shows that meiofaunal communities can respond to changes in the quantity of food abundance with changes in ingestion rates.

Only one study has come to the conclusion that meiofauna grazing has no impact on bacterial dynamics (Epstein and Shiaris, 1992). They concluded that meiofauna consumption only accounted for about 0.03 % of the bacterial standing stock per day. This is equivalent to a meiofaunal grazing rate of 0.0000125 h^{-1}, which is about three orders of magnitude lower than all other estimates reported in the literature (Table III). In fact, the estimate is comparable to rates of individual organisms, and not communities (Tables I and II). This result is at odds with most published literature. The difference must be attributed to one or more unique circumstances, e.g., the use of a different technique, the specific location, or the bacteria used. The technique, fluorescence-labeled bacteria, appears to be a very powerful method. However, rather than staining a broad natural community, four strains of coliform

Table III. – Meiofaunal community grazing rates from studies that label food while grazing occurs. Grazing rate was calculated as two times the fraction of radioactivity incorporated per unit time (Daro, 1978). Units are in h^{-1}, which is equivalent to % × 100 h^{-1}.

Habitat	Location	Bacteria	Microalgae	Reference
Salt marsh	North Inlet, SC	0.0337	0.0065	Montagna (1984)
Beach	San Francisco Bay, CA	0.0028	0.0008	Montagna & Bauer (1988)
Subtidal	San Antonio Bay, TX	0.0099	0.0411	Montagna & Yoon (1991)
Ocean 18m	Santa Barbara, CA	0.0058	0.0018	Montagna *et al.* (1994)
Oyster pond	La Rochelle, France	-	0.0070	Blanchard (1991)
Mesocosm	Tjärnö, Sweden	0.003	0.004	Nilsson *et al.* (1991)
Mud flat	Ems-Dollard, Netherlands	-	0.003[a]	Admiraal *et al.* (1983)
Mud flat	Marennes-Oléron Bay, France	-	0.0014	Montagna *et al.* (1995)
Mud flat	Boston Harbor, MA	0.00001[b]	-	Epstein & Shiaris (1992)

[a]Different technique: estimate based on laboratory cultures

[b]Different technique: estimate based on fluorescence-labeled-bacteria

bacteria were used, two extracted from sewage treatment plant effluent. The natural community was reported to be dominated by rod-shaped bacteria. There are one of two possibilities that must be explored in future research : technology and spatial variability. Perhaps grazing rate results are a function of the technique used and one technique (or the application of it) may be flawed. Alternatively, grazing rate results are always unique to the study area and not general (in this case Boston Harbor just represents the low end of the spectrum). In either case, much more work is needed.

Results from freshwater studies are in the same ranges as those found in marine studies. *Attheyella* sp. in freshwater streams ingest bacterial carbon at rates of 0.03-0.47 $\mu g \cdot ind^{-1} \cdot d^{-1}$ (Perlmutter and Meyer, 1991). This is comparable to rates of 0.05-0.12 $\mu g \cdot ind^{-1} \cdot d^{-1}$ that is reported for *Tisbe* sp. (Vanden Berghe and Bergmans, 1981). The total stream community is grazing bacteria at rates that range from 0.0004 to 0.0092 h^{-1}, which is in the low range for marine meiofauna (Table III). The lower rates are concordant with the densities of meiofauna in streams, which is about an order of magnitude lower than in marine sediments. The comparability of these rates indicate that the conclusions drawn from marine habitats have generality to all aquatic habitats that support meiofauna communities.

Metazoan meiofauna are not the only grazers in the benthos (Lee *et al.*, 1966). Protozoans are also important in some environments (Fenchel, 1977 ; Epstein and Shiraris, 1992), but perhaps not others (Kemp, 1988). Temporary meiofauna, and taxa other than harpacticoids and nematodes can also be important grazers. For example, polychaetes (Montagna, 1984), mollusks (Montagna and Yoon, 1991), and oligochaetes and turbellarians (Meyer-Reil and Faubel, 1980) have been shown to be dominant grazers in some ecosystems. The total impact of meiofauna on microbes is a complex issue.

CONCLUSIONS

Metazoan meiofaunal grazing rates appear to be functional responses to available food. Grazing rates increase when meiofauna are offered increased abundances of microbial food as predicted by optimization models. Since ingestion varies as a function of food, it is imperative that all future studies measure and report the total amount of food available in the experiment or the concentration of food available in the field. Meiofauna taxa apparently have different grazing rates on different food, as well as different selectivity of different foods. Ontogenetic grazing rate differences also exist. Intrageneric grazing rates may exist, but more work is needed on this issue. It is possible that most bacteria ingested by meiofauna in shallow water, is coincidental ingestion. There is much variability from site to site worldwide, because of differing amounts of food available due to different environmental conditions and meiofauna community structure differences. Despite this variability, grazing rates only vary by an order of magnitude, and on average meiofauna graze at a rate of $0.01 \cdot h^{-1}$, or 1 % of the standing stock of both heterotrophic bacteria and autotrophic microalgae. As long as the average global microbial turnover time is about 4 days or less, meiofauna grazing will be roughly in equilibrium with microbial production. This suggests that meiofaunal communities are tightly coupled to microbial communities. This coupling implies that meiofauna have a significant global impact on microbially mediated processes by allowing microbial growth rates to be maintained in log phase.

ACKNOWLDGEMENTS – I am grateful to the anonymous reviewers who gave me many helpful suggestions. This is University of Texas Marine Science Institue Contribution number 919.

REFERENCES

ADMIRAAL W., L.A. BOUWMAN, L. HOEKSTRA and K. ROMEYN, 1983. Qualitative and quantitative interactions between microphytobenthos and herbivorous meiofauna on a brackish intertidal mudflat. *Int. Revue ges. Hydrobiol.* **68** : 175-191.

BLANCHARD G.F., 1991. Measurement of meiofauna grazing rates on microphytobenthos : is primary production a limiting factor?. *J. Exp. Mar. Biol. Ecol.* **147** : 37-46.

CARMAN K.R., 1990. Mechanisms of uptake of radioactive labels by meiobenthic copepods during grazing experiments. *Mar. Ecol. Prog. Ser.* **68** : 71-83.

CARMAN K.R. and D. THISTLE, 1985. Microbial food partitioning by three species of benthic copepods. *Mar. Biol.* **88** : 143-148.

COULL B.C. and S.S BELL, 1979. Perspectives of marine meiofaunal ecology. *In* Ecological Processes in Coastal and Marine Systems. Edited by R.J. Livingston, Plenum Publ. Corp., New York, 189-216.

DARO M.H., 1978. A simplified ^{14}C method for grazing measurements on natural planktonic populations. *Helgol. wiss. Meeresunters.* **31** : 241-248.

DECHO A.W., 1988. How do harpacticoid grazing rates differ over a tidal cycle? Field verification using chlorophyll-pigment analyses. *Mar. Ecol. Prog. Ser.* **45** : 263-270.

DECHO A.W. and J.W. FLEEGER, 1988. Ontogenetic feeding shifts in the meiobenthic harpacticoid copepod *Nitocra lacustris*. *Mar. Biol.* **97** : 191-197.

DUNCAN A., F. SCHIEMER and R.Z. KLEKOWSKI, 1974. A preliminary study of feeding rates on bacterial food by adult females of a benthic nematode, *Plectus palustris* DeMan, 1880. *Pol. Arch. Hydrobiol.* **21** : 249-258.

DYE A.H. and L.P. FURSTENBURG, 1981. Estuarine meiofauna. *In* Estuarine Ecology. Edited by J.H. Day, Balkema, AA, Rotterdam, 179-186.

EPSTEIN S.S. and M.P. SHIARIS, 1992. Rates of microbenthic and meiobenthic bactivory in a temperate muddy tidal flat community. *Appl. Environ. Microbiol.* **58** : 2426-2431.

FENCHEL T., 1977. The significance of bactivorous Protozoa in the microbial community of detrital particles. *In* Aquatic Microbial Communities. Edited by J. Carins, Jr., Garland Publ., New York, 529-544.

FENCHEL T., 1978. The ecology of micro– and meiobenthos. *Ann. Rev. Ecol. Syst.*, **9** : 99-121.

GUIDI L., 1984. The effect of food consumption on ingestion, development, and survival of a harpacticoid copepod, *Tisbe cucumariae* Humes. *J. Exp. Mar. Biol. Ecol.* **84** : 101-110.

HANDY J.F., 1971. An in situ method for measurement of zooplankton grazing rates. *Limnol. Oceanogr.* **16** : 970-977.

HEINLE D.R., R.P. HARRIS, J.F. USTACH and D.A. FLEMMER, 1977. Detritus as food for estuarine copepods. *Mar. Biol.* **40** : 341-353.

HERMAN M.J. and G. VRANKEN, 1988. Studies of the life-history and energetics of marine and brackish-water nematodes. II. Production, respiration and food uptake by *Monohystera disjuncta*. *Oecologia* **77** : 457-463.

HICKS G.R.F. and B.C. COULL, 1983. The ecology of marine meiobenthic harpacticoid copepods. *Oceanogr. Mar. Biol. Ann. Rev.* **21** : 67-175.

JENSEN P., 1984. Food ingestion and growth of the diatom-feeding nematode *Chromadorita tenuis*. *Mar. Biol.* **81** : 307-310.

JENSEN P., 1987. Feeding ecology of free-living aquatic nematodes. *Mar. Ecol. Prog. Ser.* **35** : 187-196.

KEMP P.F., 1988. Bactivory by benthic ciliates : significance as a carbon source and impact on sediment bacteria. *Mar. Ecol. Prog. Ser.* **49** : 163-169.

LEAR D.W. Jr., and C.H. OPPENHEIMER, Jr., 1962. Consumption of microorganisms by the copepod *Tigriopus californicus*. *Limnol. Oceanogr.* **7** : 63-65.

LEE J.J, M. McENERY, S. PIERCE, H.D. FREUDENTHAL and W.A. MULLER, 1966. Tracer experiments in feeding littoral Foraminifera. *J. Protozoology* **13** : 659-670.

LOPEZ G., F. RIEMANN and M. SCHRAGE, 1979. Feeding biology of the brackish-water oncholaimid nematode *Adoncholaimus thalassophygas*. *Mar. Biol.* **54** : 311-318.

LOPEZ G.R. and J.S. LEVINTON, J.S. Ecology of deposit-feeding animals in marine sediments. *Quart. Rev. Biol.* **62** : 235-260.

McINTYRE A.D., 1969. Ecology of marine meiobenthos. *Biol. Rev.*, **44** : 245-290.

MEYER-REIL L.-A. and A. FAUBEL, 1980. Uptake of organic matter by meiofauna organisms and interrelationships with bacteria. *Mar. Ecol. Prog. Ser.* **3** : 251-256.

MONTAGNA P.A., 1983. Live controls for radioisotope food chain experiments using meiofauna. *Mar. Ecol. Prog. Ser.* **12** : 43-46.

MONTAGNA P.A., 1984. In situ measurement of meiobenthic grazing rates on sediment bacteria and edaphic diatoms. *Mar. Ecol. Prog. Ser.* **18** : 119-130.

MONTAGNA P.A. 1993. Radioisotope technique to quantify *in situ* microbivory by meiofauna in sediments. *In* Handbook of Methods in Aquatic Microbial Ecology. Edited by P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole, Lewis Publishers, Boca Raton, 745-753.

MONTAGNA P.A. and J.E. BAUER, 1988. Partitioning radiolabeled thymidine uptake by bacteria and meiofauna using metabolic blocks and poisons in benthic feeding studies. *Mar. Biol.* **98** : 101-110.

MONTAGNA P.A., J.E. BAUER, D. HARDIN and R.B. SPIES, 1994. Meiofaunal and microbial trophic interactions in a natural hydrocarbon seep. *Vie Milieu*, 45 (1) : 17-25.

MONTAGNA P.A., G.F. BLANCHARD and A. DINET, 1995. Affect of production and biomass of intertidal microphytobenthos on meiofaunal grazing rates. *J. Exp. Mar. Biol. Ecol.* in press.

MONTAGNA P.A. and W.B. YOON, 1991. The effect of freshwater inflow on meiofaunal consumption of sediment bacteria and microphytobenthos in San Antonio Bay, Texas, U.S.A. *Estuar. Coast. Shelf Sci.* **33** : 529-547.

NILSSON P., B. JONSSON, I.L. SWANBERG and K. SUNDBACK, 1991. Response of a marine shallow-water sediment system to an increased load of inorganic nutrients. *Mar. Ecol. Prog. Ser.* **71** : 275-290.

PERLMUTTER D.G. and J.L. MEYER, 1991. The impact of a stream-dwelling harpacticoid copepod upon detritally associated bacteria. *Ecology* **72** : 2170-2180.

PLATT H., 1981. Meiofaunal dynamics and the origin of the metazoa. *In* The Evolving Biosphere. Edited by P.H. Greenwood, and P.L. Forey, P.L., British Museum (Natural History), Cambridge, 207-216.

RIEPER M., 1978. Bacteria as food for marine harpacticoid copepods. *Mar. Biol.* **45** : 337-345.

RIVKIN R.B. and T.E. DeLACA, 1990. Trophic dynamics in antarctic benthic communities. I. *In* situ ingestion of microalgae by Foraminifera and metazoan meiofauna. *Mar. Ecol. Prog. Ser.* **64** : 129-136.

SCHIEMER F., 1982. Food dependence and energetics of free living nematodes. I. Respiration, growth and reproduction of *Caenorhabditis briggsae* (Nematoda) at different levels of food supply. *Oecologia* **54** : 108-121.

SCHIEMER F., 1983. Comparative aspects of food dependence and energetics of free living nematodes. *Oikos* **41** : 32-42.

SCHIEMER F., A. DUNCAN and R.Z. KLEKOWSKI, 1980. A bioenergetic study of a benthic nematode, *Plectus palustris* de Man 1880, throughout its life cycle. II. Growth, fecundity and energy budgets at

different densities of bacterial food and general ecological considerations. *Oecologia* **44** : 205-212.

SYVITSKI J.P. and A.G. LEWIS, 1980. Sediment injestion by *Tigriopus californicus* and other zooplankton : mineral transformation and sedimentological considerations. *J. Sed. Petrol.* **50** : 869-880.

TAGHON G.L. and R.R. GREENE, 1990. Effects of sediment-protein concentration on feeding and growth rates of *Abarenicola pacifica* Healy et Wells (Polychaeta : Arenicolidae). *J. Exp. Mar. Biol. Ecol.* **136** : 197-216.

USTACH J.F., 1982. Algae, bacteria and detritus as food for the harpacticoid copepod, *Heteropsyllus pseudonunni* Coull and Palmer. *J. Exp. Mar. Biol. Ecol.* **64** : 203-214.

VANDEN BERGHE W. and M. BERGMANS, 1981. Differential food preferences in three co-occurring species of *Tisbe* (Copepoda, Harpacticoida). *Mar. Ecol. Prog. Ser.* **4** : 213-219.

Reçu le 5 septembre 1994; received September 5, 1994
Accepté le 28 septembre 1994; accepted September 28, 1994

VIE MILIEU, 1995, 45 (1) : 11-15

INGESTION RATES OF BATHYAL DEEP-SEA MEIOBENTHOS COLLECTED FROM SURUGA BAY, CENTRAL JAPAN

Y. SHIRAYAMA

Ocean Research Institute, University of Tokyo, 1-15-1, Minami-dai, Nakano-Ku, Tokyo 164, Japan

MEIOBENTHOS
INGESTION RATE
DEEP SEA
ENERGY BUDGET

ABSTRACT – Ingestion rates of bathyal deep-sea meiobenthos collected from Suruga Bay, central Japan, were measured using either ^{14}C-amino acid mixture or ^{3}H-thymidine as radioactive tracers. When ^{14}C-amino acid mixture was used, active incorporation of the tracer by the meiofauna was not detectable. On the other hand, if ^{3}H-thymidine was used, labels of specimen in control were constant, whereas those in feeding experiments increased with the meiofaunal body mass. Ingestion rate of a meiofaunal individual of 1 nl in body mass was estimated as 3.51 nl of sediment/hr assuming non-selective deposit feeding. This result is enigmatic because it means the animal feeds on 3.5 times more volume of sediment than its own body mass every hour. Assuming selective feeding on bacteria, the ingestion rate was estimated as 18,900 cells/hr, or 0.378 ng of C/hr. This value is close to the estimated energy consumption through respiration by the deep-sea meiofauna.

MEIOBENTHOS
TAUX D'INGESTION
ZONE BENTHIQUE PROFONDE
BUDGET ÉNERGÉTIQUE

RÉSUMÉ – Les taux d'ingestion du méiobenthos de la zone bathyale profonde récolté dans la Baie de Suruga, Japon Central, ont été mesurés à l'aide de mélanges C^{14}-amino-acide ou de H^{3}-thymidine comme traceurs radioactifs. L'utilisation de mélanges C^{14}-amino-acide ne permet pas de détecter l'incorporation active du marqueur. D'autre part, avec la thymidine H^{3}, le marquage des spécimens témoins reste constant, tandis que celui de la méiofaune en expérience croit avec le poids du corps. Le taux d'ingestion par individu de la méiofaune d'un poids du corps de 1 nl est estimé à 3,51 nl de sédiment à l'heure pour des mangeurs de dépôts non sélectifs. Ce résultat est énigmatique car il signifie que l'animal se nourrit d'un volume de sédiment égal à plus de 3,5 fois son poids à l'heure. Si la nutrition est sélective chez les Bactéries, le taux d'ingestion peut être estimé à 18 900 cellules/heure ou 0,378 ng C à l'heure. Cette valeur est proche de la consommation d'énergie due à la respiration par la méiofaune profonde.

INTRODUCTION

The deep sea is an energy-limited environment (Thiel, 1975, 1979; Shirayama, 1983, 1984). Thus one of the most important subjects in the deep-sea biology is understanding how the organisms have adapted to such a severe environment. Extremely low respiration rates of deep-sea fishes suggest that conserving energy by reducing metabolic activities is one way to adapt to the deep-sea condition (Smith and Hessler, 1974; Smith and Brown, 1983). The reported respiration rates of meiofauna are, however, similar to those of shallow-water relatives (Shirayama, 1992). These results make it questionable as to whether or not deep-sea meiofauna ingest foods as actively as their shallow-water counterparts even though the former inhabit an energy-limited environment.

The technique of measuring ingestion rates of meiofauna using radioactive tracers was developed by Montagna (1984 a, b), and has been applied in several studies of shallow-water meiofauna (Carman, 1990 a, b; Carman and Thistle, 1985). Shirayama (1991) used the technique to measure the ingestion rate of a single species of deep-sea nematode, and emphasized the importance of dissolved organic matter in addition to the particulate organic matter as an energy source for the nematode. The study involved only one species of nematode, but in this study, ingestion rates were measured for not only nematodes but also harpacticoid copepods and polychaetes. In addition, the size range of organisms was extended from an order of 0.1 µg to more than 100 µg. On the basis of the results, energy balance of deep-sea meiofauna will be discussed.

MATERIALS AND METHODS

Deep-sea sediment was collected using a box corer of USNEL type (Hessler and Jumars, 1974) modified to collect 1/10 m^2 of the sediment (Rigosha Co., Tokyo). The sampling was carried out once on November 1, 1986 at station A3 of R/V Tansei Maru cruise KT8601. The sampling station was established at the bay head of Suruga Bay, Central Japan (35°00.96'N 138°41.18' E) at a depth of 1214 m (Fig. 1).

Immediately after hauling up the corer on the board, five subcores were taken by inserting plexiglass tubes (34 mm inner diameter and 300 mm long) into the sediment. The topmost 1 cm layers of the sediment in these subcores were transferred into glass vials separately and 10 ml of the overlying water was added into each vials gently such that the disturbance of the sediment is minimal.

One of these vials was used to determine the abundance of bacteria in the sediment. To fix bacteria, 20 ml of 10 % V/V neutralized formalin was added into the vial on board, and kept refrigerated (5°C). In the laboratory on land, six replicates of 1 ml of the material in the vial were taken out, diluted 10 to 10 000 times with filtered seawater and 0.1 ml of the material was filtered using nucleopore filter of 0.2 µm opening, which had been stained using Irgalan Black. The filter was then stained using DAPI, and the number of bacteria on the filter was counted under a fluorescent microscope.

The sediments in the other glass vials were kept refrigerated at ambient temperature (5°C), and transferred to the radioisotope laboratory on land. Tracer experiments were carried out 48 hours after the collection. I basically followed the method described in Montagna (1984 a) with several modifications. Followings are the brief description of the present method. Into the glass vials which contain the deep-sea sediment, [U-^{14}C] L-amino acid mixture (ICN Radiochemicals, specific activity : 65 mCi/mmole) or [Methyl-^{3}H] Thymidine (ICN Radiochemicals, specific activity : 1.89 mCi/mg) was inoculated so as the radioactivities will be 0.526 and 9.85 µCi/ml, respectively, and the overlying water was stirred so gently as to avoid disturbance of the sediment surface. After culturing at ambient temperature (5°C) for 4.5 hours, 20 ml of 10 % V/V neutralized formalin in seawater was added into each vial and stirred vigorously so as to make the content of the vial uniform in order to stop the experiment. In the control experiments, addition of formalin was done immediately after the inoculation of radioactive tracer.

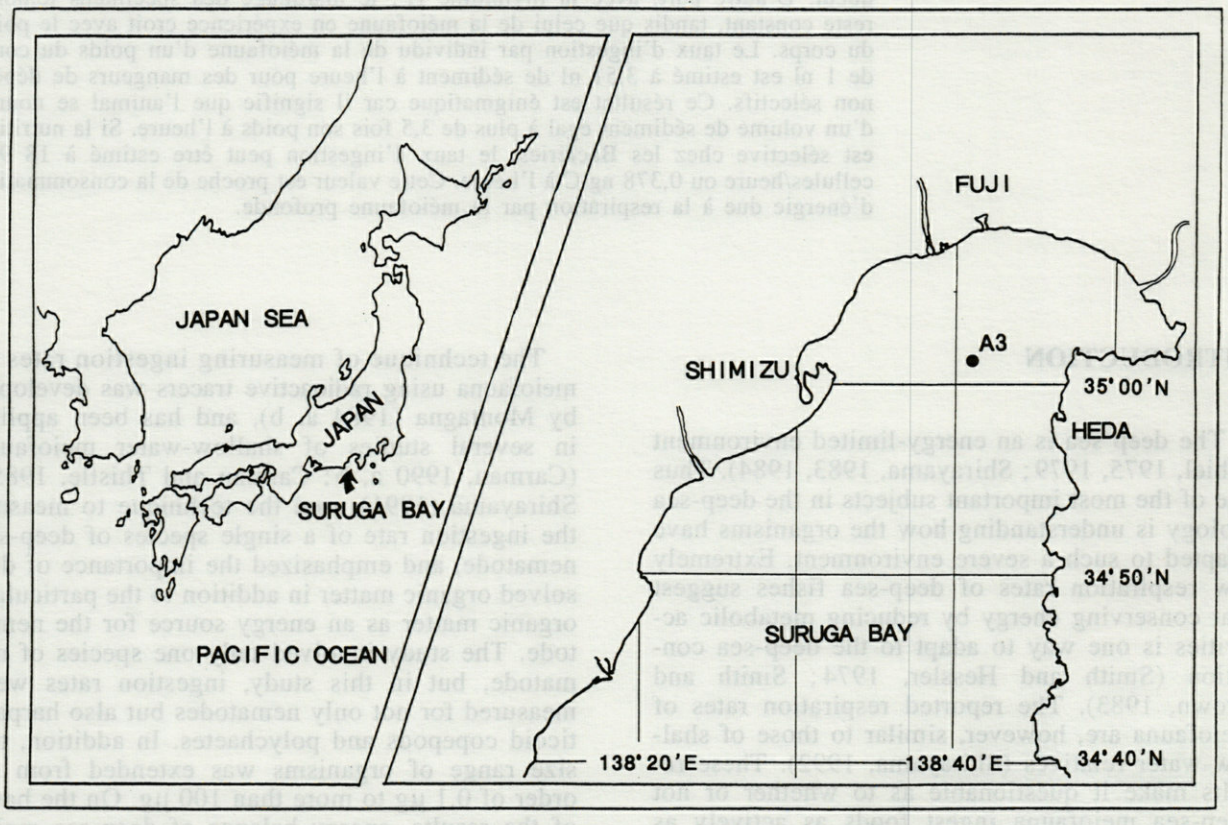

Fig. 1. – Map of sampling station.

To measure the radioactivity of the sediment, nine replicates of 10 µl of the fixed material were taken out from each vial and filtered using millipore filters of 0.2 µm opening. Each filter was washed with seawater, air dried, and placed in a vial for liquid scintillation counting. By adding the liquid scintillator (Aquasol II, Packard) into the vial, the filter became translucent, and the radioactivity of the filter was measured using a liquid scintillation counter (LKB 1215).

The rest of the sediment which had been used for the tracer and the control experiments was washed in a sieve of 63 µm opening, and from the material retained on the sieve, meiofaunal organisms were sorted out under a dissecting microsocope. Each meiofaunal individual was washed with seawater, and its body volume was measured following the method of Warwick and Price (1979). Then it was placed in a vial for liquid scientillation counting, dissolved using 1 ml of Soluence 100s (Packard), and its radioactivity was measured using the liquid scintillation counter (LKB 1215).

RESULTS

The surface sediment was mud, but at the layer of about 3 cm deep, it suddenly changed to coarse sand, suggesting that the area may have been affected by a turbidity current recently. Even though the environment seems to be unstable, a variety of meiofauna was present, and like other meiofaunal communities, nematodes were the most dominant whereas polychaetes and harpacticoids were the subdominant taxonomic groups. In the present study, the ingestion rates of these three major taxonomic groups were measured.

The results of tracer experiments differed depending on the species of tracer. When ^{14}C-amino acid mixture was used as a tracer, label of meiofauna increased with body mass of meiofauna in both control and feeding experiments (Fig. 2A). In this case, regressions for control and experiments were not significantly different, and active ingestion of meiofauna on sediment was not detectable.

In the ^{3}H-thymidine experiment, the radioactivities of meiofauna were constant regardless of body size in the control experiment (Fig. 2B). On the other hand, the label increased significantly with increase of body weight in the feeding experiment. Thus, the average of all results obtained in the control experiments was considered as spontaneous absorption of ^{3}H-thymidine by meiofauna. After substituting this average value from all the experimental results, the following significant regression ($r = 0.64$, $n = 26$, $p < 0.001$) was obtained.

$$\log DPM = 0.144 \log Wt + 0.051 \quad (1)$$

where DPM denotes the label of meiofauna (Decay/min) and Wt the weight of meiofauna (µg). Assuming that the specific density of meiofauna is 1.13, the above equation was converted to

$$\log DPM = 0.144 \log V + 0.059 \quad (2)$$

where V (nl) denotes body mass of meiofauna.

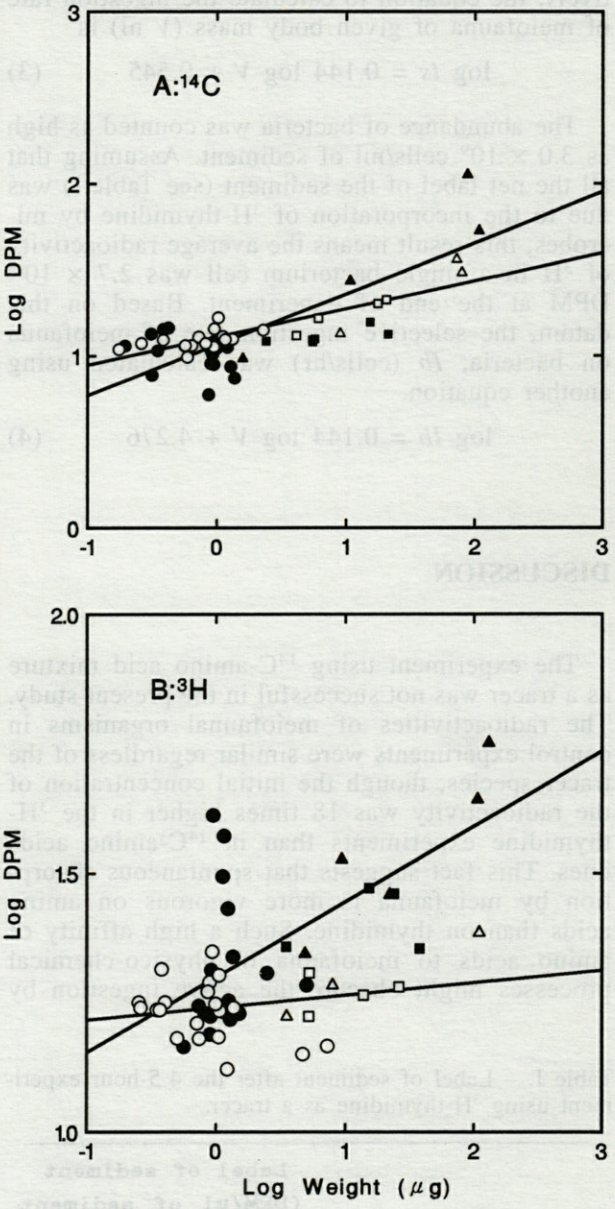

Fig. 2. – Relationships between label of meiofauna and body weight. Two species of radioactive material (A : ^{14}C-amino acid mixture; B : ^{3}H-thymidine) were used as tracer. Filled and open symbols denote experimental and control data, respectively. Circle, square and triangle symbols denote nematode, copepod and polychaete data, respectively.

After the 4.5-hour experiment, the sediment used for the feeding experiment incorporated ^{3}H-thymidine significantly more than that for the control one (Table I), suggesting active growth of microbes in the present sediment, even though they experienced rapid decompression when sampled from the deep sea.

Ingestion rates of meiofauna on sediment, *Is* (nl of sediment/hr), were calculated based on the label of ^{3}H in sedimentary particles (see Table I) using the formula described in Montagna (1984 a). If the animals fed on the sediment non-selectively, the equation to calculate the ingestion rate of meiofauna of given body mass (V nl) is

$$\log Is = 0.144 \log V + 0.545 \quad (3)$$

The abundance of bacteria was counted as high as 3.0×10^{9} cells/ml of sediment. Assuming that all the net label of the sediment (see Table I) was due to the incorporation of ^{3}H-thymidine by microbes, this result means the average radioactivity of ^{3}H in a single bacterium cell was 2.7×10^{-5} DPM at the end of experiment. Based on this datum, the selective ingestion rate of meiofauna on bacteria, *Ib* (cells/hr) was calculated using another equation,

$$\log Ib = 0.144 \log V + 4.276 \quad (4)$$

DISCUSSION

The experiment using ^{14}C-amino acid mixture as a tracer was not successful in the present study. The radioactivities of meiofaunal organisms in control experiments were similar regardless of the tracer species, though the initial concentration of the radioactivity was 18 times higher in the ^{3}H-thymidine experiments than in ^{14}C-amino acids ones. This fact suggests that spontaneous absorption by meiofauna is more vigorous on amino acids than on thymidine. Such a high affinity of amino acids to meiofauna in physico-chemical processes might obscure the active ingestion by the meiofauna on the particulate matter labelled by ^{14}C-amino acids.

Table I. – Label of sediment after the 4.5-hour experiment using ^{3}H-thymidine as a tracer.

	Label of sediment (DPM/µl of sediment ± S.D.)
Control	64.56 ± 6.76
Feeding Experiment	145.48 ± 20.85
Net incorporation	80.92 ± 21.92

The equation (3) indicates that a meiofaunal individual of 1 nl in body mass ingests 3.51 times more volume of sediment than its own per hour. Such an enigmatic result was obtained because non-selective deposit feeding was supposed. In the deep sea, flux of particulate organic matter is limited, and most sedimentary particles are not valuable as food. In such environment, non-selective deposit feeding would be not a suitable feeding strategy, because the animals will waste energy by ingesting sedimentary particles that do not contain any nutrients.

According to the equation (4), 18,900 cells of bacteria per hour were ingested by a meiofauna of 1 nl in body volume. Taking the general size of the marine bacteria ($3.6 - 7.3 \times 10^{-8}$ nl : Lee and Fuhrman, 1987) into account, the volume of food ingested is reasonably small compared with the body size of meiofauna. This ingestion rate is equivalent to 0.378 ng of C/hr, assuming a bacterium cell contains 2×10^{-14} g of C (Lee and Fuhrman, 1987). This value is distinctively lower than those reported for shallow-water nematode species (6 – 17 ng of C/hr; Tietjen, 1980).

Assuming respiration of 1 nl O_2 is equivalent to the energy consumption of 0.4 ng of C (Heip *et al.*, 1985), an individual of deep-sea nematode and meiofauna other than nematodes (i.e. harpacticoid copepods, polychaetes and aplacophorans) of 1 nl in body volume will consume 0.304 and 0.159 ng of C/hr by respiration, respectively (Shirayama, 1992). Based on these values as well as ingestion rates obtained in the present study, budget of energy in deep-sea meiofauna seems to be just balanced. However, it should be noted that all the matter ingested will not necessarily be assimilated by the organisms. Tietjen (1980) reviewed that assimilation efficiency of ingested bacteria is 18.3 – 25.8 % in nematodes, and even lower for algae (Tietjen *et al.*, 1970).

It also should be noted that the ingestion rate estimated here is the maximal one, and several factors can be pointed out as a reason for overestimation. It is impossible for organisms to perfectly discriminate bacteria from inorganic sedimentary particle. A part of the label of meiofauna thus should come from ingesting inorganic particles that absorbed ^{3}H-thymidine through physico-chemical processes.

Another possible reason for overestimation is direct absorption of dissolved radioactive tracer by the meiofauna. Meiofauna may take up ^{3}H-thymidine dissolved in seawater by means of active absorption through its body surface, though Shirayama (1991) argued it is less likely. Meiofauna also might actively drink interstitial water which contained radioactive tracer (Lopez *et al.*,

1979), though Carman (1990 a) reported negative results using an autoradiography technique that uptake of ^{14}C-acetate by shallow-water copepods was entirely due to activity by epibiotic bacteria, and no radioactivity was detected inside of copepods. Some workers tried to overcome the problem related to the direct uptake of dissolved tracer by meiofauna using food items labelled separately before the ingestion experiment (Epstein and Shiaris, 1992; Taylor and Sullivan, 1984). This method however is not possible to use in the study of deep-sea meiofauna, because it is difficult to prepare the labelled deep-sea bacteria before the collection of deep-sea meiofauna.

Carman *et al.* (1989) showed that disturbance of sediment integrity alters grazing rates of benthic copepods significantly. If one plans to do experiments using the deep-sea sediment on board the ship or in the laboratory on land, it is not possible to carry out it without any disturbing of the sediment. Decompression also may affect on physiology of either meiofauna or microbes. Taking these potential artifacts into account, it is desired to develop a novel method that can be carried out *in situ* at depth in the future study regarding energy uptake of deep-sea meiofauna.

ACKNOWLEDGEMENTS – The author expresses his sincere gratitude to captain, officers and crew of RV Tansei Maru of his institute for their help in sampling. Thanks are also extended to the anonymous reviewer for the valuable comments. Dr. Robert P. Higgins kindly read the early draft of the manuscript. Part of the study was supported by the grant-in-aid of the Ministry of Education, Science and Culture, Japan, No. 59480004, 61740371, 62740371 and 02740317.

REFERENCES

CARMAN K.R., 1990 a. Mechanisms of uptake of radioactive labels by meiobenthic copepods during grazing experiments. *Mar. Ecol. Prog. Ser.* **68** : 71-83.

CARMAN K.R., 1990 b. Radioactive labeling of a natural assemblage of marine sedimentary bacteria and microalgae for trophic studies : an autoradiographic studies. *Microbiol. Ecol.* **19** : 279-290.

CARMAN K.R., F.C. DOBBS and J.B. GUCKERT, 1989. Comparison of three techniques for administering radiolabeled substrates to sediments for trophic studies : uptake of label by harpacticoid copepods. *Mar. Biol.* **102** : 119-126.

CARMAN K.R. and D. THISTLE, 1985. Microbial food partitioning by three species of benthic copepods. *Mar. Biol.* **88** : 143-148.

EPSTEIN S.S. and M.P. SHIARIS, 1992. Rates of microbenthic and meiobenthic bacteriovory in a temperate muddy tidal flat community. *Appl. Environ. Microbiol.* **58** : 2426-2431.

HEIP C., M. VINCX and G. VRANKEN, 1985. The ecology of marine nematodes. *Oceanogr. Mar. Biol. Ann. Rev.* **23** : 399-489.

HESSLER R.R. and P. JUMARS, 1974. Abyssal community analysis from replicate box cores in the central North Pacific. *Deep-Sea Res.* **21** : 185-209.

LEE S. and J.A. FUHRMAN, 1987. Relationships between biovolume and biomass of naturally derived marine bacterioplankton. *Appl. Environ. Microbiol.* **53** : 1298-1303.

LOPEZ G., F. RIEMANN and M. SCHRAGE, 1979. Feeding biology of the brackish-water oncholaimid nematode *Adoncholaimus thalassophygas*. *Mar. Biol.* **54** : 311-318.

MONTAGNA P.A., 1984 a. *In situ* measurement of meiobenthic grazing rates on sediment bacteria and edaphic diatoms. *Mar. Ecol. Prog. Ser.* **18** : 119-130.

MONTAGNA P.A., 1984 b. Competition for dissolved glucose between meiobenthos and sediment microbes. *J. exp. mar. Biol. Ecol.* **76** : 177-190.

SHIRAYAMA Y., 1983. Size-structure of deep-sea meio– and macrobenthos in the Western Pacific. *Int. Revue ges. Hydrobiol.* **68** : 799-810.

SHIRAYAMA Y., 1984. The abundance of deep-sea meiobenthos in the Western Pacific in relation to environmental factors. *Oceanol. Acta* **7** : 113-121.

SHIRAYAMA Y., 1991. Preliminary studies on the energy budget of a deep-sea nematode. *La mer* **29** : 170-173.

SHIRAYAMA Y., 1992. Respiration rates of bathyal meiofauna collected using a deep-sea submersible « SHINKAI 2000 ». *Deep-Sea Res.* **39** : 781-788.

SMITH K.L. Jr. and N.O. BROWN, 1983. Oxygen consumption of pelagic juveniles and demarsal adults of the deep-sea fish *Sebastolobus altivelis*, measured at depth. *Mar. Biol.* **76** : 325-392.

SMITH K.L. Jr. and R.R. HESSLER, 1974. Respiration of benthopelagic fishes : *in situ* measurement at 1230 meters. *Science* **184** : 72-73.

TAYLOR G.T. and C.W. SULLIVAN, 1984. The use of ^{14}C-labeled bacteria as a tracer of ingestion and metabolism of bacterial biomass by microbial grazers. *J. Microbiol. Meth.* **3** : 101-124.

THIEL H., 1975. The size structure of the deep sea benthos. *Int. Revue ges. Hydrobiol.* **60** : 575-606.

THIEL H., 1979. Structural aspects of the deep-sea benthos. *Ambio Spec. Rep.* **6** : 25-31.

TIETJEN J.H., 1980. Microbial-meiofaunal interrelationships : a review. *Microbiol.* **1980** : 335-338.

TIETJEN J.H., J.J. LEE, J. RULLMAN, A. GREENGART and J. TROMPETER, 1970. Gnotobiotic culture and physiological ecology of the marine nematode *Rhabditis marina* Bastian. *Limnol. Oceanogr.* **15** : 535-543.

WARWICK R.M. and R. PRICE, 1979. Ecological and metabolic studies on free-living nematodes from an estuarine mudflat. *Est. Coast. Mar. Sci.* **9** : 257-271.

Reçu le 6 septembre 1994; received September 6, 1994
Accepté le 10 octobre 1994; accepted October 10, 1994

VIE MILIEU, 1995, 45 (1) : 17-25

MEIOFAUNAL AND MICROBIAL TROPHIC INTERACTIONS IN A NATURAL SUBMARINE HYDROCARBON SEEP

P.A. MONTAGNA[a], J.E. BAUER[b], D. HARDIN[c] and R.B. SPIES[d]

[a]University of Texas at Austin, Marine Science Institute, P.O. Box 1267, Port Aransas, Texas, 78373, USA
[b]School of Marine Science, College of William and Mary, Gloucester Point, Virginia, 23062, USA
[c]Applied Marine Sciences, P.O. 8346, Santa Cruz, California 9506-8346, USA
[d]Applied Marine Sciences, 2155 Los Positas Court, Suite V, Livermore, California, 94550, USA

ORGANIC ENRICHMENT
OIL SEEP
BACTERIA
MICROALGAE
FOOD WEB

ABSTRACT – The Isla Vista, California, hydrocarbon seep is an organically enriched environment. Microbial biomass and production are enhanced relative to non-seep sediments in the Santa Barbara Channel. This study was performed to determine the rate at which microbial carbon is transferred to higher trophic levels in natural seep sediments. Grazing rates of meiofauna on microbes were studied at three coastal stations representing a gradient of natural hydrocarbon seepage, from very active, to moderate, to none. Sampling was performed in April, July, and December, 1986 to examine possible seasonal differences between the three major oceanographic seasons (upwelling, mixed and Davidson respectively). Samples were limited to the top 1 cm of the sediment, where photosynthesis and most of the meiofauna occur. Meiofaunal community grazing rates were dominated by small polychaetes, and were slightly higher in July. There were no differences in grazing rates between stations, indicating that petroleum exposure had no effect on the feeding behavior of meiofauna. On average, meiofauna grazing rates were $0.0058 \cdot h^{-1}$ on bacteria and $0.0018 \cdot h^{-1}$ on microalgae in the sandy, subtidal environment. The higher rates on bacteria reflect the importance of heterotrophic processes in the seep environment. Since, the biomass of microalgae is much higher than that of bacteria, the consumption of microalgae ($4 \text{ mg C} \cdot m^{-2} \cdot h^{-1}$) is higher than on bacteria ($0.04 \text{ mg C} \cdot m^{-2} \cdot h^{-1}$), indicating the importance of benthic primary production in shallow marine ecosystems. The rates in coastal sediments are comparable to those found in estuarine sediments.

ENRICHISSEMENT ORGANIQUE
SUINTEMENT PÉTROLIER
BACTÉRIES
MICROALGUES
RÉSEAU TROPHIQUE

RÉSUMÉ – La zone de suintements d'hydrocarbure de l'Isla Vista en Californie est un milieu enrichi organiquement. La biomasse et la production des microorganismes sont augmentées par rapport aux sédiments non situés dans la zone d'émanation du détroit de Santa Barbara. Cette étude a été menée pour déterminer à quel taux le carbone des microorganismes est transféré aux niveaux trophiques supérieurs dans les sédiments infiltrés d'hydrocarbure. Le broutage des microorganismes par la méiofaune a été étudié dans trois stations de la côte montrant un gradient d'infiltration d'hydrocarbure naturel, à partir d'un site très actif, vers un site modérément actif, jusqu'à une station inactive. L'échantillonnage a eu lieu en avril, juillet et décembre 1986 en vue d'examiner d'éventuelles différences saisonnières entre les trois saisons océanographiques majeures (upwelling, mixte et Davidson, respectivement). Les prélèvements étaient limités au centimètre supérieur de sédiment où se localisent la photosynthèse et la plus grande partie de la méiofaune. Le taux de broutage par la communauté méiofaunique est dominé par les petites Polychètes et est légèrement plus élevé en juillet. Il n'y a pas de différence de consommation entre les stations, ce qui indique que l'exposition au pétrole n'a aucun effet sur le comportement de nutrition de la méiofaune. En moyenne, le taux de broutage de la méiofaune est de $0{,}0058 \cdot h^{-1}$ sur les bactéries et $0{,}0018 \cdot h^{-1}$ sur les microalgues dans les sables de la zone subtidale. La consommation plus élevée des bactéries reflète l'importance des processus hétérotrophiques dans le milieu d'émanation. La biomasse des microalgues étant beaucoup plus élevée que celle des bactéries, la consommation des microalgues ($4 \text{ mg C} \cdot m^{-2} \cdot h^{-1}$) est plus importante que celle des bactéries ($0{,}04 \text{ mg C} \cdot m^{-2} \cdot h^{-1}$), ce qui montre l'importance de la production primaire benthique dans les écosystèmes marins peu profonds. Les taux des sédiments côtiers sont comparables à ceux des sédiments des estuaires.

INTRODUCTION

The Isla Vista, California hydrocarbon seep is an organically enriched environment (Spies and Davis, 1979; Spies and DesMarais, 1983; Montagna *et al.*, 1986, 1989). The seep lies in 18 m of water, off Coal Oil Point in the Santa Barbara Channel. Seeping hydrocarbons enrich the coastal sediments with carbon (Spies *et al.*, 1980). This carbon is utilized by seep microbes, and there are very high rates of heterotrophic metabolism in these sediments (Montagna *et al.*, 1986; Bauer *et al.*, 1988). The carbon metabolized by microbes is also incorporated into the benthic food web (Spies and DesMarais, 1983). The high carbon content and heterotrophic activity of seep sediments correlate with high abundances of macrofauna (Spies and Davis, 1979) and meiofauna (Montagna *et al.*, 1987; 1989). The high amount of microbial biomass in seep sediments is fueling an active benthic food web.

Organic matter deposited in sediments is decomposed by heterotrophic bacteria. These bacteria form the basis of a detrital food chain with benthic invertebrates and epibenthic fish at the top. The hydrocarbon seep may simply represent an "upside down benthos," where organic matter comes from below to the surface. In addition to heterotrophy there are two forms of autotrophy present in seep sediments. Benthic microalgae are present, as are chemosynthetic bacteria (Spies and DesMarais, 1983; Montagna and Spies, 1985). Chemoautotrophic production by bacteria and primary production by microphytobenthos forms the basis of the grazing food chain. Together, the detrital and grazing food chains can fuel secondary production by benthic invertebrates.

Benthic bacteria (Zobell and Feltham, 1935) and microalgae (Leach, 1970) have long been hypothesized as major food and carbon sources for benthic invertebrates. Diatoms and bacteria are eaten by meiofaunal taxa such as nematodes (Jensen, 1982; Romeyn and Bouwman, 1983) and harpacticoid copepods (Sellner, 1976; Rieper, 1978, 1982, 1984). Previous work in estuaries, has shown that meiofauna grazing rates on bacteria and microalgae are close to the natural growth rates of those populations (Montagna, 1984). Thus, meiofauna can maintain microbial populations in log phase growth by their grazing pressure. Meiofauna should not be limited by food abundance, but by food production. If this hypothesis is true, then there should be concomitantly high rates of meiofaunal grazing when turnover times are high.

The purpose of this study was to examine meiofauna-microbial trophic interactions in a shallow, coastal, hydrocarbon seep. The seep is a good environment to study benthic trophic dynamics, because it is an organically enriched environment. The rate at which microbial carbon was being passed to higher trophic levels was assessed by measuring microbial productivity and meiofaunal grazing rates on microbial populations. These data can be used to test if meiofauna are having an important impact on the growth rates of microbes, by comparing meiofaunal grazing rates and microbial turnover times. The relative importance of the grazing and detrital food chains in seep sediments can also be determined.

The current study was performed at the same time as two other studies. One examining the biogeochemical relationships within vertical profiles of sediments was performed at the same time as this study (Bauer *et al.*, 1988), and the other examining vertical profiles of meiofaunal and microbial biomass (Montagna, *et al.*, 1989). The data on microbial biomass and productivity presented in the present study are derived from these two studies.

MATERIALS AND METHODS

The hydrocarbon seep is in the Santa Barbara Channel between Coal Oil Point and Goleta Point about 800 m offshore of Isla Vista, California, USA (Fig. 1). We sampled three stations with different rates of petroleum seepage. Station A is within the Isla Vista seep and in the center of large quantities of fresh oil and natural gas are seeping from the sediments. In our previous studies, we sampled at the edge of station A, where there was less fresh petroleum in the samples (Montagna *et al.*, 1986; 1987). In the current study we sampled the center of the seep with concentrations of total extractable hydrocarbons (TEH) averaging 2.2 $mg \cdot cm^{-3}$ at the surface and increasing to 25 $mg \cdot cm^{-3}$ at 7 cm below the surface (Bauer *et al.*, 1988). Station B, about 20 m north of A, has much less fresh oil seepage but has large quantities of weathered asphalt-like tar 4-12 cm below the sediment surface. The average TEH concentration of sediments at station B was 0.4 $mg \cdot cm^{-3}$ at the surface and increased to 3.5 $mg \cdot cm^{-3}$ (Bauer *et al.*, 1988) at 5 cm depth. Station C is about 1.4 km east of the Isla Vista seep and has about 4.8 times less weathered tar than station B (Stuermer *et al.*, 1982). Station C averaged only about 0.1 $mg \cdot cm^{-3}$ over the entire top 7 cm of sediment (Bauer *et al.*, 1988). Stations B and C have similar granulometry with a median grain size of about 160 µm (Spies & Davis, 1979; Palmer *et al.*, 1988). Stations A and B are within the Isla Vista seep but station C is not (Fig. 1). All stations were at a depth of 18 m in fine-sand sediments.

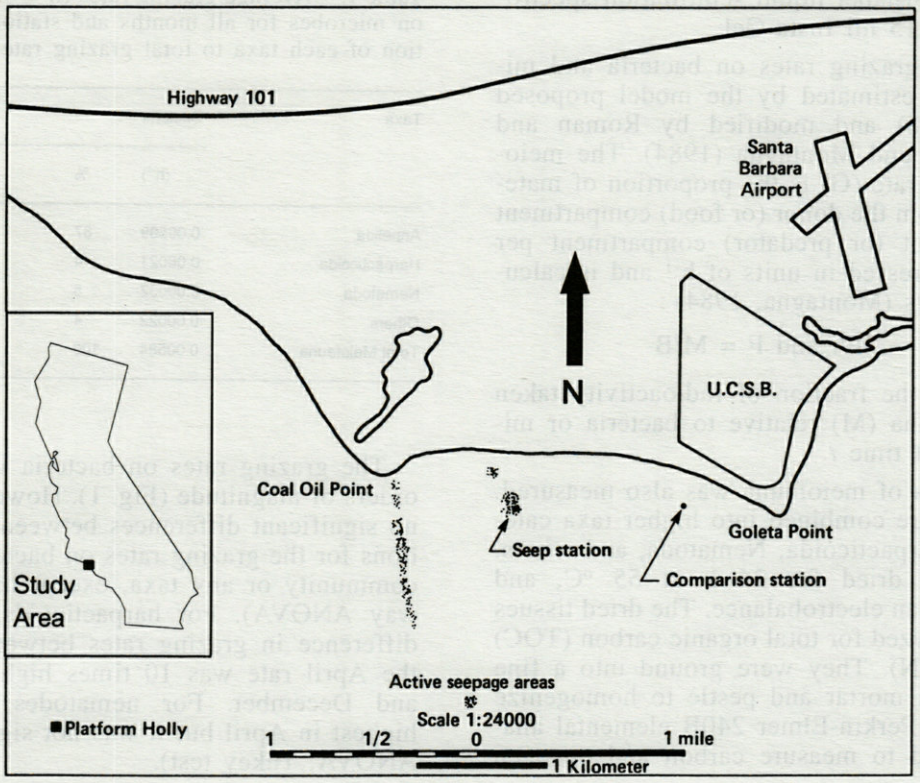

Fig. 1. – Location of sampling sites. The seep stations (A and B) are located east of Coal Oil Point. The comparison station (C) is located south of the University of Santa Barbara (U.C.S.B.) campus. Inset shows location of Santa Barbara in California, USA.

Three sampling periods were chosen to examine different environmental conditions. The average bottom water temperatures during April, July and December 1986 were 13.5 °C, 14.6 °C, and 17.0 °C respectively. During the December sampling period there was a very unusual storm. Although the skies were very clear, wave height was around 3 m and there was a great deal of storm surge at the bottom of the sampling sites. Sand ripples were also greatly pronounced relative to other times.

In situ meiofaunal grazing rates on bacteria and microalgae were measured by incubating sediment slurries with two radiolabeled substrates, tritiated thymidine (^{3}HTdR) and ^{14}C-bicarbonate ($H^{14}CO$) (Montagna and Bauer, 1988). The top 1 cm (~5.5 cm^3) of 60-cm^3 sediment cores were placed in 60 cm^3 clear centrifuge tubes. Two µCi of ^{3}HTdR and 2 µCi of $H^{14}CO_3^-$ were added to the slurries and samples were incubated for 2 h at *in situ* temperature and light conditions.

Live controls were used to assess label uptake in meiofauna not due to grazing on microbes. A saturated solution of nalidixic acid (200 µg ml^{-1}) plus 5'-deoxythymidine (2 µg ml^{-1}) (hereafter referred to as ND) was used to inhibit prokaryotic uptake of thymidine (Findlay *et al.*, 1984; Montagna and Bauer, 1988). The live controls for this experiment consisted of 3 replicate slurries with $H^{14}CO_3^-$, ^{3}HTdR and ND added. These were incubated in the dark to inhibit photosynthetic fixation of CO_2.

After 2 h, incubations were terminated by adding 2% formalin. A 1-ml subsample was withdrawn from the slurries. The subsample was filtered onto a 0.2 µm Millipore filter and rinsed 3 times with filtered seawater to estimate uptake of $H^{14}CO_3^-$ by microalgae and ^{3}HTdR by bacteria. The subsample was dispersed and suspended in 5 ml distilled water and 15 ml Insta-Gel for dual-label liquid scintillation counting. Meiofauna were separated from sediments by diluting samples with 2% formalin, swirling to suspend the animals, and decanting them and the supernate onto 63 µm Nitex screen filters. Meiofauna were then rinsed into jars and kept in refrigerated 2% formalin until sorting (1 to 2 d).

Sorting was performed using a dissecting microscope. Meiofauna were sorted by major taxa into the following groups : Harpacticoida, Nematoda, Polychaeta, and other meiofauna taxa. The sorted organisms were placed into scintillation vials containing 1 ml distilled water. After sorting, meiofauna were dried at 60 °C to evaporate the water and solubilized by adding 100 µl Soluene tissue solubilizer for 24 h. Samples were

counted by dual-label liquid scintillation spectrophotometry in 15 ml Insta-Gel.

Meiofaunal grazing rates on bacteria and microalgae were estimated by the model proposed by Daro (1978) and modified by Roman and Rublee (1981) and Montagna (1984). The meiofaunal grazing rate (G) is the proportion of material flowing from the donor (or food) compartment to the recipient (or predator) compartment per hour. G is expressed in units of h^{-1} and is calculated as follows (Montagna, 1984) :

$$G = 2F/t \text{ and } F = M/B$$

where F is the fraction of radioactivity taken up by meiofauna (M) relative to bacteria or microalgae (B) at time *t*.

The biomass of meiofauna was also measured. Individuals were combined into higher taxa categories, i.e., Harpacticoida, Nematoda, and others. Samples were dried for 24 h at 55 °C, and weighed using an electrobalance. The dried tissues were also analyzed for total organic carbon (TOC) and nitrogen (N). They were ground into a fine powder with a mortar and pestle to homogenize the sample. A Perkin-Elmer 240B elemental analyzer was used to measure carbon and nitrogen content.

Statistical analyses were performed using 2-way analysis of variance (ANOVA) where stations and seasons were the two main treatment effects. Tukey multiple comparison tests were performed to find post hoc differences among sampling means.

RESULTS

Meiofaunal grazing on both bacteria and microalgae was dominated by small annelid polychaetes (Table I). Juvenile polychaetes were responsible for 87 % of the bacteria, and 55 % of the microalgae consumed by meiofauna. Although juvenile polychaetes are only temporary meiofauna, that is they will grow out of the meiofaunal size class, they apparently have a great energetic impact on microbes. Of the permanent meiofauna, nematodes were the dominant grazers, responsible for 5 % of the bacteria and 27 % of the microalgae consumed by meiofauna.

The overall mean grazing rate for the total meiofaunal community 0.00584 h^{-1} on bacteria and 0.00181 h^{-1} on microalgae (Table I). Therefore, 0.584 % of the bacteria were removed by meiofauna in 1 h, and the turnover time (i.e., the inverse of the grazing rate) that bacteria would require to maintain their population size is 7.1 days. For microalgae, 0.181 % were removed by meiofauna in 1 h, and that the turnover time microalgae would require to maintain their population size is 23 days.

Table I. – Average grazing rates (h^{-1}) of meiofaunal taxa on microbes for all months and stations, and contribution of each taxa to total grazing rate (%).

Taxa	Bacteria		Microalgae	
	(h^{-1})	%	(h^{-1})	%
Annelida	0.00509	87	0.00099	55
Harpacticoida	0.00021	4	0.00015	8
Nematoda	0.00032	5	0.00049	27
Others	0.00022	4	0.00018	10
Total Meiofauna	0.00584	100	0.00181	100

The grazing rates on bacteria varied over two orders of magnitude (Fig. 1). However, there were no significant differences between months or stations for the grazing rates on bacteria by the total community or any taxa, except harpacticoids (2-way ANOVA). For harpacticoids, there was no difference in grazing rates between stations, but the April rate was 10 times higher than in July and December. For nematodes, the rate was highest in April but it was not significant (2-way ANOVA, Tukey test).

The grazing rates on microalgae also varied over two orders of magnitude (Fig. 3A). Again, there were no differences in grazing rates on microalgae for either stations or months for the community as a whole. Harpacticoids and nematodes had different grazing rates in different months. Both had higher grazing rates in July than in April and December. The nematode grazing rate on microalgae at station A was 5 times higher than at B and C. This was the only taxa that had significantly different grazing rates among stations for either bacteria or microalgae.

The total amount of microbial biomass consumed per hour is calculated by the product of the microbial biomass and the meiofaunal grazing rate. These rates varied considerably. Consumption of bacterial biomass varied over 2 orders of magnitude (Fig. 2B), averaging 41.2 µg $C \cdot m^{-2} \cdot h^{-1}$. The total amount of microalgal biomass consumed by meiofauna varied over 3 orders of magnitude (Fig. 3B). The average consumption of microalgal biomass by meiofauna was 3840 µg $C \cdot m^{-2} \cdot h^{-1}$.

Total meiofaunal biomass was always highest at station A (Table II). This was caused by the large amount of nematodes at station A, which dominated community biomass. There was always higher amounts of harpacticoid biomass at station C. There was not a large difference in meiofaunal biomass among the three months (Table II).

The carbon content of the harpacticoid copepods was 57.7 %, nematodes 49.2 % and the other meiofauna 38.6 %.

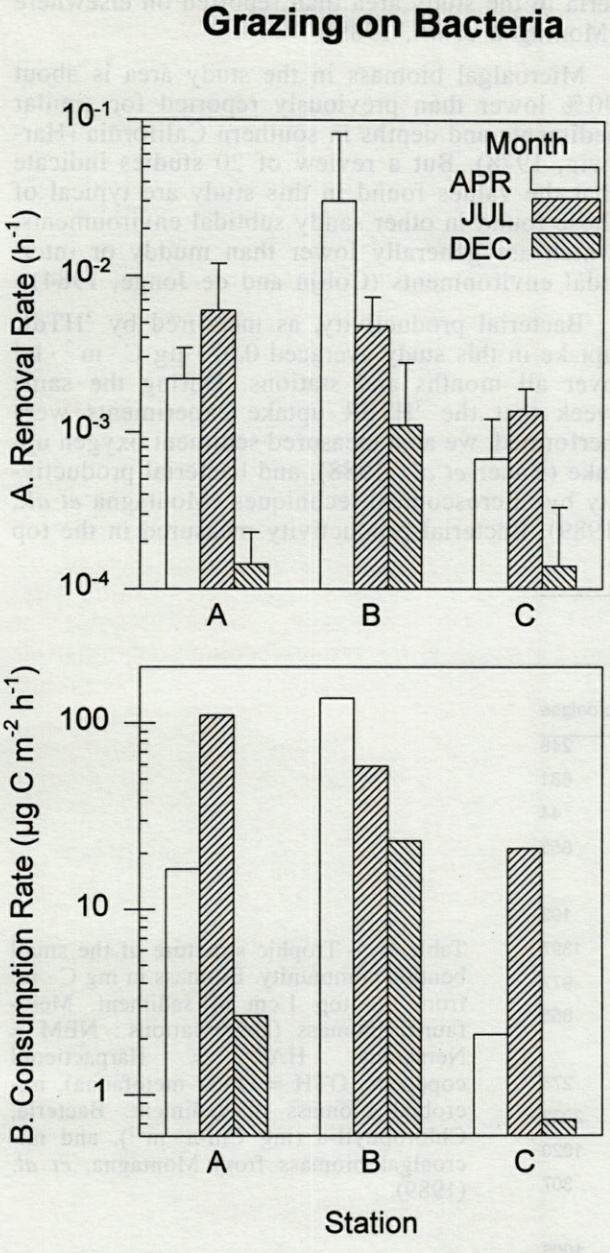

Fig. 2. – Grazing rate of the total meiofauna community on bacteria. A. The clearance rate (% of bacteria removed h^{-1}). The overall mean grazing rate was 0.520 % h^{-1}, and the C.V. was 333 %. B. The amount of bacterial biomass consumed by the total meiofauna community (µg C · m^{-2} · h^{-1}). The overall mean grazing rate was 41.2 µg C · m^{-2} · h^{-1}.

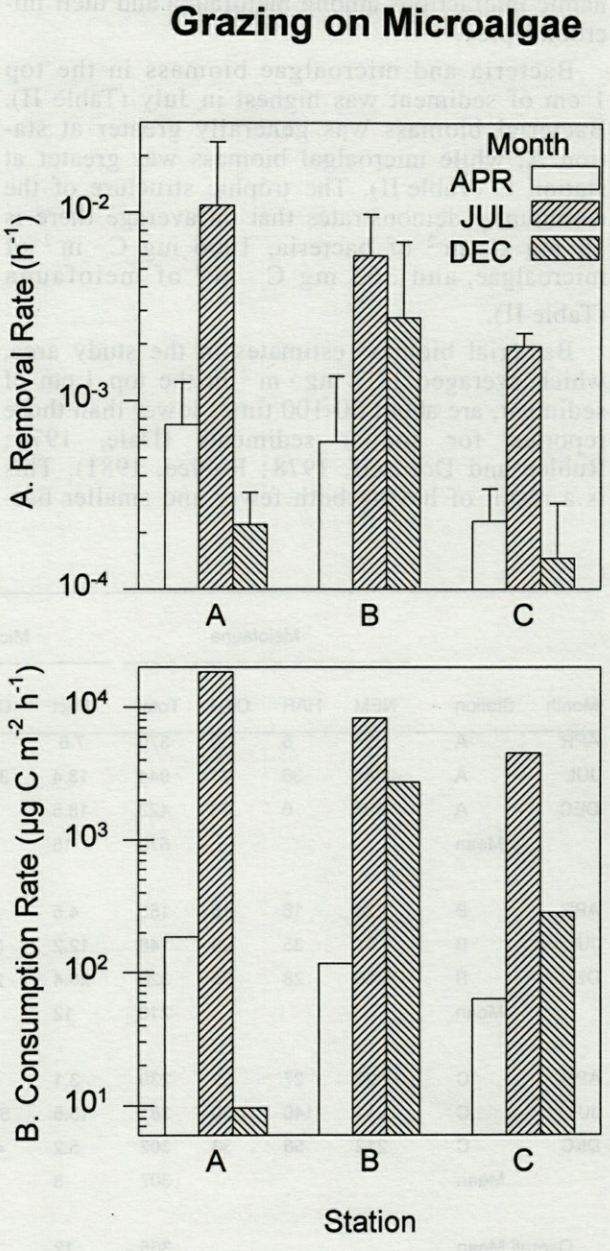

Fig. 3. – Grazing rate of the total meiofauna community on benthic microalgae. A. The clearance rate (% of microalgae removed h^{-1}). The overall mean grazing rate was 0.262 % h^{-1}, and the C.V. was 180 %. B. The amount of microalgal biomass consumed by the total meiofauna community (µg C · m^{-2} · h^{-1}). The overall mean grazing rate was 3840 (µg C · m^{-2} · h^{-1}).

DISCUSSION

Microbial biomass and productivity

Many aspects of microbial biomass and productivity were previously studied. These studies include *Beggiatoa* mats (Montagna and Spies, 1985), benthic metabolism (Montagna *et al.*, 1986), microbial biomass (Montagna *et al.*, 1987; 1989), and microbial production (Montagna *et al.*, 1989; Bauer *et al.*, 1988). A review of these studies and other published studies allows us to compare meiofaunal feeding behavior with microbial dynamics. This is required to assess the dynamics of the "small benthic food web," i.e., trophyc dy-

namic interactions among meiofauna and their microbial prey.

Bacteria and microalgae biomass in the top 1 cm of sediment was highest in July (Table II). Bacterial biomass was generally greater at station A, while microalgal biomass was greater at station C (Table II). The trophic structure of the community demonstrates that on average there is 12 mg C · m^{-2} of bacteria, 1006 mg C · m^{-2} of microalgae, and 365 mg C · m^{-2} of meiofauna (Table II).

Bacterial biomass estimates in the study area, which averaged 11.7 mg · m^{-2} in the top 1 cm of sediment, are about 50-100 times lower than those reported for similar sediments (Dale, 1974; Rublee and Dornseif, 1978; Rublee, 1981). This is a result of having both fewer and smaller bacteria in the study area than reported on elsewhere (Montagna *et al.*, 1989).

Microalgal biomass in the study area is about 30% lower than previously reported for similar sediments and depths in southern California (Hartwig, 1978). But a review of 20 studies indicate that the values found in this study are typical of those found in other sandy subtidal environments, which are generally lower than muddy or intertidal environments (Colijn and de Jonge, 1984).

Bacterial productivity, as measured by ^{3}HTdR uptake in this study averaged 0.073 µg C · m^{-2} · h^{-1} over all months and stations. During the same week that the ^{3}HTdR uptake experiments were performed, we also measured sediment oxygen uptake (Bauer *et al.*, 1988), and bacterial productivity by microscopical techniques (Montagna *et al.*, 1989). Bacterial productivity measured in the top

		Meiofauna				Microbes		
Month	Station	NEM	HAR	OTH	Total	Bact	Chl-a	Microalgae
APR	A	354	6	10	370	7.6	5.6	248
JUL	A	887	36	21	944	18.4	37.6	681
DEC	A	395	6	23	423	18.5	1.0	44
	Mean				579	15		658
APR	B	124	16	15	155	4.5	4.3	192
JUL	B	91	35	23	148	12.2	31.3	1397
DEC	B	265	28	35	328	20.4	21.9	977
	Mean				210	12		855
APR	C	190	27	22	239	3.1	6.2	275
JUL	C	211	140	30	381	15.5	51.9	2322
DEC	C	212	58	33	302	5.2	43.0	1920
	Mean				307	8		307
Overall Mean					365	12		1006

Table II. – Trophic structure of the small benthic community. Biomass in mg C · m^2 from the top 1 cm of sediment. Meiofaunal biomass (abbreviations : NEM = Nematoda, HAR = Harpacticoid copepods, OTH = Other meiofauna), microbial biomass of sediment. Bacteria, Chlorophyll-a (mg Chl-a · m^{-2}), and microalgal biomass from Montagna, *et al.* (1989).

		Heterotrophy		Chemoautotrophy		Photoautotrophy	
Month	Station	Mean	STD	Mean	STD	Mean	STD
APR	A	2085	2275	4.13	3.10	2.93	5.53
APR	B	3847	2914	1.45	0.60	0.85	1.09
APR	C	964	1454	1.73	1.42	0.08	1.70
JUL	A	4718	3879	36.77	2.63	0.07	5.85
JUL	B	1776	2270	2.00	1.01	26.50	2.12
JUL	C	1449	2167	2.54	0.51	2.02	0.98
DEC	A	8620	5109	2.61	3.02	7.76	3.22
DEC	B	32794	9512	5.32	4.05	7.43	7.73
DEC	C	13241	5351	1.63	1.77	8.08	5.78

Table III. – Microbial productivity in the top 1 cm of sediment at the three stations for three months. Bacterial secondary production (µg C · m^{-2} · h^{-1}) measured by the FDC technique (Montagna *et al.*, 1989). Chemoautotrophic production (µg C · m^{-2} · h^{-1}) and microalgal production (µg C · m^{-2} · h^{-1}) was measured by uptake of bicarbonate (Bauer *et al.* 1988).

1 cm of sediment by the microscopical frequency of dividing cells (FDC) technique averaged 7.7 mg $C \cdot m^{-2} \cdot h^{-1}$, 5 orders of magnitude larger than the ^{3}HTdR technique. When oxygen uptake is converted to carbon consumption equivalents, assuming a respiratory quotient of 1.0 (Strickland and Parsons, 1972), calculated total benthic production was on average 42.6 mg $C \cdot m^{-2} \cdot h^{-1}$. Based on oxygen measurements, bacterial production estimates based on FDC seem much more reasonable than the estimates based on ^{3}HTdR uptake and are used for the present study. This is in contrast to Atlantic Ocean estimates, where ^{3}HTdR estimates agreed with oxygen uptake, and FDC was to high (Fallon *et al.*, 1983).

Bacterial production (as measured by the FDC technique) varied over one order of magnitude and increased throughout the study period (Table III). There were differences in bacterial production between months and stations. Production was higher in December (18.2 mg $C \cdot m^{-2} \cdot h^{-1}$) than in July (2.65 mg $C \cdot m^{-2} \cdot h^{-1}$) or April (2.30 mg $C \cdot m^{-2} \cdot h^{-1}$) which were the same (Tukey multiple comparison test).

By dividing the standing stock ($\mu g\ C \cdot m^{-2}$) by the production rate ($\mu g\ C \cdot m^{-2} \cdot h^{-1}$), we can calculate the turnover time (h). The turnover time for the FDC technique is 1.5 h. The FDC rates appear to be high. Other studies indicate that bacterial turnover times range from .75 to 21 days (Moriarty and Pollard, 1982; Riemann *et al.*, 1984).

Autotrophic production was calculated from bicarbonate uptake rates (Bauer *et al.*, 1988). Samples were incubated in the dark and under simulated in situ light conditions (180 $\mu E \cdot m^{-2} \cdot s^{-1}$). It is assumed that dark uptake is by heterotrophic and chemosynthetic bacteria, and light mediated uptake is by photosynthetic microalgae.

Chemosynthetic production was generally around 3 $\mu g\ C \cdot m^{-2} \cdot h^{-1}$, except for one large value in July at station A (37 $\mu g\ C \cdot m^{-2} \cdot h^{-1}$) (Table III). Station A had the highest production rate. Chemoautotrophic production averaged 6.46 $\mu g\ C \cdot m^{-2} \cdot h^{-1}$ over all months and stations.

Primary production by benthic microalgae varied over three orders of magnitude during the study period (Table III). There were significant interactions between months and stations (2-way ANOVA, $P = 0.0001$). Production was highest at station A in April, but lowest at A in July. In December all three stations were the same. Production averaged 2.01$\mu g\ C \cdot m^{-2} \cdot h^{-1}$ over all months and stations.

Microalgal productivity in the seep, measured by the ^{14}C technique, is yielding rates about 3 orders of magnitude lower than those found in 20 other studies referred to by Colijn and de Jonge (1984). Microalgae can fix CO_2 in the dark, and this process is correlated with nitrogen limitation (Goldman and Dennett, 1986). Since the C:N ratio increases from stations C to B to A (Bauer *et al.*, 1988), the large amount of dark bicarbonate uptake at the seep may actually be due to microalgae and not to chemosynthetic bacteria. In fact, dark productivity averaged 3 times higher than light productivity. In either case, the grazing technique measures grazing on all autotrophs, i.e., anything that will take up CO_2. If the two are combined, then total autotrophic productivity is only about one order of magnitude lower than previous studies in similar sediments (Colijn and de Jonge, 1984).

To examine the impact of meiofauna on autotrophs, we combine autotrophic production as measured in light and dark incubations. The average standing stock of (1.01 g $C \cdot m^{-2}$) divided by the average productivity (8.47 $\mu g\ C \cdot m^{-2} \cdot h^{-1}$) suggests that the microalgal turnover time is 13.6 years. This is slow. At this rate the meiofauna would soon strip the sediment of all algae. Measurement of photosynthesis in the study area based on oxygen production suggest that the correct values are in the range of 36 mg $C \cdot m^{-2} \cdot h^{-1}$ (Montagna *et al.*, 1986). A summary of trophic interactions (Table IV) suggests seep meiofauna could be limited by autotrophic food sources, even though there is an obvious dependence upon heterotrophic food sources.

Component	Process	Station			Average
		A	B	C	
Heterotrophy	Production	5141	12806	5218	7722
	Grazing	51	63	9	41
Chemoautotrophy	Production	15	3	2	6
Photoautotrophy	Production	4	12	3	6
Total autotrophy	Production	19	15	5	12
	Grazing	6195	3707	1620	3841

Table IV. – Trophic interactions of the small benthic community. Meiofaunal grazing rates, heterotrophic production, and autotrophic production ($\mu g\ C \cdot m^{-2} \cdot h^{-1}$). Average at stations for the top 1 cm of sediment from April, July, and December 1986.

Meiofaunal grazing rates

There are two ways to assess the impact of meiofauna grazing on microbial populations, one is to look at the actual amount of carbon being removed, and the other is to compare meiofaunal grazing rates with microbial turnover times. Comparing the microbial biomass consumed to the amount of biomass available is intrinsically interesting. However, biomass consumed is a function of the amount of biomass available. Comparing the grazing rate to production rates may be more valid, since both measures are arrived at independently.

On average, the total meiofaunal grazing rate on bacteria is 0.00584 h^{-1}. This would require bacteria to have turnover times of 7.1 d to maintain their populations in equilibrium under this grazing pressure. If we use the bacterial turnover times suggested by the FDC technique and literature values (reviewed above), then it is certain that meiofauna will not run out of food. Bacterial production and turnover is more than sufficient to satisfy the demands of the meiofaunal population.

Meiofauna are on average removing microalgae at a rate of 0.00181 h^{-1} (i.e., about 0.2 % per h) suggesting that the algae must turnover every 23 days to maintain their populations under the grazing pressure. In contrast, turnover times for benthic algae fall around 16 hours (Colijn and de Jonge, 1984). Microalgal production estimates, based on oxygen production and consumption, indicate that meiofauna are not grazing so fast that they are limited by microalgal food.

Meiofauna consumed bacteria biomass at a rate of about 2 orders of magnitude less than bacterial carbon is being produced by heterotrophy and chemoautotrophy (Table IV). In contrast, Meiofauna consumed microalgal biomass at a rate that was about 2 orders of magnitute larger than the production rate of microalgal carbon (Table IV). Together, these results indicate that in the organically enriched hydrocarbon seep, there is more than enough bacterial production to maintain meiofaunal populations. However, meiofauna appear to have an insatiable appetite for microalgal carbon.

There are large seasonal differences. In previous seep studies it was noted that April, during the upwelling season, has the lowest bottom temperatures, July has the highest chlorophyll and meiofaunal densities, and December has the lowest chlorophyll and meiofaunal densities (Montagna *et al.*, 1987). Harpacticoids and nematodes had highest grazing rates on microalgae in July. This trend suggests that harpacticoids and perhaps nematodes switch food sources seasonally, selecting for microalgae during blooms in July, and preferring bacteria when microalgae are not abundant. In support of this hypothesis, harapacticoids and nematodes had their highest grazing rate on bacteria during April.

The interactions among meiofauna and the microbial community demonstrate the dichotomy of strategies that are used among organisms in the two different food webs. The heterotrophic food web relies on small, rapidly turning over populations of bacteria. These populations are enhanced at the seep. The autotrophic food web is based on large, slowly turning over populations of microalgae. Regardless, of the importance of heterotrophy at the seep, autotrophy is still an important energy source for meiobenthos in shallow benthic ecosystems where light is sufficient. Even though chemoautotrophy is not important in the top 1 cm of sediment (Table IV), it is probably very important in deeper layers of the sediment, where autotrophy is limited by light. The meiofauna community (about 4 mg C · m^{-2}, Table II) is supported by a flow of carbon from microalgae (about 4 mg C · m^{-2}, Table IV) and bacteria (about 0.04 mg C · m^{-2}, Table IV).

ACKNOWLEDGEMENTS – We thank Shane Anderson, Jim McCullagh, and other members of the Biology Department and Marine Science Institute at the University of California, Santa Barbara for providing logistical support for the field work in this study. Dave Alexander and Jon Toal provided technical assistance for several aspects of this study. This work was supported by the U.S. Department of Interior, Minerals Management Service, Pacific Outer Continental Shelf Office, under contract 14-12-0001-30159 to Kinnetic Laboratories, Inc., and was performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under contract W-7405-ENG-48. This is University of Texas Marine Science Institute Contribution number 925.

REFERENCES

BAUER J.E., P.A. MONTAGNA, R.B. SPIES, M. PRIETO and D. HARDIN, 1988. Microbial biogeochemistry and heterotrophy in sediments of a marine petroleum seep in relation to the mode of organic carbon input. *Limnol. Oceanogr.* **33** : 1493-1513.

COLIJN F. and V.N. DE JONGE, 1984. Primary production on microphytobenthos in the Ems-Dollard estuary. *Mar. Ecol. Prog. Ser.* **14** : 185-196.

DALE N.G., 1974. Bacteria in intertidal sediments : factors related to their distribution. *Limnol. Oceanogr.* **19** : 509-518.

DARO M.H., 1978. A simplified ^{14}C method for grazing measurements on natural planktonic populations. *Helgolander wiss. Meeresunters.* **31** : 241-248.

FALLON R.D., S.Y. NEWELL and C.S. HOPKINSON, 1983. Bacterial production in marine sediments : will cell-specific measures agree with whole-system metabolism ? *Mar. Ecol. Prog. Ser.* **11** : 119-127.

FINDLAY S., J.L. MEYER and P.J. SMITH, 1984. Significance of bacterial biomass in the nutrition of a freshwater isopod (*Lirceus* sp.). *Oecologia* **63** : 38-42.

GOLDMAN J.C. and M.R. DENNETT, 1986. Dark CO_2 uptake by the diatom *Chaetoceros simplex* in response to nitrogen pulsing. *Mar. Biol.* **90** : 493-500.

HARTWIG E.O., 1978. Factors affecting respiration and photosynthesis by the benthic community of a subtidal siliceous sediment. *Mar. Biol.* **46** : 283-293.

JENSEN P., 1982. Diatom-feeding behaviour of the free-living marine nematode *Chromodorita tenuis*. *Nematologica* **28** : 71-76.

LEACH J.H., 1970. Epibenthic algal production in an intertidal mudflat. *Limnol. Oceanogr.* **15** : 514-521.

MONTAGNA P.A., 1983. Live controls for radioisotope food chain experiments using meiofauna. *Mar. Ecol. Prog. Ser.* **12** : 43-46.

MONTAGNA P.A., 1984. In situ measurement of meiobenthic grazing rates on sediment bacteria and edaphic diatoms. *Mar. Ecol. Prog. Ser.* **18** : 119-130.

MONTAGNA P.A. and J.E. BAUER, 1988. Partitioning radiolabeled thymidine uptake by bacteria and meiofauna using metabolic blocks and poisons in benthic feeding studies. *Mar. Biol.* **98** : 101-110.

MONTAGNA P.A., J.E. BAUER, M.C. PRIETO, D. HARDIN and R.B. SPIES, 1986. Benthic metabolism in a natural coastal petroleum seep. *Mar. Ecol. Prog. Ser.* **34** : 31-40.

MONTAGNA P.A., J.E. BAUER, J. TOAL, D. HARDIN and R.B. SPIES, 1987. Temporal variability and the relationship between benthic meiofaunal and microbial populations of a natural coastal petroleum seep. *J. Mar. Res.* **45** : 761-789.

MONTAGNA P.A., J.E. BAUER, J. TOAL, D. HARDIN and R.B. SPIES, 1989. Vertical distribution of microbial and meiofaunal populations in sediments of a natural coastal hydrocarbon seep. *J. Mar. Res.* **47** : 657-680.

MONTAGNA P.A. and R.B. SPIES, 1985. Meiofauna and chlorophyll associated with *Beggiatoa* mats of a natural submarine petroleum seep. *Mar. Environ. Res.* **16** : 231-242.

MORIARTY D.J.W. and P.C. POLLARD, 1982. Diel variation of bacterial productivity in seagrass (*Zostera capricorni*) beds measured by the rate of thymidine incorporation into DNA. *Mar. Biol.* **72** : 165-173.

PALMER M.A., P.A. MONTAGNA, R.B. SPIES and D. HARDIN, 1988. Meiofauna dispersal near natural petroleum seeps in the Santa Barbara Channel : a recolonization experiment. *Oil Chem. Pollut.* **4** : 179-189.

RIEMANN B., P. NIELSEN, M. JEPPESEN, B. MARCUSSEN and J.A. FUHRMAN, 1984. Diel changes in bacterial biomass and growth rates in coastal environments, determined by means of thymidine incorporation into DNA, frequency of dividing cells (FDC), and microautoradiography. *Mar. Ecol. Prog. Ser.* **17** : 227-235.

RIEPER M., 1978. Bacteria as food for marine harpacticoid copepods. *Mar. Biol.* **45** : 337-345.

RIEPER M., 1982. Feeding preferences of marine harpacticoid copepods for various species of bacteria. *Mar. Ecol. Prog. Ser.* **7** : 303-307.

RIEPER M., 1984. Relationships between bacteria and marine copepods. *Bacteriol. Mar.* (University Provence, Editions du CNRS, Paris), 169-172.

ROMAN M.R. and P.A. RUBLEE, 1981. A method to determine in situ zooplankton grazing rates on natural particle assemblages. *Mar. Biol.* **65** : 303-309.

ROMEYN K. and L.A. BOUWMAN, 1983. Food selection and consumption by estuarine nematodes. *Hydrobiol. Bull.* **17** : 103-109.

RUBLEE P. and B.E. DORNSEIF, 1978. Direct counts of bacteria in the sediments of a North Carolina salt marsh. *Estuaries* **1** : 188-191.

RUBLEE P.A., 1981. Bacteria and microbial distribution in estuarine sediments. *In* Estuarine Comparisons. Edited by V.S. Kennedy, Academic Press, Inc., New York, 159-182.

SELLNER B.W., 1976. Survival and metabolism of the harpacticoid copepod *Thompsonula hyaenae* (Thompson) fed on different diatoms. *Hydrobiologia* **50** : 233-238.

SPIES R.B. and P.H. DAVIS, 1979. The infaunal benthos of a natural oil seep in the Santa Barbara channel. *Mar. Biol.* **50** : 227-237.

SPIES R.B., P.H. DAVIS and D.H. STUERMER, 1980. Ecology of a submarine petroleum seep off the California coast. *In* Marine Environmental Pollution, 1. Hydrocarbons. Edited by R.A. Geyer, Elsevier, Amsterdam, 229-263.

SPIES R.B. and D.J. DESMARAIS, 1983. Natural isotope study of trophic enrichment of marine benthic communities by petroleum seepage. *Mar. Biol.* **73** : 67-71.

STRICKLAND J.D.H. and T.R. PARSONS, 1972. A practical handbook of seawater analysis. *J. Fish. Res. Board Can.* Bull. 167, (Second ed.), 1-310.

STUERMER D.H., R.B. SPIES, P.H. DAVIS, D.J. NG, C.J. MORRIS and S. NEAL, 1982. The hydrocarbons in the Isla Vista marine seep environment. *Mar. Chem.* **11** : 413-426.

ZOBELL C.E. and C.B. FELTHAM, 1938. Bacteria as food for certain marine invertebrates. *J. Mar. Res.* **1** : 312-327.

Reçu le 5 septembre 1994 ; received September 5, 1994
Accepté le 24 octobre 1994 ; accepted October 24, 1994

VIE MILIEU, 1995, 45 (1) : 27-38

INFLUENCE OF THE HETEROTROPHIC DINOFLAGELLATE *OXYRRHIS MARINA* ON THE POPULATION DYNAMICS OF *TISBE HOLOTHURIAE*

M. KIRCHNER* & J.-P. GUÉRIN**

**Biologische Anstalt Helgoland, Meeresstation Helgoland, Postfach 180, D-27498 Helgoland, Germany*
***Centre d'Océanologie de Marseille, Université Aix-Marseille II, URA CNRS n° 41, Campus de Luminy, Case 901, F-13288 Marseille Cedex 9, France*

COPÉPODES HARPACTICOÏDES
NUTRITION
DINOFLAGELLÉS

RÉSUMÉ – Le Flagellé hétérotrophe *Oxyrrhis marina* a été proposé au Copépode Harpacticoïde *Tisbe holothuriae* comme complément de différents régimes alimentaires tels que du Tétramin (nourriture pour Poissons d'aquarium), de la matière organique dissoute ou de la levure. Les paramètres pris en considération ont été : la mortalité, le sex ratio, la proportion de femelles ovigères, la production d'œufs et la vitesse de développement des Copépodes. Les résultats montrent que la valeur alimentaire de *O. marina* n'est pas identique pour les Harpacticoïdes et d'autres organismes signalés dans la littérature ; dans certains cas le Dinoflagellé apparaît défavorable pour *Tisbe*. La valeur alimentaire de *O. marina* semble varier selon la souche utilisée et les techniques de culture employées.

HARPACTICOID COPEPOD
FEEDING
DINOFLAGELLATES

ABSTRACT – Experiments are described in which the heterotrophic dinoflagellate *Oxyrrhis marina* was offered as food organism to the harpacticoid copepod *Tisbe holothuriae* under different dietary conditions such as the addition of the pet fish food TetraMin, dissolved organic matter and yeast. Variables measured were the mortality, sex ratio, proportion of ovigerous females, production of eggs and development rate of the copepods. Results indicate that *O. marina* does not have the same food value for harpacticoids, as for other organisms reported in the literature, and in some cases may even be detrimental. The possible influence of different sources and culture techniques of *O. marina* on the outcome of feeding experiments is discussed.

INTRODUCTION

The trophic relations of copepods are important first from an ecological point of view, as they play an essential role in the food web in nature, and secondly for maricultural purposes, as food in the rearing of young fish.

Because of their benthic way of life, the diet of numerous species of harpacticoids will most likely include detritus with its attached microflora as well as the surface growth on other substrates, consisting of bacteria, protozoans and diatoms. A detailed summary of our knowledge on the trophic relations of meiobenthic copepods has been compiled by Hicks and Coull (1983). While evidence has been growing that planktonic copepods may supplement their phytoplankton diets with protozoans, in particular ciliates (Berk *et al.*, 1977; Stoecker & Egloff, 1987; Gifford & Dagg, 1988, 1991; White & Roman, 1992; Sanders & Wickham, 1993; Fessenden & Cowles, 1994), there is still little known about protozoan-harpacticoid relations. *Tisbe furcata* is capable of consuming the flagellates *Monas* sp. and *Parabodo attenuatus* (Kopylov *et al.*, 1981) and the ciliate *Uronema* was taken up by *Tisbe holothuriae* and *Paramphiascella vararensis* in laboratory experiments (Rieper & Flotow, 1981 ; Rieper, 1985). The widespread dinoflagellate *Oxyrrhis marina* has often been observed in healthy cultures of harpacticoid copepods (Kirchner, unpubl.). Klein Breteler *et al.* (1990) showed that the main food requirements of second copepodid and later stages of the calanoid copepod *Temora longicornis* could be supported by *Oxyrrhis*.

The experiments described in this paper are designed to show the influence of different strains of non-axenic dinoflagellate *Oxyrrhis marina* Dujardin cultures on the development of *Tisbe holothuriae* Humes, particularly on the mortality, sex ratio, proportion of ovigerous females and production of eggs, under different dietary conditions.

MATERIAL AND METHODS

This section is divided into two parts, according to the sources of *O. marina* strains used in the experiments : in Part One, from the maceration of organic material with Mediterranean seawater, and in Part Two, with a pure culture originating from the North Sea.

Part one

Experiments

The cultivation techniques of *T. holothuriae* were those described by Gaudy and Guérin (1977). The copepods were cultivated in small, air-tight containers at a temperature of 19-20°C in natural seawater (S = 38‰) under natural illumination. Three successive experiments were performed. For each experiment, several females with large eggsacs, issued from a population fed TetraMin (a commercial pet fish food) in 1 liter aerated seawater, were selected. Each ovigerous female was isolated into a small glass vessel with small pieces of TetraMin. As soon as possible after hatching, the offspring were divided into three (Expt. 1 and 2) or four (Expt. 3) groups in small glass vessels containing filtered (0.45 µm) seawater. Three (Expt. 1 and 2) or four (Expt. 3) series were thus obtained, each series containing 1/3 or 1/4 of the nauplii of each female. Thus, series differed by diet and not by genetical origin, which is an important factor in harpacticoids (Battaglia, 1970).

An overview of the experiments with the different diets is given in Table 1. In each experiment, a series of nauplii was put into 50 ml filtered seawater and fed TetraMin, as control. This food was added at the beginning of an experiment and later whenever necessary to prevent a food deficit. From the fifth day of the experiment, each vessel was observed daily.

Each experiment was ended when the development of eggsacs obtained in the experimental generation was adequate in order to dissect them easily. The first females developing an eggsac were isolated and sacrificed; each vessel was observed during 48 hours to remove each ovigerous female with an eggsac in suitable development. After this period of time, females without eggsacs were regarded as sterile and animals remaining in each vessel were fixed. Females, males and the number of eggs in each eggsac of the ovigerous females were counted.

Cultivation and preparation of *Oxyrrhis marina*

Cultures of *O. marina* were obtained from different sources and offered to the copepods in different diet combinations. Thus the influence of the dinoflagellates could be examined under different conditions (e.g., presence of bacteria, dissolved organic matter).

For Expt. 1, *O. marina* were obtained by maceration of a handful of seagrass (*Zostera* sp.) in 10 l seawater collected simultaneously near Marseille. For this purpose fresh *Zostera* were collected and kept without water in a closed bucket 48 hours before incubation in seawater. Within a few hours bacteria developed; some days later a population (250 individuals ml^{-1}) of *O. marina* was obtained. Before being placed with nauplii of *T. holothuriae, O. marina* were filtered through a 10 µm gauze, to remove other large protists, and eventually diluted. Under these conditions, there were very few protists accompanying *O. marina*, including organic matter issued from the exudation of *Zostera*, with numerous and varied bacteria.

For Expt. 2.1 and 2.2, *O. marina* were obtained by maceration of 5 g ground TetraMin in 10 liters of gently aerated seawater. In this case *O. marina* were also filtered as above.

For Expt. 3, a new maceration of *Zostera* was performed under the same conditions as Expt. 1 and filtered before use. For this purpose, the suspension was filtered over a 5 µm pore filter. The filtrate was without *O. marina*, but with bacteria and organic material.

Experimental conditions (Table I)

In Expt. 1, a series ("Diet 1") received 10 ml of an *O. marina* culture at an approximate density

Table I. – Part One. Overview of the experimental conditions for *Tisbe holothuriae* diets in experiments 1 – 3.

	N. of females	Control	Diet 1	Diet 2	Diet 3
Expt. 1	12	TetraMin	19 O. marina/ml	19 O. marina/ml + Tet	-
Expt. 2.1	12	TetraMin	27 O. marina/ml	54 O. marina/ml	-
Expt. 2.2	9	TetraMin	42 O. marina/ml	85 O. marina/ml	-
Expt. 3	7	TetraMin	Zostera filtrate + Tet	Yeast	Zostera filtrate + Yeast

of 93 ind.ml^{-1}, to which 40 ml filtered seawater was added. The concentration of dinoflagellates was thus about 18.6 ml^{-1} in the vessels. The third series ("Diet 2") received the same amount of *O. marina* and also TetraMin, which was renewed under the same conditions as the control.

Expt. 2.1 and 2.2 began on two successive days, from two different populations of *Tisbe* and fed with two different cultures of *Oxyrrhis* issued from the maceration of TetraMin. In each case "Diet 1" and "Diet 2" received only *O. marina*, but the density in Diet 2 was twice that of Diet 1 (10 ml *O. marina* plus 40 ml filtered seawater in Diet 1, 20 ml *O. marina* plus 30 ml seawater in Diet 2, respectively). The concentration of dinoflagellates in the vessels were thus 26.8 and 53.6 ind.ml^{-1} in Expt. 2.1. and 42.4 and 84.8 ind.ml^{-1} in Expt. 2.2.

In Expt. 3, two comparisons were made : first, between two diets, TetraMin as control and yeast (dried *Saccharomyces cerevisiae*) as Diet 2, each used alone ; secondly, a comparison between these two diets after enrichment of each with 10 ml of a filtered suspension of *Zostera* maceration (Diets 1 and 3). After the fifth day,10 ml from the vessels with Diets 1 and 3 were withdrawn and replaced with freshly prepared juice.

Part two

Organisms and experimental conditions

Five additional experiments were performed with organisms originating from the North Sea. *T. holothuriae,* non-axenic but free of protozoans, was cultivated over many generations in the laboratory with a mixture of TetraMin and a fish food preparation described in Rieper (1978). *O. marina* was obtained from the Culture Collection of Algae and Protozoa CCAP, Scotland, UK (N° 1133/2). The *O. marina* culture was maintained on rice grains and contained unspecified bacteria only. The individual *O. marina* cells were counted directly under a microscope, after suitable dilution of a concentrated suspension.

Experiments were performed in the laboratory in covered glass vessels containing 50 ml autoclaved, 0.45 µm-filtered seawater, S = 30 ‰. The temperature was 20 ± 1.5 °C, with a natural light:dark cycle of 18:6 hours, that of a northern European summer. No aeration or stirring of the vessels took place. The variables measured were development time of *T. holothuriae* to the first copepodid stage, life cycle from nauplius to first new nauplius, sex ratio (defined as total number of females to total number of adults and copepodids at the end of the experiment) and mortality (copepods which died during the experiment excluding nauplii). All vessels were examined daily under a dissecting microscope. Food consisted of a mixture of TetraMin and fish food preparation (except in Expt. 3, with TetraMin alone) and was added as necessary so no deficit occurred.

The experiments performed were as follows :
Expt. 1 : 2 ovigerous females of different ages, from a stock culture, were placed into each vessel, then removed as soon as their eggs hatched. Two replicates contained the copepods alone, and two others received *O. marina*, at an initial concentration in the vessels of 65 ind.ml^{-1}.
Expt. 2 : was performed with the first females to become ovigerous, arising from the progeny of Expt. 1. The initial numbers of mother females in each vessel varied, and were removed as soon as sufficient new nauplii appeared. Thus here all mother females were of the same age. Four replicates contained copepods alone, and four with copepods and *O. marina* added. The concentration of *O. marina* was not determined.
Expt. 3 : 12 healthy ovigerous females of different ages were taken from the stock culture and added singly to each experimental vessel. Six replicates were with copepods alone, six others received *O. marina* added at an initial concentration of 15.6 ind.ml^{-1} per vessel. The nauplii at time zero were thus the offspring of the same eggsac in each vessel.
Expt. 4 : For this experiment, only young mother females arising from the progeny of Expt. 3 were used which carried their first or, at most, their second eggsac. As in Expt. 3, the ovigerous females were placed singly into each vessel. Six replicates received no *O. marina,* and six replicates received them. Since the concentration of *O. marina* at time zero was so low (< C0.10 ind.m-1 per vessel), one rice grain was added to promote its growth in each of 3 replicates, and also to 3 control replicates without *O. marina.*
Expt. 5 : Here the progeny of Expt. 4 were used : as soon as the first new nauplii appeared in all vessels (with the exception of N° 2), all other copepods present were immediately removed and the same vessels retained for Expt. 5. This became the new time zero for Expt. 5. The initial concentration of *O. marina* was not determined ; instead, it was determined at the end of Expt. 5 in each vessel, as soon as new nauplii hatched again. Bacteria counts were also made from an aliquot of seawater from each vessel at the end of the experiment, after suitable dilution. The spread plate method was used with ZoBell 2216E medium for marine bacteria.

Statistics

Comparisons on percent of mortality, sex ratio and proportions of ovigerous females under different conditions were performed with the χ^2 test using the Brandt-Snedecor formula for the entire

data in each experiment. When a significant difference was shown, a 2 × 2 test was used to compare the different groups of data (with the Yates' correction).

Comparisons on the number of adults obtained under different conditions and the mean number of eggs observed were performed with the ANOVA test.

RESULTS

Part one

Experiment 1 (Tables II, III and V) : Comparison of effect on copepods of *O. marina* alone and *O. marina* + TetraMin to controls with TetraMin alone

Mortality. At first view, the mortality appears very important (Table III). With 636 nauplii at the beginning of the experiment, the adults obtained were only 508 : the average mortality rate was thus 20 %. The mortality was greatest with TetraMin alone (29.7 %), less with *O. marina* + TetraMin (19.3 %) and lowest with *O. marina* alone (11.3 %). These differences were very highly significant ($\chi^2 = 22.44$***). Between TetraMin alone and *O. marina* + TetraMin, the difference was very significant ($\chi^2 = 5.61$**). The difference between *O. marina* and *O. marina* + TetraMin was significant ($\chi^2 = 4.65$*), and very highly significant ($\chi^2 = 20.88$***) between *O. marina* and TetraMin alone. The 100 % mortality with the nauplii of female D fed TetraMin alone, as well as only one surviving adult from female H (fed *O. marina* + TetraMin) and female J (fed TetraMin) are the possible result of contamination of the vessels which cannot be further explained.

Sex ratio. Among results obtained with the different groups of nauplii it can be seen (Table II) that, among the 10 groups of the control considered, only 6 gave more females than males; with *O. marina* alone, 6 groups gave more females than males, one gave as many females as males (group H); with *O. marina* + TetraMin (11 groups considered), 8 groups gave more females than males, 2 gave the same number of each (C and L). Sex ratio (number of females/number of adults) was disadvantageous to females with TetraMin (0.48), better for females with *O. marina* (0.53) and very good with *O. marina* + TetraMin (0.65). In Table III one can see that these differences were highly significant ($\chi 2 = 10.61$**). Between TetraMin and *O. marina*, differences were too little to be significant. On the other hand, differences between TetraMin and *O. marina* + TetraMin were highly significant ($\chi^2 = 8.28$**), differences between *O. marina* and *O. marina* + TetraMin were significant ($\chi^2 = 5.04$*).

Table II. – Part One. Results of experiment 1. The letters A-L represent the 12 females used, as stated in Table 1. In each rectangle with data :
— on the upper left : number of females ; middle : number of males ; right : number of adults (surviving from the original nauplii in each treatment, column 2) ;
— on the lower left : number of ovigerous females ; middle : total number of eggs ; right : mean number of eggs per ovigerous female.
In the three rectangles "Σ", sums of the data A-L according to the same order.

♀	Nauplii per treatment	Control TetraMin			Diet 1 19 O. m./ml			Diet 2 19 O.m./ml + Tet		
A	16 X 3	6	9	15	8	7	15	12	3	15
		2	116	58	1	67	67	11	1011	91.9
B	17 X 3	6	10	16	5	12	17	5	8	13
		6	374	62.3	3	188	62.7	2	163	81.5
C	25 X 3	0	19	19	1	16	17	12	12	24
		0	0	0	1	67	67	8	725	90.6
D	18 X 3	0	0	0	13	4	17	12	3	15
		0	0	0	5	284	56.8	10	785	78.5
E	15 X 3	12	2	14	8	4	12	8	6	14
		3	107	35.7	7	376	53.7	6	344	57.3
F	15 X 3	8	6	14	6	8	14	9	6	15
		2	128	64	3	178	59.3	3	288	96
G	18 X 3	8	6	14	13	4	17	5	2	7
		3	132	44	0	0	0	5	314	62.8
H	18 X 3	6	12	18	7	7	14	0	1	1
		0	0	0	0	0	0	0	0	0
I	15 X 3	9	1	10	11	2	13	14	0	14
		0	0	0	2	120	60	0	0	0
J	18 X 3	1	0	1	12	6	18	14	4	18
		0	0	0	5	282	56.4	14	1010	72.1
K	17 X 3	7	6	13	6	9	15	11	6	17
		4	213	53.3	6	544	90.7	10	875	87.5
L	20 X 3	9	6	15	9	10	19	9	9	18
		7	494	70.6	9	807	90.1	5	504	100.8
Σ	212X3	72	77	149	99	89	188	111	60	171
		27	1564	57.9	42	2913	69.4	74	6019	81.3

Proportions of ovigerous females. Among the adult females, only a part became ovigerous. On the whole, with 282 females, only 143 – i.e. 50.7 % – reproduced. Among these, group I, fed *O. marina* + TetraMin, gave 14 females and no males. Three of these females became ovigerous; they were not counted, since, without fecundation, development would be impossible. It is interesting to note the delay observed in the development of females fed *O. marina* compared with females fed TetraMin or *O. marina* + TetraMin. Indeed, egg-sacs appeared the eighth day after the beginning of the experiment for the series fed TetraMin and *O. marina* + TetraMin, whereas two additional days were required to obtain reproduction with *O. marina* only.

In Table III, the proportions of ovigerous females show very significant differences between the three dietary conditions ($\chi^2 = 18.59$***). Pro-

Table III. – Part One. Summary of results of experiment 1. Meaning of statistical symbols : df : degree of freedom. N.S. : non significant. * : significant. ** : highly significant. *** : very highly significant.

	Control TetraMin	Diet 1 19 O. m./ ml	Diet 2 19 O. m. /ml + Tet	Means	Statistics
N. of adults	148	188	170	168.7	F = 0.25 N.S. (df: 2; 30)
% mortality	29.7 ← 20.88***	→ 11.3 ← 4.65*	→ 19.3	20.1	χ 2 = 22.44*** (df: 2)
	5.61**				
Sex ratio	0.48 ← N.S.	→ 0.53 ← 5.04*	→ 0.65	0.55	χ 2 = 10.61** (df: 2)
	8.28**				
% ovi. females	38.0 ← N.S.	→ 42.4 ← 11.48***	→ 66.7	50.7	χ 2 = 18.59*** (df: 2)
	13.24***				
N. of eggs	1564	2913	6019	3498.7	
m/ovi.female	57.9±17.1	69.4±19.2 ← 8.56**	→ 81.3±23.4	69.5	F = 13.15*** (df: 2; 140)
	← 17.74***	→ (Diet1 + Diet 2)			

portions were almost similar with TetraMin and *O. marina* (38.0 and 42.4 % respectively, (χ^2 = 0.24, N.S.) and very highly significantly different in regard to TetraMin vs *O. marina* + TetraMin diet (66.7 %, χ^2 = 13.24***); differences between *O. marina* and *O. marina* + TetraMin were also very highly significant (χ^2 = 11.48***).

Production of eggs. The reproductive output of offspring of 143 ovigerous females gave, in total, 10496 eggs, with a general mean of 73.4 eggs/ovigerous female. Table III shows that females fed TetraMin were the less productive, with 14.9 % of the whole number of eggs, and they exhibited the lowest mean per female; females fed *O. marina*, a little more numerous, gave nearly twice as many eggs (28 % of the total), with a greater mean per female (69.4). Females fed *O. marina* + TetraMin gave 57 % of the total number of eggs, with a much larger mean per female (81.3).

Differences between total number of eggs produced by females under the three conditions were very highly significant : F = 13.15***. Differences between females fed TetraMin and those fed Diet 1 or 2, respectively, were very highly significant (F = 17.74***). The differences between Diet 1 and Diet 2 were also highly significant (F = 8.56**).

Regarding partitioning of the number of eggs in the eggsacs, females fed TetraMin gave 87 eggs at most; with *O. marina* two eggsacs included at least 100 eggs; with *O. marina* + TetraMin 13 eggsacs contained at least 100 eggs, the maximum number being 136 (data not shown).

It is interesting to note that in the series of copepods fed *O. marina* + TetraMin, two groups (A and J) gave 1 011 and 1 010 eggs, respectively (Table II), i.e. 1/3 of the total number of eggs in the series. This result was obtained mainly by a large number of ovigerous females and not by very numerous eggs in the eggsacs. Thus two groups fed *O. marina* + TetraMin (L and F) produced a mean number of eggs per female greater than group A (100.8 and 96 vs 91.9, respectively); 7 groups of ovigerous females produced a larger mean number of eggs than J.

Experiment 2, with replicates 1 and 2 (Tables IV A, B and V) : Comparison of effect of two different strains of *O. marina* obtained under the same conditions, on two different populations of *T. holothuriae*

Mortality. In these two replicates there was no vessel without any survivors as in Expt. 1. However, the two groups of nauplii of female A (Expt. 2.1) fed with *O. marina* produced only 1 descendant each : this double result cannot be considered an experimental mistake, but a physiological consequence of the non-adaptation of these nauplii to the diet.

The mean result is that a fourth of the copepods died in Expt. 2.1 (27.2 % exactly) and only 1 copepod from 8 in Expt. 2.2 (12.61 % exactly). Rates of mortality appeared very highly significant in Expt. 2.1 (χ^2 = 15.91***), and highly significant in Expt. 2.2 (χ^2 = 9.83**).

In Tables IV A and B data show that rates of mortality appeared almost identical with TetraMin (16.8 % in Expt. 2.1 and 18.9 % in Expt. 2.2). With *O. marina*, mortality increased very highly significantly in Expt. 2.1 in comparison with the control (χ^2 = 11.14***), and decreased in Expt. 2.2 (χ^2 = 6.71**).

Between Diet 1 and Diet 2 mortality increased in the same manner in the two replicates, but these increases were too little to be significant. The result was that the difference between the control

Table IV. A. – Part One. Summary of results of experiment 2.1. B. – Part One. Summary of results of experiment 2.2. C. – Part One. Summary of results of experiment 3. For symbols see legend to Table III.

A

	Control TetraMin	Diet 1 27 O.m./ml	Diet 2 54 O.m./ml	Means	Statistics
N. of adults	159	130	128	139	F = 1.71 N.S. (df: 2; 33)
% mortality	16.8 ← 11.14*** →	31.9 ← N.S. →	33	27.2	χ 2 =15.91*** (df: 2)
	← 12.61*** →				
Sex ratio	0.50 ← N.S. →	0.39 ← 11.30*** →	0.61	0.50	χ 2 = 12.16** (df: 2)
	← N.S. →				
% ovi. females	61.3	52,.9	60.3	58.2	χ 2 = 0.99 N.S. (df: 2)
N. of eggs	2637	1010	1792	1813	
m/ovi.female	53.8±22.2	37.4±23.6 ← N.S. →	38.1±22.2	44.2	F = 7.41*** (df: 2; 120)
	← 14.80*** →	(Diet1 + Diet 2)			

B

	Control TetraMin	Diet 1 42 O.m./ml	Diet 2 85 O.m./ml	Means	Statistics
N. of adults	146	164	162	157.3	F = 1.20 N.S. (df: 2; 24)
% mortality	18.9 ← 6.71** →	8.9 ← N.S. →	10	12.6	χ 2 = 9.83** (df: 2)
	← 5.06* →				
Sex ratio	0.25	0.32	0.27	0.28	χ 2 = 2.09 N.S. (df: 2)
% ovi. females	72.2 ← N.S. →	65.4 ← 5.58* →	88.4	75.3	χ 2 = 6.78* (df: 2)
	← N.S. →				
N. of eggs	1492	1826	2848	2055.3	
m/ovi.female	57.4±25.0	53.7±31.6 ← 9.12** →	74.9±31.1	62.9	F = 5.17** (df: 2; 95)
	← N.S. →	(Diet1 + Diet 2)			

C

	Control TetraMin	Diet 1 Zostera filtrate + TetraMin	Diet 2 Yeast	Diet 3 Zostera filtrate + Yeast	Means	Statistics
N. of adults	126	121	115	116	119.5	F = 0.24 N.S. (df: 3; 23)
% mortality	13.1	16.6	20.7	20.0	17.6	χ 2 = 4.59 N.S. (df: 3)
Sex ratio	0.67	0.66	0.67	0.75	0.69	χ 2 = 2.77 N.S. (df: 3)
% ovi. females	68.2	55.0	58.4	57.5	59.8	χ 2 = 3.54 N.S. (df: 3)
N. of eggs	3294	2753	942	3042	2507.8	
m/ovi.female	56.8±23.5	62.6±15.8	20.9±11.1	60.8±24.9	50.3	F = 44.02*** (df: 3;196)
	← 7.02**	(Diet 1 + Diet 2 + Diet 3) →				
		← 62.53*** →				

and diet 2 increased in Expt. 2.1 (χ^2 = 12.61***) and decreased in Expt. 2.2 (χ^2 = 5.06*).

Sex ratio. In Expt. 2.1 and 2.2 data obtained about the sex ratio appeared very different : differencies were highly significant in Expt. 2.1 (χ^2 = 12.16**) and non-significant in Expt. 2.2 (χ^2 = 2.09). With TetraMin, the total number of females and males was identical in 2.1. Conversely, in 2.2 the sex ratio was unfavourable to females : only 0.25. In Expt. 2.1, with Diet 1 the sex ratio was lower than in the control (0.39 vs 0.50 : this difference was not significant), with Diet 2, higher (0.61 vs 0.50, the difference was also not significant). Between Diet 1 and 2, the difference is very highly significant (0.39 vs 0.61, χ = 11.30***).

Both replicates contained *O. marina* in Diets 1 and 2, but in Expt. 2.2, variations in the sex ratio were too little to be significant; they were always unfavourable to females.

Proportions of ovigerous females. In Expt. 2.1, all the control groups included at least one ovigerous female. In groups fed *O. marina* there were 3 groups without ovigerous females in the series fed Diet 1, and 4 in the series fed Diet 2. In Expt. 2.2 two control groups were without females car-

Table V. – Summary of data of the different experiments of Part One. Order of data as in Table II.

	Control			Diet 1			Diet 2			Diet 3		
	TetraMin			19 O. marina/ml			19 O. marina/ml + TetraMin					
Expt 1	72	77	149	99	89	188	111	60	171			
	27	1564	58	42	2913	70	74	6019	81			
	TetraMin			27 O. marina/ml			54 O. marina/ml					
Expt 2.1	80	79	159	51	79	130	78	50	128			
	49	2637	54	27	1010	37	47	1792	38			
	TetraMin			42 O. marina/ml			85 O. marina/ml					
Expt 2.2	36	110	146	52	112	164	43	119	162			
	26	1492	57	34	1826	54	38	2848	75			
	TetraMin			Zostera filtrate + TetraMin			Yeast			Zostera filtrate + Yeast		
Expt 3	85	41	126	80	41	121	77	38	115	87	29	116
	58	3294	57	44	2753	62	45	942	21	50	3042	61

rying eggsacs, only 1 in the series fed Diet 1, and 2 in the series fed Diet 2.

In all series the mean proportions of ovigerous females were high; differences between series were not significant in Expt. 2.1 and significant in Expt. 2.2 (χ^2 = 6.78*). Differences were significant only between Diet 1 and Diet 2 (χ^2 = 5.58*).

In these two replicates the mean value of proportion of ovigerous females was 58.2 % in Expt. 2.1 and 75.3 % in Expt. 2.2; the difference between these proportions is very significant (χ^2 = 8.33**).

Production of eggs. The productivity of the 123 ovigerous females in Expt. 2.1 resulted in 5 439 eggs with a mean of 44.2 eggs per female; differences were very highly significant (F = 7.41***). Females fed TetraMin were the most productive, with 48 % of the amount of eggs, and a mean of 53.8 eggs per ovigerous female. Copepods fed Diet 1 were the least productive (18.6 % of the amount of eggs, 37.4 per female). With Diet 2, females produced a mean number of eggs almost identical to those fed Diet 1 (38), and gave 33 % of the total amount. Regarding the total number of eggs, there was only a very highly significant difference between the control and Diet 1 (F = 14.80***).

In Expt. 2.2 the productivity of 98 ovigerous females gave a total of 6 166 eggs, with a mean of 63 eggs per ovigerous female; differences were highly significant (F = 5.17**). Table IV B shows that females fed TetraMin were the least productive, with 24 % of the whole number of eggs, but they did not exhibit the lowest mean per ovigerous female (57.4). Females fed Diet 1, somewhat more numerous, gave a few more eggs (30 %), but with a lower mean per female (53.7). Females fed Diet 2 gave the greatest amount of eggs (46 %) and a very large mean per female (75). Regarding the total number of eggs, there was only a highly significant difference between Diet 1 and Diet 2 (F = 9.12**).

Experiment 3 (Tables IV C and V) : Effect of yeast and organic matter issued from maceration of *Zostera* on reproduction of *Tisbe*

Mortality. As in the two replicates of Expt. 2 there was no 100 % mortality in any vessel of this experiment. The percentage of mortality was very low in all series; the differences between values were not significant (χ^2 = 4.59).

Sex ratio. The values were remarkably constant in all series; thus differences were not significant (χ^2 = 2.77)

Proportions of ovigerous females. In all groups more than half of the females became ovigerous. The observed variations were of little importance and were not significant (χ^2 = 3.54).

Production of eggs. The 197 ovigerous females gave a total of 10 031 eggs, with a mean of 50.9 eggs per female. Table IV C shows the partitioning of these eggs. Differences were very highly significant (F= 44.02***). The highest value was obtained with TetraMin alone (control) which gave 32.8 % of the amount of the eggs, the lowest with Diet 2 (yeast alone) which gave only 9.4 % of the eggs. Diet 1 (TetraMin + filtrate) gave 27.4 % of the eggs and Diet 4 (yeast + filtrate), 30.3 %. Differences between TetraMin and the three other diets were highly significant (F = 7.O2**). Differences between the three diets among themselves were very highly significant (F = 62.53***).

Part two

The results of 5 experiments with organisms originally isolated from the North Sea are pre-

sented in Table VI. As described above, the experiments differed from one another mainly in the ages of the ovigerous females used at the start and also in the initial concentrations of *O. marina* added to the vessels. The source of *O. marina* and food offered were the same in all cases (except in Expt. 3 whereby only TetraMin was offered, without the fish food preparation). The results are summarized with regard to the following parameters :

Development of T. holothuriae. The time elapsed between the appearance of the first nauplius to the first copepodid stage was generally 2 to 3 days, in rare cases 4. There was no apparent correlation with the presence or absence of *O. marina* at time zero. The vessels in Expt. 4 received the lowest concentrations of *O. marina* (< 10 ind.ml^{-1}), the highest *O. marina* concentrations were achieved in the vessels of Expt. 4 and 5 after rice grains had been added (see below). The length of time required to complete one life cycle (nauplius to nauplius) varied greatly between the five experiments and between the individual vessels of the same experiment, ranging from 9 to 15 days. Shorter average life cycle development times were required where *O. marina* was present in Expt. 1, 4 and 5 : 10 vs 11.5 days in Expt. 1, 11.7 vs 12.6 days in Expt. 4 and 12.2 vs 13.2 days in Expt. 5 (Table VI). Little difference was noted in Expt. 2 (11.0 days with *O. marina*, and 10.5 days without). In contrast, clearly longer development times were required for copepods with *O. marina* in Expt. 3 (13.5 vs 11 days without *O. marina*); here no females reached maturity in 2 out of 6 replicates without *O. marina* and also in 2 out of 6 with *O. marina*. These vessels were not included in the evaluations in Table VI.

Sex ratio. With the exception of Expt. 1 and 3, the sex ratio was higher in vessels which did not contain the dinoflagellates. Noteworthy is the preponderance of males particularly in Expt. 4 and 5, resulting in the relatively low sex ratios. In vessels with rice grains added to enhance the growth of *O. marina*, the sex ratio was lower in Expt. 4 and higher in Expt. 5, compared to controls without *O. marina*.

Proportion of ovigerous females. The numbers of ovigerous vs non-ovigerous females are given in Table VI. The percentage of females with eggsacs compared to the total number of females varied greatly. The greatest differences were found in Expt. 1 ; 36.8 % in the controls compared to 86.2 % in vessels with *O. marina* added. In all other experiments, however, lower proportions were found in the vessels with *O. marina*.

Mortality. The mortality rates of copepods (naupliar death excluded) were low in Expt. 1 and 2, in all vessels with and without *O. marina.* In Expt. 4 and 5 the mortality rate ranged from 0 to 7.6 % (mean values are shown in Table VI); in only one case was it higher than 10 % (Expt. 5). Expt. 3 was an exception, with an average rate of 11.8 % in vessels without *O. marina* and 31.4 % with *O. marina*. The high mortality rates as well as the large number of vessels in which no females produced eggsacs indicate that the conditions for growth of *Tisbe* in Expt. 3 were extremely unfavourable.

Bacteria. The numbers of heterotrophic bacteria (cfu) were determined at the end of Expt. 5 only.

Table VI. – Part Two. Overview of results of 5 experiments with *Tisbe holothuriae* under different dietary conditions. Symbols used : Tet : TetraMin. m : mean value. N : nauplius stage. C : copepodid. Σ : sum. * : 2 replicates of each treatment were not evaluated; see text.

Experiment N°	Vessel number	Diet	Days to first C	Days to complete life cycle (N to N)	Number of animals alive at end of experiment					Sex ratio	% mortality
					Females		Males	C	Total		
					ovi	non ovi					
			(m)	(m)	(Σ)	(Σ)	(Σ)	(Σ)	(Σ)	(m)	(m)
1	2	Tet + fishfood	2.5	11.5	7	12	45	0	64	0.284	0
	2	Tet + fishfood + O. marina	2.5	10	25	4	29	0	58	0.504	0
2	4	Tet + fishfood	2.5	10.5	157	31	219	7	414	0.463	0
	4	Tet + fishfood + O. marina	2.5	11	147	76	482	13	718	0,337	0.14
3	4*	Tet + fishfood	2.5	11	13	12	72	1	98	0.271	11.8
	4*	Tet + fishfood + O. marina	2.75	13.5	10	11	56	0	77	0.279	31.4
4	3	Tet + fishfood	3	12	25	6	118	3	152	0.192	2.67
	3	Tet + fishfood + rice	2.67	13	15	2	150	0	167	0.087	1.47
	3	Tet + fishfood + O. marina	2.67	11.7	15	13	133	7	168	0.172	2.87
	3	Tet + fishfood + O.m.+rice	3	11.7	9	3	188	0	200	0.058	3.40
5	2	Tet + fishfood	3	13	65	12	270	19	366	0.198	1.25
	3	Tet + fishfood + rice	3	13.3	25	14	81	3	123	0.327	5.33
	3	Tet + fishfood + O. marina	3	12.5	22	10	168	13	213	0.104	2.83
	3	Tet + fishfood + O.m.+rice	3	12	19	14	124	1	158	0.247	2.67

Results gave the following concentrations :
Replicates with TetraMin + fish food : 10.7 and 0.91×10^6 ml^{-1} ;
TetraMin + fish food + rice : 1.02 and 1.6×10^6 ml^{-1}, and 2.3×10^5 ml^{-1} ;
TetraMin + fish food + *O. marina* : 1.1 and 0.83×10^6 ml^{-1} (not determined in one replicate) ;
TetraMin + fish food + *O. marina* + rice : 3.6, 4.3 and 2.3×10^6 ml^{-1}.

The bacteria numbers were thus consistently highest in the three replicates which contained *O. marina* and rice grains to enhance its growth. The extremely high bacteria counts in one of the two replicates with TetraMin and fish food cannot be explained ; in this vessel, however, a larger number of copepods had developed, than in all other vessels of this experiment.

Numbers of O. marina. The concentrations of *O. marina* in the 5 experiments of Part Two were as follows :
Expt. 1 : 65 ind.ml^{-1} per vessel at time zero
Expt. 2 : not determined
Expt. 3 : 15.6 ind.ml^{-1} at time zero
Expt. 4 : < 10 ind.ml^{-1} at time zero
Expt. 5 : not determined at time zero ; the same vessels used in Expt. 4 were also used for Expt. 5, after removal of the first generation of copepods but with the *O. marina* left in. The numbers of *O. marina* at the end of Expt. 5 were, in the replicates with TetraMin + fishfood, 54, 81 and 68 ind.ml^{-1}, and in vessels with rice added to these, 995, 778 and 617 ind.ml^{-1}.

Summary of results of Part Two. The source and treatment of *O. marina* was the same in all five experiments, so differences in results were not due to differences in culture techniques. The accompanying bacterial populations would thus not be expected to differ widely, nor did variations in their numbers appear to affect the parameters measured, e.g. in Expt. 5.

The relatively higher concentrations of *O. marina* used in Expt. 1 and 5 were neither of advantage nor disadvantage to the growth and development of *Tisbe*, when the parameters are compared to vessels with low or no numbers of *O. marina* present. A comparison of Expt. 4 and 5 indicates that the presence of *O. marina* may have been detrimental, with regard to the percentage of ovigerous females (of the total females) and the sex ratio, under the given conditions.

Considering all five experiments as a whole, only in one case were positive results obtained in the presence of the flagellates. In the other cases, a tendency to a negative influence on the copepods cannot be excluded, whether *O. marina* here were actually ingested or rejected as food organisms. The different ages of the mother females used were not a determining factor in the results.

DISCUSSION

The dinoflagellate *Oxyrrhis marina* is ubiquitous in nature. In the laboratory, it has been successfully cultivated with yeast (Droop, 1953) and various species of algae (Goldman *et al.*, 1989, and many others) as well as in seawater with rice grains and the accompanying bacteria (this paper). *O. marina* has itself been used as food for Bryozoans (Schneider, 1959 ; Jebram, 1968 ; Kitamura & Hirayama, 1984). Larval stages of the calanoid copepod *Temora longicornis* are able to ingest *O. marina* (Klein Breteler *et al.*, 1990). Laboratory cultures of *Acartia clausi*, although fed sufficient algae, failed in brood production when *O. marina* was lacking in their diet (Klein Breteler, 1980). Despite this evidence for the food value of *O. marina* in the experiments described here in Part Two, the presence of the dinoflagellates was generally not beneficial to *Tisbe holothuriae*, although the sex ratio was greater with *O. marina* in Expt. 1.

The results of Part One, on the oher hand, are often very different with regard to possible effects of *O. marina*, according to the manner of production of the dinoflagellates – whether from maceration of *Zostera* or from TetraMin. These results are discussed as follows.

Mortality

In the controls, mortality was similar between the different experiments. These values were always lower than the rates observed in preceding experiments with TetraMin alone (Gaudy and Guérin, 1977). The absence of significant variations between the control and the different conditions of Expt. 1 and 3 permits the conclusion that mortality was not modified by maceration of *Zostera* alone, nor maceration with addition of TetraMin, nor by addition of the filtrate of this maceration without *O. marina*.

In Expt. 2, however, very highly significant differences appeared on the one hand, in the replicate 2.1, between the control and Diet 1 – mortality being greater with Diet 1 ; and on the other hand, in the replicate 2.2, also between the control and Diet 1, but with a mortality rate lower with Diet 1 than with the control. Thus the strain of *O. marina* issued from maceration of TetraMin may be able to induce very different results in comparison with that from maceration of *Zostera*.

Sex ratio

The reason for determination of sex in copepods is still generally unknown. In the genus

Tisbe numerous factors have been discussed, but none of them can explain observed variations (Guérin and Rieper-Kirchner, 1991). In the controls, the mean values of the sex ratio were 0.48, 0.50, 0.25 and 0.67 for the different experiments, respectively (Part One). In Expt. 2.2 and 3 no significant differences could be observed between the controls and treatments. But in Expt. 2.2, the sex ratio was always unfavourable to females, and just the opposite in Expt. 3. In these two cases it seems possible to assume that experimental conditions did not act on sex ratio values – or, in other words, the uniformity of the results reflects more the action of genetical characteristics which took precedence over experimental conditions. In Expt. 1, however, there was a highly significant difference between the TetraMin control (0.48) and Diet 2 (*O. marina* + TetraMin) (0.65). Here it seems possible to assume that the experimental conditions took precedence over the genetical factors.

Proportion of ovigerous females

Only a part of the produced females became ovigerous; this proportion has a direct effect on the potentiality of the population to grow. Between the controls, variations in the number of ovigerous females appeared important: 38.0, 61.3, 72.2 and 68.2 % respectively, in the different experiments. Under the various conditions, the only noticeable differences appeared in Expt. 1: these were highly significant between the control and Diet 2 (*O. marina* + TetraMin) and also between Diet 1 (*O. marina* alone) and Diet 2.

Production of eggs

The production of eggs is the most important parameter for the development of a population. This parameter is directly under the influence of genetical factors (Battaglia, 1970), but numerous works have shown that both quality and quantity of food can significantly affect offspring production in harpacticoids (Harris, 1973, 1977; Heinle *et al.*, 1977; Gaudy and Guérin, 1977; Uhlig, 1984; Schwenzer, 1985; Wen Yuh Lee *et al.*, 1985; Guérin and Rieper-Kirchner, 1991).

Between controls, variations in the mean numbers of eggs produced by females appeared of little importance (57.9, 53.8, 57.4 and 56.8 for the different experiments, respectively); these values are very similar to the mean value observed by Gaudy and Guérin (1977): 58.2. If one considers the mean numbers of eggs produced in each experiment, variations appear noticeable between the experiments (3 499, 1 813, 2 055 and 2 508, respectively). Statistically, however, in each experiment variations in the total number of eggs produced appear of little importance; there were only 3 cases with significant variations: increase between the control and Diet 2 (*O. marina* + TetraMin) in Expt. 1, decrease between the control and Diet 1 (*O. marina* alone) in Expt. 2.1, and the decrease between the control and Diet 2 (yeast alone) in Expt. 3. In Expt. 1, where there were large differences between groups, this result of the statistical evaluation seems to be a consequence of the dispersion of values. There were cases in which either no males or females were produced in the progeny fed the different diets. On the other hand, it has already been mentioned that two groups alone in Expt. 1 (fed Diet 2, *O. marina* + TetraMin) produced more than 1 000 eggs each.

From these results it can be concluded that *O. marina* did not represent the same trophic value for the harpacticoid *Tisbe holothuriae,* as for calanoid copepods and other organisms reported in the literature. Possibly *O. marina* was partially or totally rejected as food, under the conditions offered. Variations in the concentrations of *O. marina* with or without TetraMin added, presence of dissolved organic matter from the macerations, the accompanying bacteria and small protists, all did not prove to be of consistent reproducible advantages to *Tisbe*, as compared to controls with standard laboratory food. However, although care was taken that food offered was in excess, uncontrolled variations in its components may have affected the reproductive output of *Tisbe*.

Among other factors, different culture techniques may conceivably be an important element in determining the effect of flagellates on copepods: changes in the composition of the prey organism could influence the outcome of feeding experiments. The food quality of an organism, namely, comprises not only its size, morphology and motility, but also its chemical composition, e.g., storage of starches and oils (Gifford & Dagg, 1991, and literature therein). This may have been a factor in the results of experiments by Kitamura and Hirayama (1984). These authors gave *O. marina* as food organism to the bryozoan *Bugula neritina*; they used two different strains of flagellates, the first one ("D type") fed on *Dunaliella tertiolecta* cultured in a modified Erdschreiber medium, the second ("Y type") was cultured on this medium enriched with yeast extract. With "Y type" they observed the formation of 48 zooids and only 27 zooids with "D type", after ten days' culture.

It is thus conceivable that not only the particulate food of a designated prey organism but also the dissolved organic matter in the medium could influence its chemical composition and thus its food value; in addition to phytoplankton and microorganisms, *O. marina* is also capable of meeting most of its nutritional requirements by

osmotrophy (Provasoli, 1977). Conceivably also the faeces of this flagellate could play a role in feeding experiments (Elbrächter, 1991 ; Barlow *et al.*, 1988).

If the trophic relations of copepods and protozoans are greatly influenced by culture techniques, this could have interesting implications regarding food web and microbial loop studies. Copepods ingesting *O. marina* at high rates in nature could reduce the grazing pressure of these protozoans on algae and enhance an algal bloom (Hansen *et al.*, 1993). *O. marina* is considered a dinoflagellate, many representatives of which are capable of causing red tide phenomena. Some members of this group are poisonous. Therefore it is important to understand what factors may influence the relationship of dinoflagellates with other organisms in the food web.

REFERENCES

BARLOW R.G., P.H. BURKILL and R.F.C. MANTOURA, 1988. Grazing and degradation of algal pigments by marine protozoan *Oxyrrhis marina*. *J. Exp. Mar. Biol. Ecol.* **119** : 119-129.

BATTAGLIA B., 1970. Cultivation of marine copepods for genetic and evolutionary research. *Helgoländer wiss. Meeresunters.* **20** : 385-392.

BERK S.G., D.C. BROWNLEE, D.R. HEINLE, H.J. KLING and R.R. COLWELL, 1977. Ciliates as a food source for marine planktonic copepods. *Microbial Ecology* **4** : 27-40.

DROOP M.R., 1953. Phagotrophy in *Oxyrrhis marina* Dujardin. *Nature* **172** : 250-251.

ELBRÄCHTER M., 1991. Faeces production by dinoflagellates and other small flagellates. *Mar. Microb. Food Webs* **5** : 189-204.

FESSENDEN L. and T.J. COWLES, 1994. Copepod predation on phagotrophic ciliates in Oregon coastal waters. *Mar. Ecol. Prog. Ser.* **107** : 103-111.

GAUDY R. et J.-P. GUERIN, 1977. Dynamique des populations de *Tisbe holothuriae* (Crustacea : Copepoda) en élevage sur trois régimes artificiels différents. *Mar. Biol.* **39** : 137-145.

GIFFORD D.J. and M.J. DAGG, 1988. Feeding of the estuarine copepod *Acartia tonsa* Dana : carnivory vs herbivory in natural microplankton assemblages. *Bull. mar. Sci.* **43** : 458-468.

GIFFORD D.J. and M.J. DAGG, 1991. The microplankton-mesoplankton link : consumption of planktonic protozoa by the calanoid copepods *Acartia tonsa* and *Neocalanus plumchrus* Murukawa. *Mar. Microb. Food Webs* **5** : 161-177.

GOLDMAN J.C., M.R. DENNETT and H. GORDIN, 1989. Dynamics of herbivorous grazing by the heterotrophic dinoflagellate *Oxyrrhis marina*. *J. Plankton Res.* **11** : 391-407.

GUERIN J.-P. and M. RIEPER-KIRCHNER, 1991. Influence of three bacteria strains on the population dynamics of *Tisbe holothuriae* (Copepoda, Harpacticoida). *Helgoländer Meeresunters.* **45** : 493-511.

HANSEN F.C., M. RECKERMANN, W.C.M. KLEIN BRETELER and R. RIEGMAN, 1993. *Phaeocystis* blooming enhanced by copepod predation on protozoa : evidence from incubation experiments. *Mar. Ecol. Prog. Ser.* **102** : 51-57.

HARRIS R.P., 1973. Feeding, growth, reproduction and nitrogen utilization by the harpacticoid copepod, *Tigriopus brevicornis*. *J. Mar. Biol. Ass. U.K.* **35** : 785-800.

HARRIS R.P., 1977. Some aspects of the biology of the harpacticoid copepod, *Scottolana canadensis* (Willey), maintained in laboratory culture. *Chesapeake Sci.* **18** : 245-252.

HEINLE D.R, R.P. HARRIS, J.F. USTACH and D.A. FLEMER, 1977. Detritus as food for estuarine copepods. *Mar. Biol.* **40** : 341-353.

HICKS G.R.F. and B.C. COULL, 1983. The ecology of marine meiobenthic harpacticoid copepods. *Oceanogr. mar. Biol.* **21** : 67-175.

JEBRAM D.,1968. A cultivation method for saltwater *Bryozoa* and an example for experimental biology. *Atti Soc. Ital. Sci. Natur. Mus. Civico Storia Natur. Milano*, **108** : 119-128.

KITAMURA H. and K. HIRAYAMA, 1984. Suitable food plankton for growth of a bryozoan *Bugula neritina* under laboratory conditions. *Bull. Jap. Soc. Sci. Fish.* **50** : 973-977.

KLEIN BRETELER W.C.M., 1980. Continuous breeding of marine pelagic copepods in the presence of heterotrophic dinoflagellates. *Mar. Ecol. Prog. Ser.* **2** : 229-233.

KLEIN BRETELER W.C.M., N. SCHOGT and S.R. GONZALEZ, 1990. On the role of food quality in grazing and development of life stages, and genetic change in body size during cultivation of pelagic copepods. *J. Exp. Mar. Biol. Ecol.* **135** : 177-189.

KOPYLOV A.I., A.F. PASTERNAK and E.V. MOISEEV, 1981. On consumption of zooflagellates by planktonic organisms. *Okeanologia.* **21** : 375-379. (In Russian, English summary).

PROVASOLI L., 1977. Axenic cultivation. *In* Marine Ecology, Vol. III, Cultivation, Part 3. Edited by O. Kinne, Wiley, Chichester, 1295-1319.

RIEPER M., 1978. Bacteria as food for marine harpacticoid copepods. *Mar. Biol.* **45** : 337-345.

RIEPER M., 1985. Some lower food web organisms in the nutrition of marine harpacticoid copepods : an experimental study. *Helgoländer Meeresunters.* **39** : 357-366.

RIEPER M. and C. FLOTOW, 1981. Feeding experiments with bacteria, ciliates and harpacticoid copepods. *Kieler Meeresforsch. Sonderh.* **5** : 370-375.

SANDERS R.W. and S.A. WICKHAM, 1993. Planktonic protozoa and metazoa : predation, food quality and population control. *Mar. Microb. Food Webs* **7** : 197-223.

SCHNEIDER D., 1959. Der Aufbau der *Bugula* –Tierstöcke und seine Beeinflussung durch Aussenfaktoren. *Biol. Zentralbl.* **78** : 250-283.

SCHWENZER D., 1985. Untersuchungen zur Produktionsbiologie von *Tisbe holothuriae* (Copepoda : Harpacticoidea) bei unterschiedlichen Temperatur-, Salinitäts– und Nahrungsbedingungen. Diss. Univ. Berlin, 198 pp.

STOECKER D.K. and D.A. EGLOFF, 1987. Predation by *Acartia tonsa* Dana on planktonic ciliates and rotifers. *J. Exp. Mar. Biol. Ecol.* **110** : 53-68.

UHLIG G., 1984. Progress in mass culture of harpacticoid copepods for mariculture purposes. *Spec. Publ. Eur. Maricult. Soc.* **8** : 261-273.

WEN YUH LEE, X.K. ZHANG, C. VAN BAALEN and C.R. ARNOLD, 1985. Feeding and reproductive performance of the harpacticoid *Tisbe carolinensis* (Copepoda, Crustacea) in four algal cultures. *Mar. Ecol. Prog. Ser.* **24** : 273-279.

WHITE J.R. and M.R. ROMAN, 1992. Egg production by the calanoid copepod *Acartia tonsa* in the mesohaline Chesapeake Bay : the importance of food resources and temperature. *Mar. Ecol. Prog. Ser.* **86** : 239-249.

Reçu le 27 juillet 1994; received July 27, 1994
Accepté le 9 septembre 1994; accepted September 9, 1994

VIE MILIEU, 1995, 45 (1) : 39-48

EVALUATION OF ABYSSAL METAZOAN MEIOFAUNA FROM A MANGANESE NODULE AREA OF THE EASTERN SOUTH PACIFIC

C. BUSSAU, G. SCHRIEVER & H. THIEL

Institut für Hydrobiologie und Fischereiwissenschaft der Universität Hamburg, Zeiseweg 9, 22765 Hamburg, Germany

SOUTH-EAST PACIFIC
DEEP SEA
MANGANESE NODULE AREA
METAZOAN MEIOFAUNA
FEEDING ECOLOGY

ABSTRACT – The metazoan meiofauna from a manganese nodule area of the eastern South Pacific (Peru Basin) was investigated. Nodule coverage in this area ranged from 5-30 % with many highly mammillated, botryoidal nodules exceeding 10 cm in diameter. Metazoan meiofaunal density in the sediment was about 147 ind./10 cm^2. The predominant taxa in the sediment (excluding Foraminiferida) were Nematoda (82 %) and Harpacticoida (15 %); other taxa occurred sporadically (3 %). While breaking-up some of the manganese nodules during an inspection for epifauna, a crevice fauna was discovered, living in the sediment-filled crevices and cracks between the mammillae. Metazoan meiofaunal density in the nodules was about 116 ind./nodule (diameter 8-12 cm) or 29 ind./10 cm^2 nodule plane, respectively. The predominant taxa in the nodules were Nematoda (89 %) and Harpacticoida (10 %); other taxa were very rare (1 %). Most of the nematode species (66 %) from this manganese nodule area possess minute buccal cavities without teeth; these nematodes are assumed to feed on bacteria and bacterial secretions. Many nematode species build sediment tubes and structure the uppermost sediment layer.

PACIFIQUE SUD-EST
GRANDS FONDS
AIRES À NODULES DE MANGANÈSE
MÉTAZOAIRES MÉIOFAUNIQUES
ÉCOLOGIE DE LA NUTRITION

RÉSUMÉ – Les Métazoaires de la méiofaune d'une aire à nodules de manganèse du Pacifique sud-est (Bassin de Pérou) ont été étudiés. Les nodules couvrent de 5 à 30 % de cette aire avec des formes mamelonnées et botriloïdes dépassant 10 cm de diamètre. La densité des Métazoaires méiofauniques du sédiment est d'environ 147 ind./10 cm^2. Les taxons dominants du sédiment sont les Nématodes (82 %) et les Harpacticoïdes (15 %) à l'exclusion des Foraminifères. Les autres taxons restent sporadiques (3 %). La fragmentation de quelques nodules de manganèse pendant la recherche de l'épifaune a permis de mettre en évidence une faune de crevasse vivant dans le sédiment qui remplit les crevasses et les fentes entre les mamelons. La densité des métazoaires de la méiofaune dans les nodules est d'environ 116 ind./nodule (diamètre de 8-12 cm) ou 29 individus/10 cm^2 de nodule-plan. Les taxons prédominants dans les nodules sont les Nématodes (89 %) et les Harpacticoïdes (10 %). Les autres taxons sont très rares (1 %). La plupart des espèces de Nématodes (66 %) de cette aire à nodules de manganèse possèdent de minuscules cavités buccales sans dent; ces Nématodes doivent se nourrir de bactéries ou de secrétions bactériennes. De nombreuses espèces de Nématodes construisent des tubes sédimentaires et structurent la strate la plus supérieure du sédiment.

INTRODUCTION

World-wide, nematodes are the predominant metazoan meiofauna (excluding foraminifera) in deep-sea sediments. Meiofaunal associations from manganese nodule areas are known from very few studies (Hessler & Jumars 1974; Mullineaux 1987; Renaud-Mornant & Gourbault 1990; Snider *et al.* 1984; Wilson & Hessler 1987).

In our 'Disturbance and recolonization (DISCOL) experiment in a manganese nodule area of the abyssal eastern South Pacific' we considered nematodes and harpacticoids as indicator taxa (Schriever *et al.* 1991; Thiel *et al.* 1992) assuming that their closer taxonomic study (Bussau 1993) would allow the evaluation of an experimental disturbance. The DISCOL project is the first long-term, large-scale, disturbance-recolonization experiment relating to the environmental effects

of future deep-sea mining (Foell *et al.* 1990, 1992; Thiel & Schriever 1989, 1990).

To date, no published reports exist on the metazoan meiofaunal assemblages from the manganese nodules (nodule endofauna and epifauna). This paper presents information on the metazoan meiofauna from a manganese nodule area; it turns attention to the recently discovered nodule crevice fauna and to the feeding ecology of nematodes.

MATERIAL AND METHODS

Work at sea was carried out in February-March 1989 (Cruise DISCOL 1). The study site was a manganese nodule area in the Peru Basin, centered upon 07°04′40″S-88°27′60″W in water depths of 4100-4200 m. Sediment samples were collected with a multiple corer, manganese nodules with a box corer. The nodules were inspected for epifauna and endofauna. The matèrial was preserved in 4 % formaldehyde-seawater solution. Numbers and percentages were calculated on twenty sediment samples (each 10 cm^3) from different sediment layers (0-1 cm, 1-2 cm, 2-4 cm, 4-6 cm; five samples from each layer) and six nodules (five from the sea floor and one from the underlying sediment). Detailed information on material and methods may be found in Thiel & Schriever (1989) and Bussau (1993).

RESULTS

1. Biotope

The biotope 'manganese nodule area' is constituted of two sub-biotopes : the manganese nodules and the sediment around them (Fig. 1). The metazoan meiofauna populates the nodule surface (nodule epifauna), the sediment-filled crevice systems of the manganese nodules (nodule endofauna) and the sediment (sediment fauna).

Nodule coverage in our study area ranged from 5-30 % with many highly mammillated, botryoidal nodules exceeding 10 cm in diameter. The nodules are of the 'cauliflower type' (Fig. 2). The shape of the nodules (diameter 3-18 cm) is globular to conical. The lower two-thirds of the nodule (the frustum of the cone with the largest diameter) is in the sediment, the upper third (the top of the cone) projects over the sediment surface. The lower surface of the nodules is almost smooth. In contrast, the upper surface is rough and composed of independently growing subnodules (mammillae) with coursely porous to gritty surface textures. By approaching each other through growth, the subnodules build up an irregular and interconnected, sediment-filled labyrinth between them. This constitutes the sediment-filled crevice system. About 95 % of the nodules are found on the sea floor and a few in the underlying sediment. Photographic evidence showed that the nodules were covered with a thin, irregular layer of sediment, concentrated in the recesses between the subnodules, leaving their upper surfaces rather bare.

The sediment showed the following stratification : the 0-6 cm layer (brown) was very soft, the 6-10 cm layer (brown) was semiliquid and the 10-40 cm layer (grey) had a stiff clay consistency.

2. Faunal compositions and densities

2.1. Sediment fauna

The major taxa in the sediment (excluding Foraminiferida) are Nematoda (average 82 %; range 77-87 %) with Harpacticoida (average 15 %; range 11-19 %) second in dominance. Gastrotricha, Kinorhyncha, Loricifera, Tardigrada, Polychaeta and Ostracoda are of sporadic occurrence (average 3 %, range 1-4 %) (Fig. 3 A).

The average density of metazoan meiofauna in the sediment is 147 ind./10 cm^2 (range 76-216 ind./10 cm^2), with the main groups Nematoda (average 121 ind./10 cm^2; range 66-177 ind./10 cm^2) and Harpacticoida (average 22 ind./10 cm^2; range 8-34 ind./10 cm^2). The other meiofaunal groups are very rare (average 4 ind./10 cm^2; range 2-5 ind./10 cm^2) (Fig. 3 B).

2.2. Nodule endofauna

Both, surficial and buried nodules possess a crevice fauna. Nematoda (average 89 %; range 83-100 %) and Harpacticoida (average 10 %; range 0-15 %) are the most dominant taxa inhabiting the nodule crevices; Kinorhyncha, Tardigrada and Polychaeta make up the rest of the metazoan meiofauna (average 1 %, range 0-4 %) (Fig. 3 A).

Meiofaunal density in the manganese nodules (diameter 8-12 cm) averages 116 ind./nodule (range 67-150 ind./nodule), the main groups are Nematoda (average 104 ind./nodule; range 67-124 ind./nodule) and Harpacticoida (average 12 ind./nodule; range 0-23 ind./nodule); the rest of the meiofauna occurs sporadically (2 ind./nodule, range 0-5 ind./nodule) (Fig. 3 B). A slightly conical nodule which has a diameter of 8-12 cm covers about 40 cm^2 of the sediment surface, therefore the meiofaunal density in this sub-biotope is about 29 ind./10 cm^2 nodule plane.

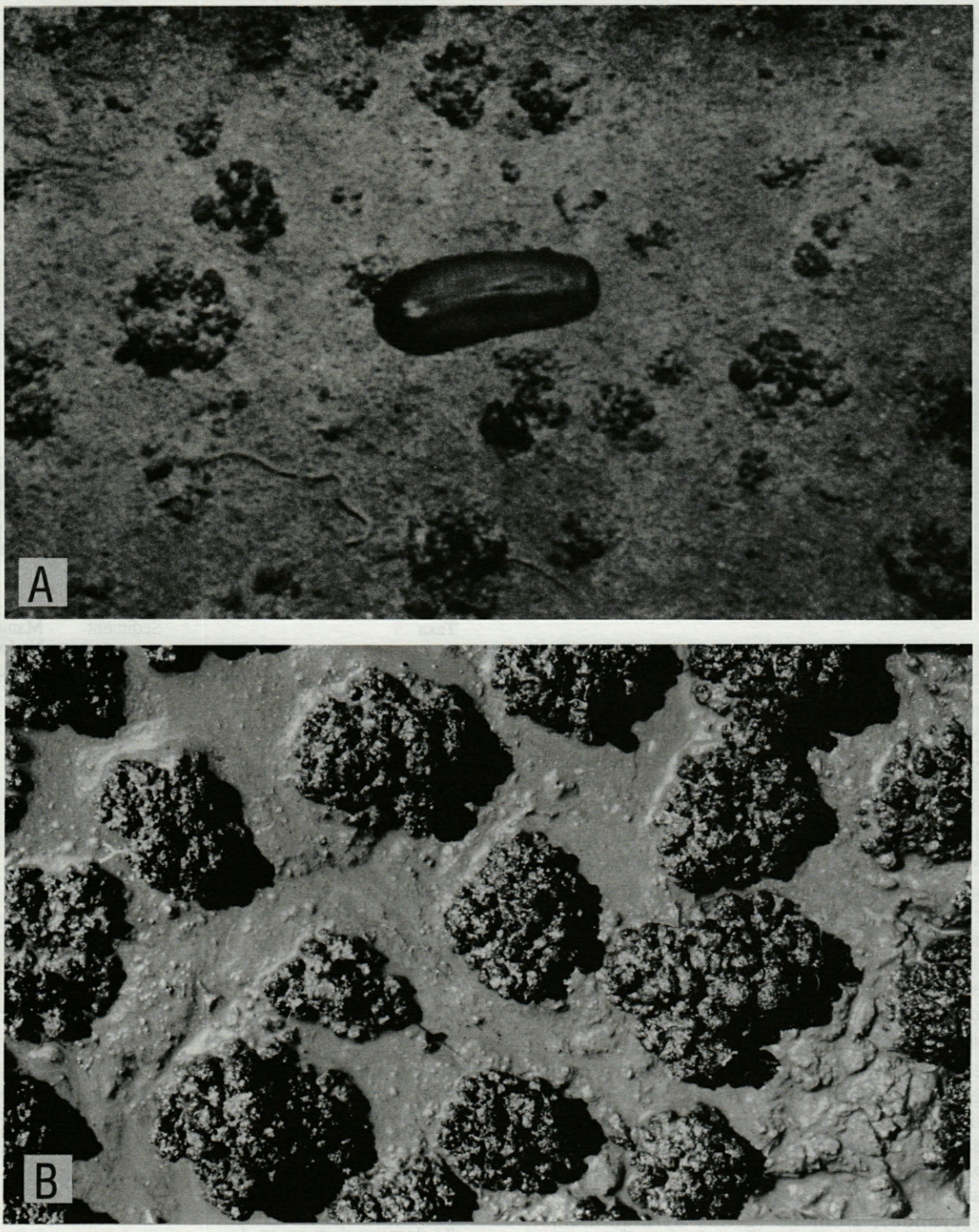

Fig. 1. – A, the manganese nodule area in the Peru Basin, this photo shows manganese nodules, some lebensspuren and a holothuroid; B, a box corer sample with manganese nodules (diameters 5-10 cm).

2.3. Nodule epifauna

Very few nematodes (*Enoploides* sp., *Acantholaimus* sp.) inhabit the thin sediment layer which covers each manganese nodule.

3. Nematoda

The nematodes include about 300 species belonging to some 60 genera; each species has a low density, and nearly all of the species are new

Fig. 2. – A manganese nodule of the 'cauliflower type', diameter 10 cm.

to science, as is typical in newly investigated areas in the deep sea (Bussau 1993).

Distinct differences are encountered between the nodule fauna and the sediment fauna. Thirty-five species have relative abundances ⩾ 1 % of the total number of individuals in one or both of the sub-biotopes. The distribution of these species (Table I) shows their habitat preferences.

The sediment is largely dominated by genera belonging to the following major families : Chromadoridae (genus *Acantholaimus*), Microlaimidae (genus *Microlaimus*), Monhysteridae (genus *Thalassomonhystera*), Xyalidae, Diplopeltidae (genus *Diplopeltula*), Desmoscolecidae (genera *Desmoscolex, Tricoma*), Oxystominidae (genus *Halalaimus*).

The nodules are largely dominated by genera belonging to the following major families : Chromadoridae (genus *Acantholaimus*), Cyatholaimidae (genus *Paracyatholaimus*), Desmodoridae (genus *Molgolaimus*), Leptolaimidae (genus *Camacolaimus*), Monhysteridae (genus *Thalassomonhystera*), Xyalidae, Ironidae (genus *Syringolaimus*), Trefusiidae (genus *Trefusia*).

Thus the Microlaimidae, Diplopeltidae, Desmoscolecidae and Oxystominidae are mainly or solely found in the sediment, whereas the Cyatholaimidae, Desmodoridae, Leptolaimidae, Ironidae and Trefusiidae occur mainly or solely in the nodules.

Table I. – Habitat preferences of 35 nematode species (with relative abundances 1 % of the total number of individuals) from a manganese nodule area of the eastern South Pacific; lines = frequent occurrence, points = sporadic occurrence, without lines or points = no occurrence.

Taxa	Sediment	Manganese Nodules
Acantholaimus sp. 1	---	
Chromadorita sp. 1	---	
Microlaimidae gen. n., sp. 1	---	
Microlaimus sp. 1	---	
Thalassomonhystera sp. 1	---	
Thalassomonhystera sp. 2	---	
Xyalidae gen. n., sp. 1	---	
Desmoscolex sp. 1	---	
Chromaspirina sp. 1	---	.....
Microlaimus sp. 2	---	.....
Diplopeltoides sp. 1	---	.....
Xyalidae gen. n., sp. 2	---	.....
Xyalidae gen. n., sp. 3	---	.....
Thalassomonhystera sp. 3	---	---
Acantholaimus sp. 2	.....	---
Acantholaimus sp. 3	.....	---
Chromadorita sp. 2	.....	---
Cyatholaimidae gen. n., sp. 1	.....	---
Paracanthonchus sp. 1	.....	---
Paracyatholaimus sp. 1	.....	---
Molgolaimus sp. 1	.....	---
Molgolaimus sp. 2	.....	---
Camacolaimus sp. 1	.....	---
Syringolaimus sp. 1	.....	---
Syringolaimus sp. 2	.....	---
Acantholaimus sp. 4		---
Camacolaimus sp. 2		---
Thalassomonhystera sp. 4		---
Thalassomonhystera sp. 5		---
Thalassomonhystera sp. 6		---
Thalassomonhystera sp. 7		---
Theristus sp. 1		---
Syringolaimus sp. 3		---
Trefusia sp. 1		---
Trefusia sp. 2		---

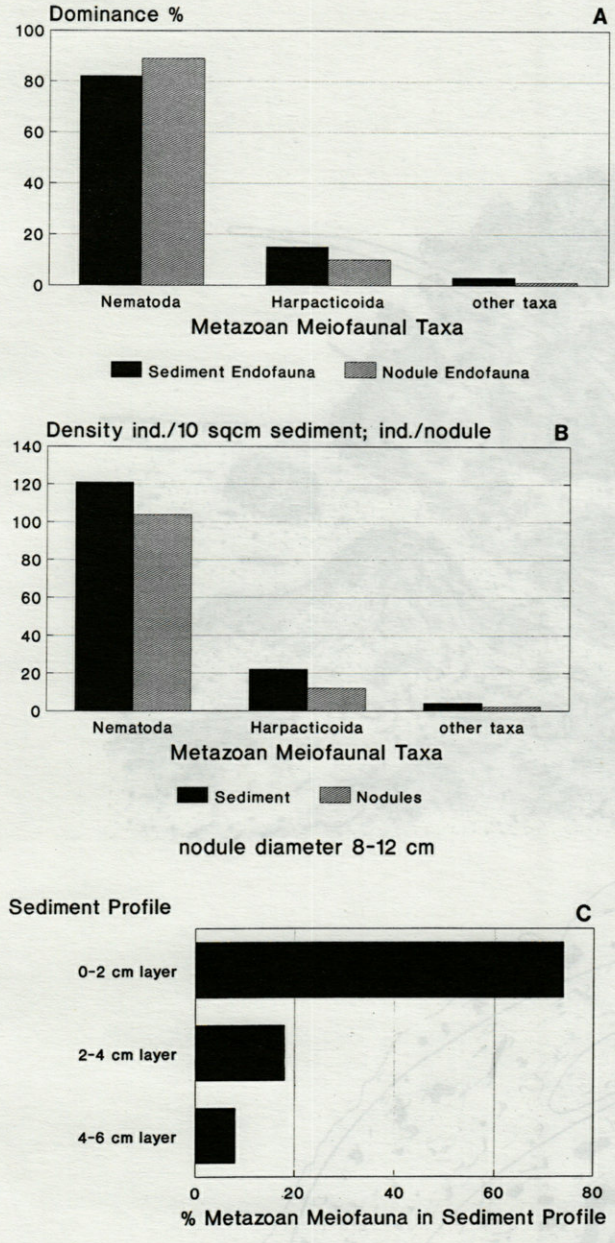

Fig. 3. A, dominance (%) of metazoan meiofaunal taxa in the sediment and manganese nodules of the eastern South Pacific. B, density of metazoan meiofaunal taxa in 10 cm^2 of sediment and per manganese nodule (diameter 8-12 cm). C, vertical distribution of metazoan meiofauna in different sediment profiles.

4. Harpacticoida

The most dominant family in the sediment is the Cletodidae followed by the Ectinosomatidae, Ameiridae and Tisbidae. Also present are the families Diosaccidae, Cerviniidae and Tetragonicipitidae. As in nearly all deep sea investigations most of the species are unknown to science. The description of four new species of the Cletodidae and eight of the Diosaccidae is in preparation.

The harpacticoid specimens of the nodule infauna are very small (< 400 µm) and not yet identified.

5. Tardigrada

The examination (Bussau 1992) revealed three species (*Moebjergarctus manganis, Angursa capsula, A. lingua*), all belonging to the family Halechiniscidae.

6. Vertical distribution

The majority of Nematoda (72%) and Harpacticoida (82%) inhabit the uppermost 0-2 cm sediment layer; 19% of the Nematoda and 14% of the Harpacticoida occur in the 2-4 cm layer; only 9% of the Nematoda and 4% of the Harpacticoida are found in the 4-6 cm sediment layer. The Gastrotricha, Kinorhyncha, Loricifera, Tardigrada, Polychaeta and Ostracoda live exclusively in the 0-2 cm sediment layer. Very few nematodes are found down to 20 cm depth (Fig. 3 C).

7. Horizontal dispersal

The densities of the nematodes in the uppermost 0-1 cm sediment layer vary from 39-107 ind./10 cm^2 (harpacticoids: 8-21 ind./10 cm^2). The meiofauna has a patchy, small-scale spatial distribution. Consequently, attractive spots which are densely populated by meiofauna are assumed to exist in the sediment; in contrast, the meiofauna might avoid unattractive spots. Attractive spots could be present for a short period (minutes, e.g., a particle of food) or for a long time (weeks, months, years, e.g., macrofauna burrows). Many nematode species from our study site build mucus sediment conglomerations (200-1000 µm in diameter) which are assumed to be densely populated by bacteria (Riemann 1986). These structures can attract nematodes which may feed on bacteria. We investigated the sediment surface in detail (multiple corer samples) and realized that innumerable mucus sediment aggregates were structuring the sediment surface (Fig. 4 A).

8. Tube-building nematodes

Many nematode species from our study site (*Acantholaimus* spp., *Microlaimus* spp., *Thalassomonhystera* spp., *Halalaimus* spp.) were lying in obvious self-made sediment conglomerations which surround the nematodes like tube-shaped overcoats. These nematodes are assumed to secrete large amounts of mucus, paste sediment

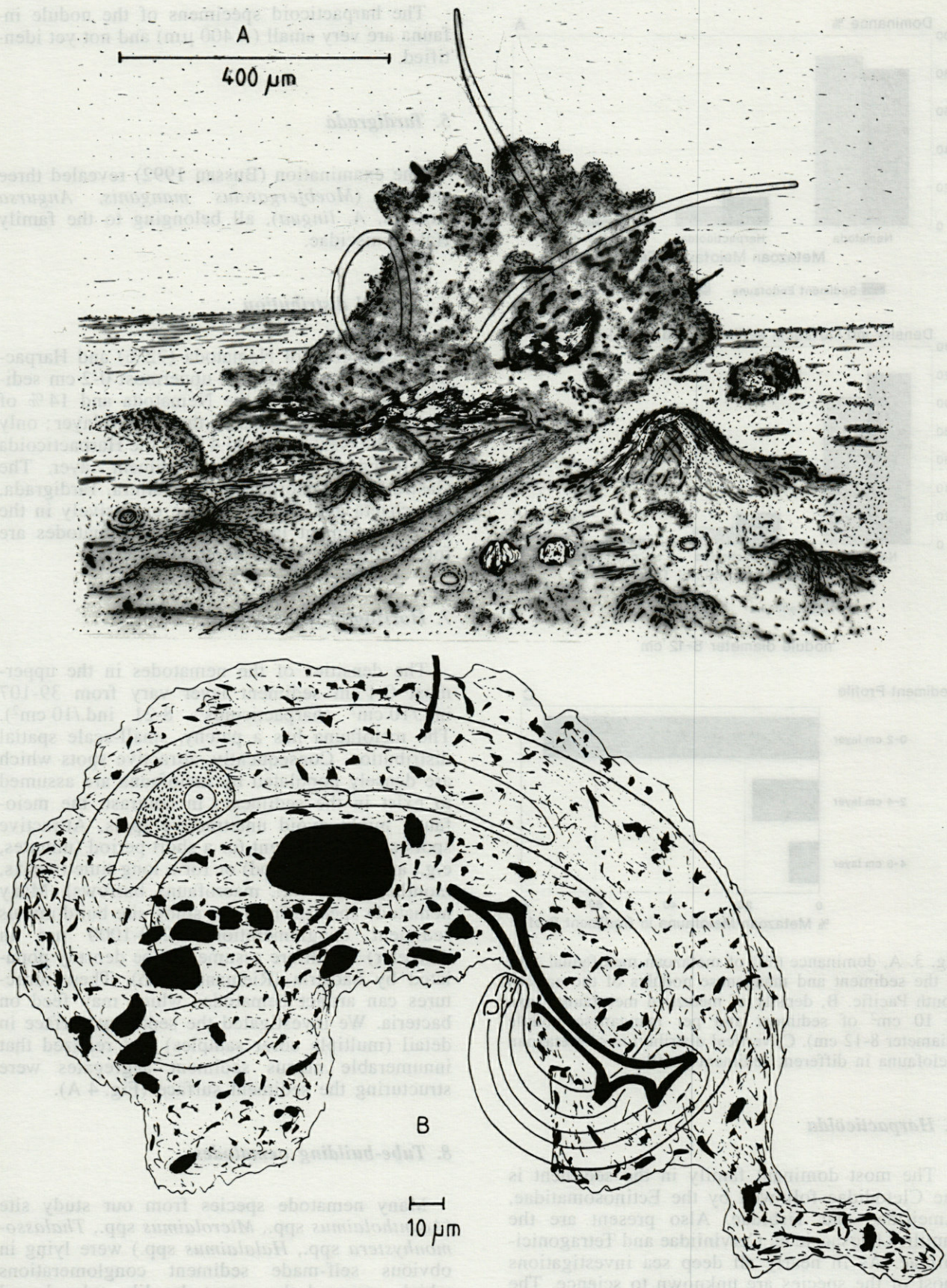

Fig. 4. – A, sediment surface with a mucus-sediment-structure containing three nematodes; B, a nematode of the family Microlaimidae within a sediment tube.

particles together and build light, lump-shaped or tube-shaped sediment structures, in which they might be able to move easily. The tubes seem to be elastic and the bending of the nematode body could cause the shape of the flexible tubes; i.e. in the case of a nematode which is curled up, a lump-shaped sediment structure arises (Fig. 4 B). The tube-building species are assumed to lead a hemisessile mode of life, and to feed on bacteria and bacterial secretions (bacterial gardening).

9. Nematode feeding types

Much of the literature on feeding and general ecology of marine nematodes is based upon Wieser's 1953 classification of four feeding types recognized from nematode buccal cavity morphology. We use a slightly modified scheme (see Bussau 1993) with five feeding types :
Feeding type 1 : without mouth
Feeding type 2 : buccal cavity weakly cuticularized, small, unarmed
Feeding type 3 : buccal cavity weakly cuticularized, small, with small teeth
Feeding type 4 : buccal cavity strongly cuticularized, large, unarmed
Feeding type 5 : buccal cavity strongly cuticularized, large, with large teeth

Most nematode species (66 %) from the manganese nodule area of the eastern South Pacific belong to feeding type 2; these species are assumed to feed on bacteria and bacterial secretions. Feeding type 3 comprised 17 % of the nematode species present; these possess small teeth for rasping and puncturing allowing for food particles to be scraped off surfaces and the food object pierced or damaged and its content sucked out. Some species (11 %) belong to feeding type 5; they are assumed to live as predators and scavengers, and may either swallow whole prey or puncture it with their teeth and suck out its contents. Species of the family Benthimermithidae (3 %) belong to feeding type 1; they are characterized by their reduced-sized mouth and anal openings and their intestine modified into a trophosome. The free-living males and females of this family are assumed to be dependent on a trans-epidermal uptake of dissolved organic matter or it is possible that they do not take up any food. Few species (3 %) belong to feeding type 4; these are assumed to be predators and scavengers which catch their prey with wide open mouths swallowing it or large particles of food whole. They may not be able to puncture food-objects. The percentages of nematodes belonging to the five different feeding categories are shown in Fig. 5.

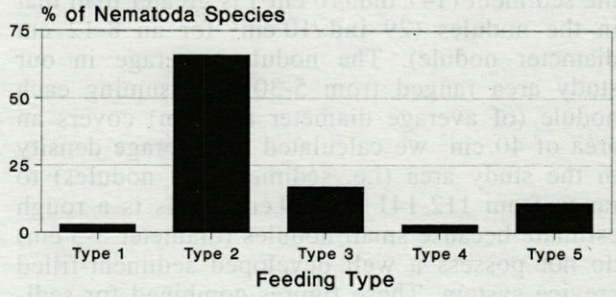

Fig. 5. – Dominance (%) of the five nematode feeding types in a manganese nodule area of the eastern South pacific.

DISCUSSION

One of us (H.T.) discovered that sediment occurs inside the nodules and that animals live in the sediment-filled crevice system of the nodules (Thiel *et al.* 1993). Additionally, although expected, it was not known that the thin sediment layer on the surface of the manganese nodules is inhabited by nematodes. Some of the nematodes are probably washed down from the nodule surface during the retrieval of the box corer and the sample processing on shipboard. Therefore not all the nematodes may have remained in their original position on the nodule surface.

The high nematode dominance at our study site shows a close correspondence with data presented by other authors (see e.g., Coull 1988, Heip *et al.* 1985, Thiel 1983). Renaud-Mornant & Gourbault (1990) reported nematode percentages of 84-100 % in the sediment from a manganese nodule area of the central Pacific in water depths of 4960-5154 m. This is close to our findings (77-87 %).

The density of the metazoan meiofauna in the sediment from the manganese nodule area of the eastern South Pacific (76-216 ind./10 cm^2) is comparable with results obtained by several authors from different ocean regions (see e.g., Thiel 1983 and references cited therein). Renaud-Mornant & Gourbault (1990) found that in the sediment of a manganese nodule area of the central Pacific (4960-5154 m) meiofaunal densities were 45-89 ind./10 cm^2. The range of nematode densities of 66-177 ind./10 cm^2 in the sediment of our study area is close to findings presented by Tietjen (1984), who reported nematode densities of 36-94 ind./10 cm^2 from the Venezuela Basin in water depths of 3517-5054 m. The higher numbers of meiofauna and nematodes in our study area compared with those in the cited papers may relate to different production and sedimentation systems. Energy input to the seafloor, for example, may be higher in the eastern South Pacific. However, the average density per unit area of the meiofauna in

the sediment (147 ind./10 cm^2) is greater than that in the nodules (29 ind./10 cm^2 for an 8-12 cm diameter nodule). The nodule coverage in our study area ranged from 5-30 %. Assuming each nodule (of average diameter 8-12 cm) covers an area of 40 cm^2 we calculated the average density in the study area (i.e. sediment and nodules) to range from 112-141 ind./10 cm^2. This is a rough estimate because small nodules (diameter 3-5 cm) do not possess a well developed sediment-filled crevice system. These figures combined for sediment and nodule faunas are closer to those reported by Renaud-Mornant & Gourbault (1990) and Tietjen (1984).

Any comparison of faunal densities must be undertaken with great care, specifically, when limited size classes such as the meiofauna are solely considered and when biotope structures are different. Comparisons are generally based on the assumption that community structures are similar. This is certainly not the case, when pure sediment areas are compared with those with sediment and hard substrate, like the manganese nodule areas, because biotopes with hard substrates are inhabited additionally by a diverse epifauna. Our samples and photographs have shown Porifera, Hydrozoa, Scyphozoa, Anthozoa, Brachiopoda, Bryozoa, Polychaeta, Crustacea, Echinodermata and Ascidia living attached to the nodules. The density of these metazoans is generally low in the deep sea but almost all nodules are inhabited by some specimens of those taxa, competing for the settling food particles. Additionally, Foraminiferida live in high densities on the nodule surfaces, as they do in the sediment. Theoretically, both sub-biotopes of the manganese nodule area, the sediment and the nodules, get equal amounts of energy (i.e. food particles) settling to the seabottom. In the nodules the density of the metazoan meiofauna is much lower than in the sediment. Thus, the energy input in the nodule sub-biotope is partitioned to a higher degree to the nodule epifauna.

The nematode composition at our study site generally corresponds with the data of Dinet & Vivier (1979), Bay Biscayne, 1920-4725 m; Jensen (1988), Norwegian Sea, 970-3284 m; Renaud-Mornant & Gourbault (1990), eastern central Pacific, 4960-5154 m; Wilson & Hessler (1987), eastern central Pacific, 4400-4600 m; Thistle & Sherman (1985), North Atlantic, 4626 m; Tietjen (1971, 1976), West Atlantic, off North Carolina, 600-2500 m; Tietjen (1984), Venezuela Basin, 3517-5054 m; Tietjen (1989), West Atlantic, Puerto Rico Trench, Hatteras Abyssal Plain, 2217-8380 m. The families Chromadoridae, Microlaimidae, Leptolaimidae, Monhysteridae, Xyalidae, Diplopeltidae, Desmoscolecidae, Ironidae, Oxystominidae seem to be dominant in the deep sea world-wide.

That nematodes do occur in greatest numbers close to the surface of the sediment has been demonstrated in many studies (see e.g., Heip *et al.* 1985, Thiel 1983). Thistle & Sherman (1985) found that 64 % of the nematodes from a deep-sea site (4626 m) in the North Atlantic occurred in the upper 2 cm of the sediment. This is very close to our results (72 %).

The deep-sea floor is structurally complex at small scales. Organisms alter the surface of soft bottom seafloors in a variety of ways : they make burrows, tracks, feeding traces, build tubes and tests and produce piles of fecal material (Thistle & Eckman 1988, 1990). At our study site many nematodes build mucus sediment conglomerations and innumerable aggregates of this kind are structuring the sediment surface. In the deep sea these biologically produced structures are assumed to persist for long periods, even after they are vacated. The mucus sediment conglomerations may be a major source of patchiness in the meiofaunal communities, play an important role in creating biotope heterogeneity and be important in maintaining the high diversity that characterizes the deep sea.

Less information is available about the feeding of harpacticoids in the deep sea. Potential food sources are obviously mucus together with its incorporated bacteria, ciliates and particulate organic matter as well as marine 'snow' (see Hicks & Coull 1983 and references therein). Therefore the tubes built by nematodes (see this paper) which can also be inhabited by harpacticoids set up an important food resource for harpacticoids. Some harpaticoids are able to build sediment tubes or coats, as previously mentioned in the literature, but we found no tube-dwelling specimens in our samples. Information on feeding mechanisms and food availability for harpacticoids in situ is still a gap in deep-sea harpacticoid biology.

Riemann (1974) gives the first record of tube-building deep-sea nematodes. What influence does the mucus of the nematodes have on the sediment texture of the deep-sea bottom ? The deep-sea mud consists of very fine sediment particles which would be densely packed if organisms were absent. In this hypothetical case (no organisms in the sediment) an extensive interstitial system would be absent. As a result of the mucus secreted by the nematodes, the sediment texture could change (Bussau 1993). Nematodes are assumed to build a network of closely spaced burrows in the uppermost (0-1 cm) layer of the very soft deep-sea sediment. Sediment particles may be agglutinated with the binding mucus and lump-shaped or tube-shaped conglomerations can arise. As a result, a narrow, imperfect lacunary system might develop which could enable an interstitial, non-boring meiofaunal community to thrive (Riemann 1974). The mucus secreting and tube building nematodes

may thus play an important role at the sediment-water interface by increasing pore water exchange, improving the 0_2-provision of the uppermost sediment layer (the movements of the nematodes within their tubes produce water transport in the uppermost sediment layer), stabilizing newly sedimented detritus with binding mucus, and counteracting resuspension. Of what use is the mucus secretion to the nematodes themselves? Sediment tubes afford many potential advantages to nematodes including avoidance of drift into unfavourable areas, a refuge in case of danger, and a place to outlast unfavourable environmental factors (dormant life period). The sediment tube could also be part of the feeding strategy (mucus-trap hypothesis, Riemann & Schrage 1978) because the tube could be densely populated with bacteria (bacterial gardening). It is further possible that the sediment tube is related to some form of maternal brood-care which has not yet been observed (Blome & Riemann 1987).

Tietjen (1971) states, "For deep-sea benthos there are essentially two main sources of food; detritus, either derived from the euphotic zone or formed in situ, and bacteria". Bacteria are the basic producers in the deep-sea benthos, producing the nutrient matrix, and most deep-sea animals are assumed to feed on bacteria or bacterial products (Thiel 1973). Therefore it is not surprising that most deep-sea nematode species (66%) in our study area possess weakly cuticularized, small buccal cavities without teeth; such species are considered to lead a more or less passive, hemisessile mode of life, to feed on bacteria and bacterial secretions, to build sediment tubes and to practise bacterial gardening (Bussau 1993). Jensen (1988), Renaud-Mornant & Gourbault (1990) and Tietjen (1984) made similar observations of the nematode feeding types in other deep-sea regions. The few nematodes (14%) which have strongly cuticularized, large buccal cavities with or without large teeth (predators and scavengers) in the present study, which also can be deduced from other deep-sea benthos studies (Tietjen 1971, 1976, 1989), could be taken as an indication of the low densities of carcasses and prey organisms. Deep-sea benthic ecosystems are governed by constantly limited food availability (Thiel 1975) and are fuelled by organic matter produced in surface waters (Pfannkuche 1985). The organic matter is recycled in the food web, partitioned in surface waters and reaches the abyss mostly in a refractory stage as small-sized, probably aggregated particles which sink down to the bottom and concentrate in the sediment surface. From the energy point of view, the active mode of life as predators or scavengers seems to be too costly because the density of their food is too low. Thus, a cost-effective way of food aquisition is the passive waiting for particles as exhibited by most deep-sea organisms.

ACKNOWLEDGEMENTS – We thank Dr C. Maybury for streamlining the English. The DISCOL-project is funded by the Ministry of Science and Technology of the Federal Republic of Germany under contract number 03F-0010F. The authors are responsible for the content of this paper. This is DISCOL publication number 35.

REFERENCES

BLOME D. & F. RIEMANN, 1987. A sediment agglutination on females of the free-living marine nematode *Desmodora schulzi*. *Helgoländer Meeresunters.* **41** : 113-119.

BUSSAU C., 1992. New deep-sea Tardigrada (Arthrotardigrada, Halechiniscidae) from a manganese nodule area of the eastern South Pacific. *Zoologica Scripta* **21** (1) : 79-91.

BUSSAU C., 1993. Taxonomische und ökologische Untersuchungen an Nematoden des Peru-Beckens. Dissertation, Universität Kiel, 1-621.

COULL B.C., 1988. Ecology of the marine meiofauna. *In* Introduction to the study of meiofauna. Edited by R.P. Higgins and H. Thiel, Smithsonian Institution Press, Washington, D.C., London, 18-38.

DINET A. & M.-H. VIVIER, 1979. Le méiobenthos abyssal du Golfe de Gascogne. II. Les peuplements de Nématodes et leur diversité spécifique. *Cah. Biol. Mar.* **20** : 109-123.

FOELL E.J., H. THIEL & G. SCHRIEVER, 1990. DISCOL : A long-term, large-scale, disturbance-recolonization experiment in the abyssal eastern tropical South Pacific Ocean. *Proc. Offshore Technol. Conf.* (OTC paper 6328) : 497-503.

FOELL E.J., G. SCHRIEVER, H. BLUHM, C. BOROWSKI, C. BUSSAU & H. THIEL, 1992. Disturbance and recolonization experiment in the abyssal South Pacific Ocean (DISCOL) : An update. *Proc. Offshore Technol. Conf.* (OTC paper 6805) : 25-34.

HEIP C., M. VINCX & G. VRANKEN, 1985. The ecology of marine nematodes. *Oceanogr. Mar. Biol. Ann. Rev.* **23** : 399-489.

HESSLER R.R. & P.A. JUMARS, 1974. Abyssal community analysis from replicate box cores in the central North Pacific. *Deep-Sea Research* **21** : 185-209.

HICKS G.R.F. & B.C. COULL, 1983. The ecology of marine meiobenthic harpacticoid copepods. *Oceanogr. Mar. Biol. Ann. Rev.* **21** : 67-175.

JENSEN P., 1988. Nematode assemblages in the deep-sea benthos of the Norwegian Sea. *Deep-sea Research* **35** : 1173-1184.

MULLINEAUX L.S., 1987. Organisms living on manganese nodules and crusts : distribution and abundance at three North Pacific sites. *Deep-Sea Research* **34** : 165-184.

PFANNKUCHE O., 1985. The deep-sea meiofauna of the Porcupine Seabight and abyssal plain (NE Atlantic) : population structure, distribution, standing stocks. *Oceanol. Acta* **8** : 343-353.

RENAUD-MORNANT J. & N. GOURBAULT, 1990. Evaluation of abyssal meiobenthos in the eastern central Pacific (Clarion-Clipperton fracture zone). *Prog. Oceanog.* **24** : 317-329.

RIEMANN F., 1974. On hemisessile nematodes with flagelliform tails living in marine soft bottoms and on micro-tubes found in deep sea sediments. *Mikrofauna Meeresboden* **40** : 1-15.

RIEMANN F., 1986. Berichte der Abteilungen Nematodenkunde. *Veröff. Inst. Meeresforsch. Bremerh.* **21** : 195-201.

RIEMANN F., & M. SCHRAGE, 1978. The Mucus-Trap Hypothesis on feeding of aquatic nematodes and implications for biodegration and sediment texture. *Oecologia* **34** : 75-88.

SCHRIEVER G., C. BUSSAU & H. THIEL, 1991. Discol-precautionary environmental impact studies for future manganese nodule mining and first results on meiofauna abundance. *Pro. Adv. Mar. Tech. Conf.* **4** : 47-57.

SNIDER L.J., B.R. BURNETT & R.R. HESSLER, 1984. The composition and distribution of meiofauna and nanobiota in a central North Pacific deep-sea area. *Deep-Sea Research* **31** : 1225-1249.

THIEL H., 1973. Der Aufbau der Lebensgemeinschaft am Tiefseeboden. *Natur und Museum* **103** : 39-46.

THIEL H., 1975. The size structure of the deep-sea benthos. *Int. Revue ges. Hydrobiol.* **60** : 575-606.

THIEL H., 1983. Meiobenthos and nanobenthos of the deep sea. *In* Deep-Sea Biology ; volume 8 : The Sea. Edited by G.T. Rowe, John Wiley & Sons, New York, 167-230.

THIEL H. & G. SCHRIEVER, 1989. Cruise report DISCOL 1, SONNE-Cruise 61. *Ber. Zentrum Meeres– u. Klimaforsch. Univ. Hamburg* **3** : 1-91.

THIEL H. & G. SCHRIEVER, 1990. Deep-sea mining, environmental impact and the DISCOL project. *Ambio* **19** : 245-250.

THIEL H., H. BLUHM, C. BOROWSKI, C. BUSSAU, A.J. GOODAY, C. MAYBURY & G. SCHRIEVER, 1992. The impact of mining on deep-sea organisms. *Ocean Challenge* **3** : 40-46.

THIEL H., G. SCHRIEVER, C. BUSSAU & C. BOROWSKI, 1993. Manganese nodule crevice fauna. *Deep-Sea Research* **40** : 419-423.

THISTLE D. & K.M. SHERMAN, 1985. The nematode fauna of a deep-sea site exposed to strong near-bottom currents. *Deep-Sea Research* **32** : 1077-1088.

THISTLE D. & J.E. ECKMAN, 1988. Response of harpacticoid copepods to habitat structure at a deep-sea site. *Hydrobiologia* **167/168** : 143-149.

THISTLE D. & J.E. ECKMAN, 1990. The effect of biologically produced structure on the benthic copepods of a deep-sea site. *Deep-Sea Research* **34** : 541-554.

TIETJEN J.H., 1971. Ecology and distribution of deep-sea meiobenthos off North Carolina. *Deep-Sea Research* **18** : 941-957.

TIETJEN J.H., 1976. Distribution and species diversity of deep-sea nematodes off North Carolina. *Deep-sea Research* **23** : 755-768.

TIETJEN J.H., 1984. Distribution and species diversity of deep-sea nematodes in the Venezuela Basin. *Deep-sea Research* **31** : 119-132.

TIETJEN J.H., 1989. Ecology of deep-sea nematodes from the Puerto Rico Trench area and Hatteras Abyssal Plain. *Deep-Sea Research* **36** : 1579-1594.

WIESER W., 1953. Die Beziehung zwischen Mundhöhlengestalt, Ernährungsweise und Vorkommen bei freilebenden marinen Nematoden. *Ark. Zool.* **4** : 439-484.

WILSON G.D.F., R.R. HESSLER, 1987. The effects of manganese nodule test mining on the benthic fauna in the North Equatorial Pacific. *In* Evironmental effects of deep sea dredging. Edited by F.N. Spiess, R. Hessler, G. Wilson & M. Weydert, Scripps Inst. Oceanogr. Ref. 87-85, La Jolla, Calif : Scripps Inst. Oceanogr, 24-86.

Reçu le 29 juillet 1994 ; received July 29, 1994
Accepté le 16 septembre 1994 ; accepted September 16, 1994

VIE MILIEU, 1995, 45 (1) : 49-59

NON-SELECTIVE INGESTION OF MEIOBENTHOS BY JUVENILE SPOT (*LEIOSTOMUS XANTHURUS*) (PISCES) AND THEIR DAILY RATION

R.J. FELLER, B.C. COULL

Department of Biological Sciences, Marine Science Program, and Belle W. Baruch Institute for Marine Biology and Coastal Research, University of South Carolina, Columbia, SC 29208 U.S.A.

SPOT
LEIOSTOMUS XANTHURUS
MEIOFAUNA
SALTMARSH
DAILY RATION
PREDATION
DIET
SOUTH CAROLINA

ABSTRACT – Saltmarsh meiobenthos along a subtidal-intertidal-marsh gradient was collected every 2-h concurrently with seine collections of juvenile spot during May of 1986 and 1988 in North Inlet, South Carolina. Feeding occurred primarily during ebb tides, with reduced numbers of prey in gut contents during low and flooding tides. Contrary to previous reports that copepods dominate their diet, comparison of meiobenthos prey abundances in the sediment with foregut and hindgut contents of the fish revealed that spot ingest nematodes and copepods in the same proportion that they exist in the sediment. With gut evacuation – more of 6 h – times measured by serial sacrifice of field-collected fish, 2 to 4 gut fillings per day, and counts of the number of prey in freshly filled foreguts, an estimate of spot daily ration was possible. Juvenile spot (27-33 mm) ate, on average, between 100 and 500 copepods and forams and up to 4000 nematodes per day. Because sediment densities of meiofauna are high, spot can obtain their daily ration from just a few thousandths of a square meter. Therefore spot predation alone probably is not a significant source of mortality for meiofauna in North Inlet.

LEIOSTOMUS XANTHURUS
MÉIOFAUNE
MARAIS MARITIMES
RATION QUOTIDIENNE
PRÉDATION
RÉGIME ALIMENTAIRE
CAROLINE DU SUD

RÉSUMÉ – Le méiobenthos des marais maritimes caractérisé par un gradient « subtidal-intertidal-lagunaire » a été récolté toutes les 2 heures parallèlement à des juvéniles de *Leiostomus xanthurus* recueillis à la Senne en mai 1986 et 1988 à North Inlet, en Caroline du Sud. La nutrition a lieu principalement pendant le jusant, tandis que le nombre de proies des contenus stomacaux est plus réduit à marée montante ou à marée haute. Contrairement aux travaux antérieurs qui montrent que les Copépodes sont la principale source de nourriture, la comparaison de l'abondance des proies méiobenthiques dans le sédiment et dans les contenus du tube digestif antérieur et postérieur du Poisson révèle que *L. xanthurus* ingère les Nématodes et les Copépodes en proportion identique à celle du sédiment. La ration journalière de *L. xanthurus* a été estimée à partir d'une durée de 6 heures pour le transit digestif de Poissons récoltés dans la nature et sacrifiés, moyennant 2 à 4 prises de nourriture par jour, et à partir du décompte du nombre de proies contenus dans le tube digestif antérieur fraichement rempli. *L. xanthurus* juvénile de 27 à 33 mm consomme en moyenne entre 100 et 500 Copépodes et Foraminifères et jusqu'à 4 000 Nématodes par jour. La densité de la méiofaune du sédiment étant élevée, *L. xanthurus* peut trouver la ration quotidienne sur quelques millièmes de m^2. Ainsi, la prédation par *L. xanthurus* ne correspond probablement pas à la cause significative de la mortalité de la méiofaune de North Inlet.

INTRODUCTION

Spot (*Leiostomus xanthurus* Lacepede), one of the most abundant fishes on the U.S. southeastern and Gulf coasts, spawn offshore and enter estuaries during the late winter or early spring as postlarval planktivores (Chestnut, 1983; Ellis & Coull, 1989). At 15-22 mm SL, these larvae metamorphose into benthic feeding juveniles with subterminal months (Currin *et al.*, 1984; Archambault & Feller, 1991) and feed by taking bites of sediment 2-3 mm deep (Billheimer & Coull, 1988; Archambault & Feller, 1991). They preferentially feed in muddy sediments (Smith & Coull 1987). The sediment is manipulated in the buccal cavity and sieved through the gill rakers and pharyngeal teeth (McCall & Fleeger, 1993). Juvenile spot feed exclusively on meiofauna (Ellis

& Coull, 1989; Nelson & Coull, 1989; Scholz *et al.*, 1991; McCall & Fleeger, 1993). It is not known exactly how the prey are selected but most unwanted particles of sediment are expelled through the opercular opening (Yetman, 1979).

The stomach contents of juvenile spot have been examined by numerous investigators to determine what, when, and where these bottom-feeding predators eat. The preponderance of these studies report that meiobenthic copepods dominate their diet (Table I of Coull 1990). Gee's 1989 review also stresses the extent to which harpacticoid copepods appear to be ingested preferentially over other meiobenthic taxa, despite the numerical dominance of nematodes in marine sediments where juvenile spot feed. It would appear from the frequency with which benthic copepods are reported in fish diets that this meiofaunal taxon represents an obligatory prey item for many estuarine-dependent juvenile fishes throughout the world. The question remains are harpacticoids the only prey?

Coull (1990) presents convincing evidence from a series of field and laboratory studies on juvenile spot that epibenthic copepod movements attract the attention of this visual predator. Because it takes bites of sediment when it consumes the copepods, the spot should also ingest other meiofauna present in the sediment. As a consequence of copepod movements and availability on the sediment-water interface and differential digestion of softer-bodied meiofauna, the spot diet usually consists of preferentially-ingested copepods and incidentally ingested nematodes (Coull, 1990). The rapidity with which nematodes are digested by juvenile spot (completely in about 2 h) may explain why this numerically dominant taxon is so scarce in the spot's diet (Scholz *et al.* 1991). Because spot feeding periodicity is regulated by the tidal periods during which intertidal feeding grounds are flooded and made accessible to the spot (Archambault and Feller, 1991), the time elapsed between when spot feed and when they are collected for dietary analysis becomes critical. Dietary composition and daily ration estimates may be seriously flawed if fish are collected long after they have fed.

The literature on meiofauna as food for fish contains few examples of collections wherein both fish and meiofauna were sampled at the same time (Gee 1989). Our study was designed to examine simultaneous changes in spot diet and in the abundance of their sediment-dwelling meiofaunal prey over 24-hour periods. Additional studies of spot gut evacuation rate also allowed estimation of daily ration (Boisclair and Marchand 1993). Coupled with information on juvenile spot abundance, we conclude with comments on the potential impact of juvenile spot predation on the sediment-dwelling meiofaunal community.

METHODS AND MATERIALS

The study was conducted at Oyster Landing in North Inlet, South Carolina (33°20′N, 79°10′W), in May 1986 and spot were again collected in May 1988 at the same location. Meiofauna core samples were collected in both 1986 and 1988, but only copepods were counted from the 1988 samples. The tidal creeks at Oyster Landing have semidiurnal tides with a range of 1.5 m during spring tides. The changes of tidal height over time are published as Fig. 2 in Feller *et al.* (1990). Lows occurred at about 13:00 and 02:00 and highs at 19:00 and 08:00 each year. The tides were uniformly about 40 cm higher in 1986 than in 1988. It was dark from 20:00 to 06:30. Water is typically turbid in the creeks and salinities were in the range of 25-30 ppt during collections both years.

Spot Collection and Dietary Analysis

Fish were collected every hour for 24 hours beginning at 10:00 in the morning on 22-23 May 1986 and every 2-h again on 15-16 May 1988. The last collection made was also at 10:00 am the next day each year. A two-person seine (7.6 x 1.8 m, 0.95 cm mesh) was pulled repeatedly for 10 min in the unvegetated tidal creek adjacent to the core collection transect (see below). Twenty fish were taken directly from the seine and placed on dry ice within 15 min of their collection in the field, and 6 of these (27-33 mm SL) were selected at random for gut contents analysis. Prey were removed from the foregut (esophagus to pyloric caecum) and hindgut (pc to anus) separately with forceps under a dissecting microscope and counted to major meiobenthic taxa. Copepods from these fish were identified to species and used to trace where the fish had most recently fed (Feller *et al.* 1990).

Spot Gut Evacuation Rate

Approximately 150 spot were taken from the ebbing tide seine collection at 10:00 on 21 May 1986, placed gently into coolers and carried about 500 m to the seawater lab. Fish were released into a clean (no sediment, barebottom) flowthrough seawater table running with 1-micron filtered water at 27°C, the same temperature as ambient seawater where the fish were collected. Random samples of fish (n = 6 or 7) were serially sacrificed at 0.5-h intervals for the first 3 hours and at hourly intervals thereafter until 18:30. Fish were frozen on dry ice and later thawed for gut contents removal and prey item identifications. Each fish was measured, weighed and its entire digestive tract was removed and weighed intact.

Gut contents wet weight (± 1mg) was determined as the difference between intact and empty gut wet weights. The volume of gut contents was estimated by visually comparing the sample's volume with that of premeasured drops of glycerine ranging in volume from 1 to 25 microliters. A subjective measure of fullness (1 = empty to 10 = full) was also applied to each gut sample. Copepods and foraminifera were counted from the hindgut of each fish as well. Following suggestions from Persson (1986) and Mullen (1986), data for gut contents wet weights divided by fish wet weight (Y_i) were plotted versus time (X in hours) and fitted with linear [Y = a + bX], exponential [Y = exp (a+bX)], multiplicative [Y = aX^b], and reciprocal [1/Y = a + bX] models to estimate the loss rate of gut contents.

Meiobenthos Collections

Details of these procedures are described in Coull and Feller (1988). Three sites (subtidal, intertidal, and vegetated *Spartina alterniflora* marsh), separated by 5 m along a transect were sampled by coring. Four cores (2.66 cm internal dia, area = 5.3 cm^2) were taken at each site every

		Nematodes		Copepods		Forams		Others		Total	
Time	Tide	F	H	F	H	F	H	F	H	F	H
1000	Ebb	81	6	34	102	7	155	8	13	130	276
1200	Ebb	494	15	72	177	30	179	5	8	601	379
1300	Ebb	36	4	29	181	5	86	2	19	72	290
1400	Low	20	1	19	118	2	45	1	7	42	171
1500	Low	2	7	19	439	1	67	1	19	23	532
1600	Flood	6	7	4	98	2	65	1	8	13	179
1700	Flood	69	67	6	111	2	111	1	14	78	192
1800	Flood	80	11	26	107	28	204	8	13	142	335
1850	Flood	18	13	9	126	2	63	3	22	32	224
1900	Flood	32	27	15	86	1	77	4	10	52	200
1950	High	71	23	19	195	10	153	6	11	106	382
2100	Ebb	-	-	-	-	-	-	-	-	-	-
2200	Ebb	102	2	19	39	1	62	7	6	129	109
2250	Ebb	165	26	26	86	5	122	3	10	199	244
2300	Ebb	95	15	13	119	2	63	7	13	117	210
2400	Ebb	262	7	18	73	7	60	3	20	290	160
0100	Ebb	137	16	32	200	13	198	10	21	192	435
0200	Low	58	5	14	39	1	55	2	13	75	112
0300	Low	21	3	16	26	2	14	10	7	49	50
0400	Flood	26	1	26	54	1	11	5	9	58	75
0500	Flood	53	1	9	36	2	17	5	13	69	67
0600	Flood	61	3	17	37	2	13	7	18	87	71
0700	Flood	70	11	19	81	1	39	5	10	95	141
0800	High	113	8	48	136	3	46	4	14	168	204
0900	High	62	27	24	205	1	83	6	17	93	332
1000	Ebb	127	22	53	143	4	56	6	4	190	225
Average		87	13	23	120	5	86	5	13	120	232
Maximum in one fish		1000	170	145	725	98	680	20	10K*	1500	10K

* One fish, 32mm total length, contained about 10,000 barnacle cyprids in its hindgut at 1900 sampling time. This extreme number has been excluded from the calculations above.

Table I. – Average abundance of prey in the foregut (F) and hindgut (H) of 6 juvenile spot each sampling time, 22-23 May 1986. Numbers have been rounded. The 2100 sample was missed. The « Others » prey category includes small polychaetes, turbellaria, ostracods, barnacle cyprids, mites, oligochaetes, amphipods, kinorhynchs and insects.

2-h at the same times fish were collected. During high tide, cores were collected from a boat with a 1.5 m extension handle which held the core, but otherwise all cores were collected by hand. Samples of the uppermost 5 cm were fixed in 10% buffered formalin and stained with Rose Bengal. Sediments were sieved with a 63 micron mesh and all organisms counted and sorted to major meiofaunal taxon.

RESULTS

Prey Composition of Spot Diet

In 1986 collections nearly 35 identifiable prey categories were found in the spot, but prey were dominated by just 3 taxa – nematodes, copepods, and foraminiferans. Stomach contents were characterized by having many more nematodes in their foreguts than in their hindguts, more copepods and foraminifera in hindguts than in foreguts, and more prey ingested during ebbing tides just before low tide (Fig. 1). Average numbers of prey contained in the guts of spot over the 24-h period were 100 nematodes, 143 copepods, and 91 foraminifera (Table I). Maximum numbers of prey in a single fish examined were 1000 nematodes in the foregut and 170 in the hindgut, 145 (F) and 725 (H) copepods, and 98 (F) and 680 (H) foraminifera, but one other spot (32 mm) contained about 10,000 barnacle cyprids in its hindgut. Gut contents weight as a percentage of the fish's wet weight varied significantly from a low of 3.2% to a high of 5.7% (F = 3.009, P 0.00001), with low values occurring about 2 h after low tide and high values about 1-2 h after high tides.

In 1988 collections a similarly wide breadth of dietary items was identified in spot stomachs, but the same 3 taxa dominated again. There were typically more nematodes in foreguts than in hindguts and more copepods and foraminifera in hindguts than in foreguts (Fig. 2). Once again, prey were more abundant during the ebbing tide just before low tide and least numerous in the guts during

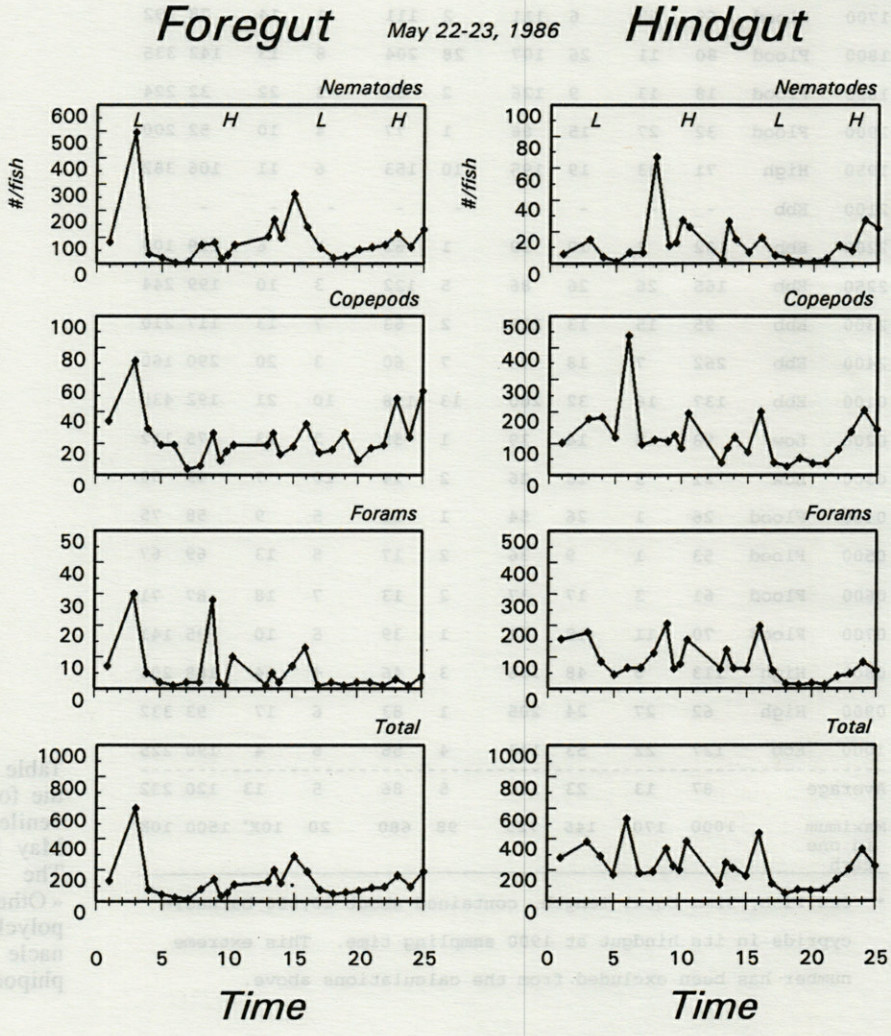

Fig. 1. – The mean number of nematodes, copepods, foraminifera, and total meiofaunal prey in foreguts and hindguts per juvenile spot. Numbers are based on an average of 6 fish collected every hour beginning at 1000 hrs on 22 May (= Time 1) and ending at 1000 hrs 23 May 1986 (= Time 25). Confidence intervals have been omitted for graphical clarity.

		Nematodes		Copepods		Forams		Others		Total	
Time	Tide	F	H	F	H	F	H	F	H	F	H
1000	Ebb	47	3	54	42	1	44	4	183	106	272
1200	Ebb	73	9	27	33	15	197	10	31	125	270
1400	Low	23	9	15	31	1	53	4	4	43	97
1600	Flood	57	7	9	24	12	116	2	3	80	150
1800	Flood	8	20	5	42	2	134	1	2	16	198
2000	High	90	33	8	47	2	92	1	6	101	178
2200	Ebb	70	13	9	27	1	26	8	5	88	71
2400	Ebb	161	32	15	41	8	154	5	6	189	233
0200	Low	120	8	15	22	10	26	5	4	150	60
0400	Flood	20	2	6	11	1	13	2	2	29	28
0600	Flood	41	11	6	61	3	69	3	7	53	148
0800	Flood	67	7	17	39	9	61	4	7	97	112
1000	High	29	22	28	91	1	263	2	34	60	410
Average		62	14	16	40	5	96	4	23	87	173
Maximum in one fish		502	111	121	164	30	601	32	500	554	777

Table II. – Average abundance of prey in the foregut (F) and hindgut (H) of 6 juvenile spot each sampling time, 15-16 May 1988. Numbers have been rounded. The «Others» prey category includes small polychaetes, turbellaria, ostracods, barnacle cyprids, mites, oligochaetes, amphipods, decapod megalopa and zoeae, zoeae tails, kinorhynchs, and insects.

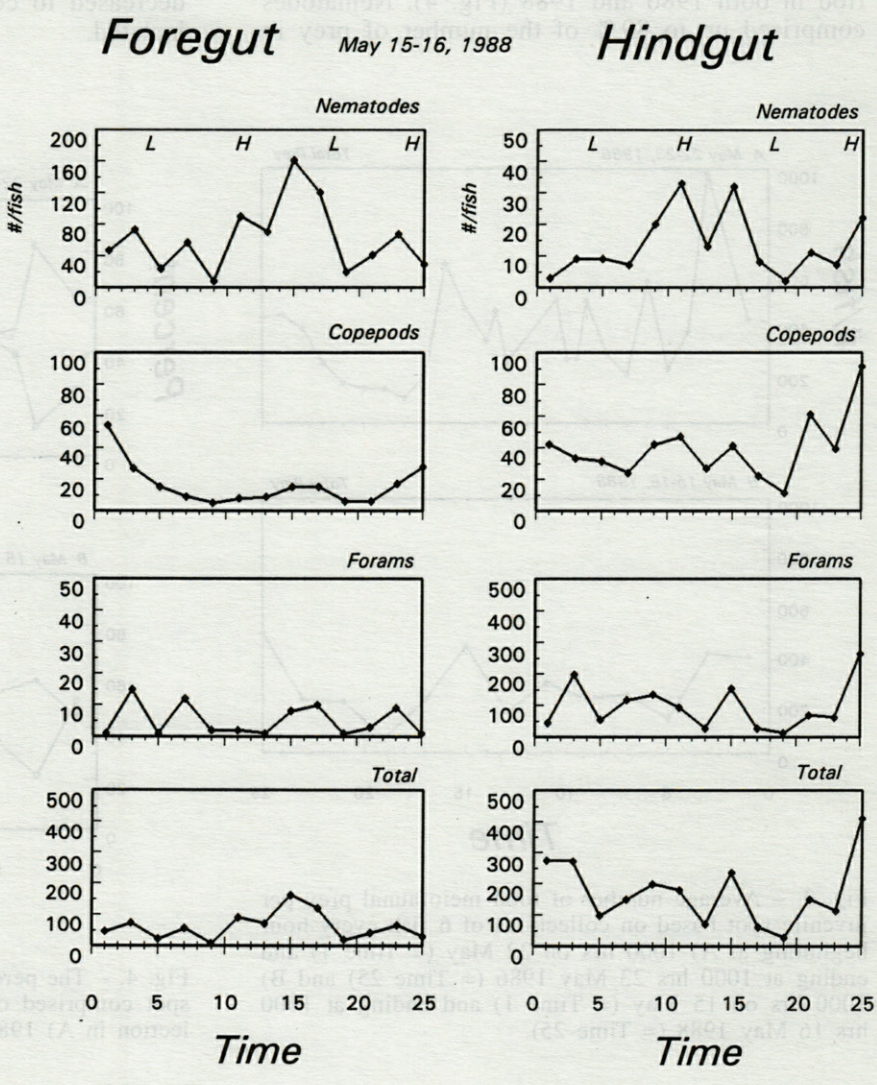

Fig. 2. – The mean number of nematodes, copepods, foraminifera, and total meiofaunal prey in foreguts and hindguts per juvenile spot. Numbers are based on an average of 6 fish collected every 2 hours beginning at 1000 hrs on 15 May (= Time 1) and ending at 1000 hrs 16 May 1988 (= Time 25). Confidence intervals have been omitted for graphical clarity.

mid-flood tides. Average numbers of prey over the 24-h period were 76 nematodes, 56 copepods, and 101 foraminifera (Table II). Maximum numbers of prey found in a single fish were 613 nematodes (502 in foregut, 111 in hindgut), 285 copepods (121 and 164), and 532 foraminiferans (32 and 500). As in 1986, these maxima were from different fish, i.e., no single fish contained the maximum number of all 3 major taxa.

The average total number of prey (all categories combined) contained in spot in 1986 was higher than in 1988 – 352 and 260, respectively (Fig. 3). These averages are not statistically significantly different, however (1-way ANOVA, within and between years, $p > 0.05$). Long-term (bi-weekly since 1984) quantitative seine collections of spot at the Oyster Landing reference station in North Inlet during May in 1986 were only about a third of the number present in May 1988 (D.M. Allen, pers. comm.).

On a proportional basis, considering the numbers of prey in foreguts only, nematodes dominated this section of the spot's digestive tract during nearly all times of the 24-h collection period in both 1986 and 1988 (Fig. 4). Nematodes comprised up to 89% of the number of prey in foreguts, and over the 24-h collection averaged 73 and 71% of foregut contents in 1986 and 1988, respectively, while copepods averaged 19 and 18% (Table III).

Gut Evacuation Rate

The wet weight of gut contents as a percentage of fish wet weight decreased nearly monotonically during the evacuation period, with essentially complete emtying or constant values attained after 6 h (Fig. 5). Of the four models tested, the exponential provided the best fit [$Y = \exp(-1.76 - 0.00126 X)$, $r^2 = 0.503$, $F = 74.8$, $P = < 0.00001$], although with such scatter in the data, the other three models fit nearly as well. The linear model [$Y = 0.086 - 0.000039X$, $r^2 = 0.462$] had a slope equivalent to a contents evacuation rate of about 9% per hour over 8 hours. Gut contents volume decreased by 3 $\mu l\ h^{-1}$, from 25 to 2 μl in 8 h, and estimated fullness declined in a nearly linear fashion also. Both fullness and volume measures decreased to constant levels 6 h after fish were isolated.

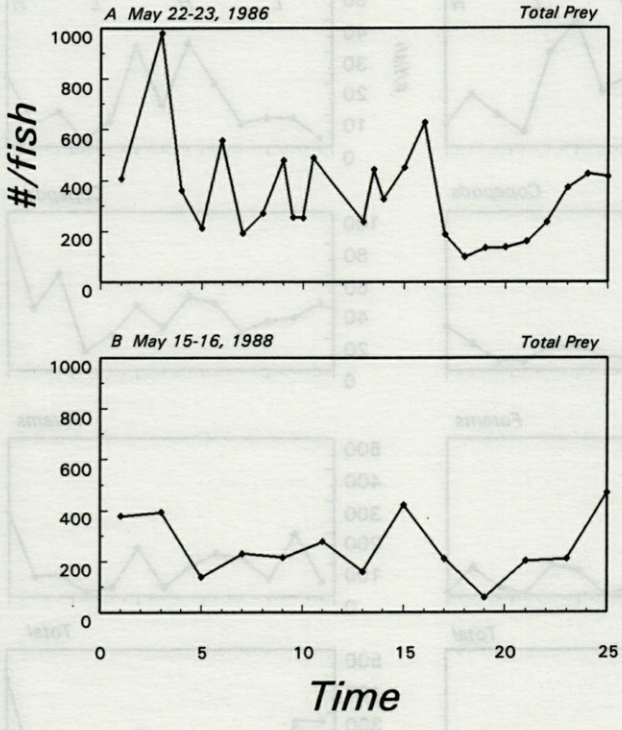

Fig. 3. – Average number of total meiofaunal prey per juvenile spot based on collections of 6 fish every hour beginning at A) 1000 hrs on 22 May (= Time 1) and ending at 1000 hrs 23 May 1986 (= Time 25) and B) 1000 hrs on 15 May (= Time 1) and ending at 1000 hrs 16 May 1988 (= Time 25).

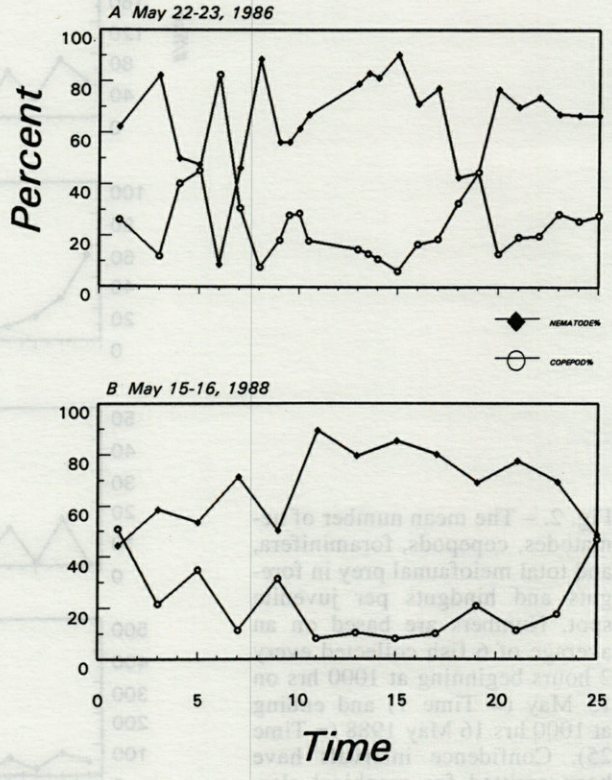

Fig. 4. – The percentage of foregut contents of juvenile spot comprised of nematodes and copepods from collection in A) 1986 and B) 1988.

	Prey Taxon	Foregut	Hindgut	Total Gut
A)	Nematodes	73 (8-88)	6 (1-35)	28 (2-52)
	Copepods	19 (8-83)	52 (36-83)	41 (20-83)
	Foraminiferans	4 (1-20)	37 (13-61)	26 (0-49)
	Others	4 (1-20)	5 (1-25)	5 (2-17)
		100	100	100
B)	Nematodes	71 (44-89)	8 (1-37)	29 (11-61)
	Copepods	18 (8-51)	23 (15-41)	22 (18-33)
	Foraminiferans	6 (1-15)	56 (16-77)	39 (17-64)
	Others	5 (1- 9)	13 (1-67)	10 (1-49)
		100	100	100

Table III. – The average percentage composition over 24-h of the gut contents of 6 juvenile spot in 1) 1986 and B) 1988. Numbers in parentheses are the minimum and maximum 6-fish averages that occurred during the 24-h collection.

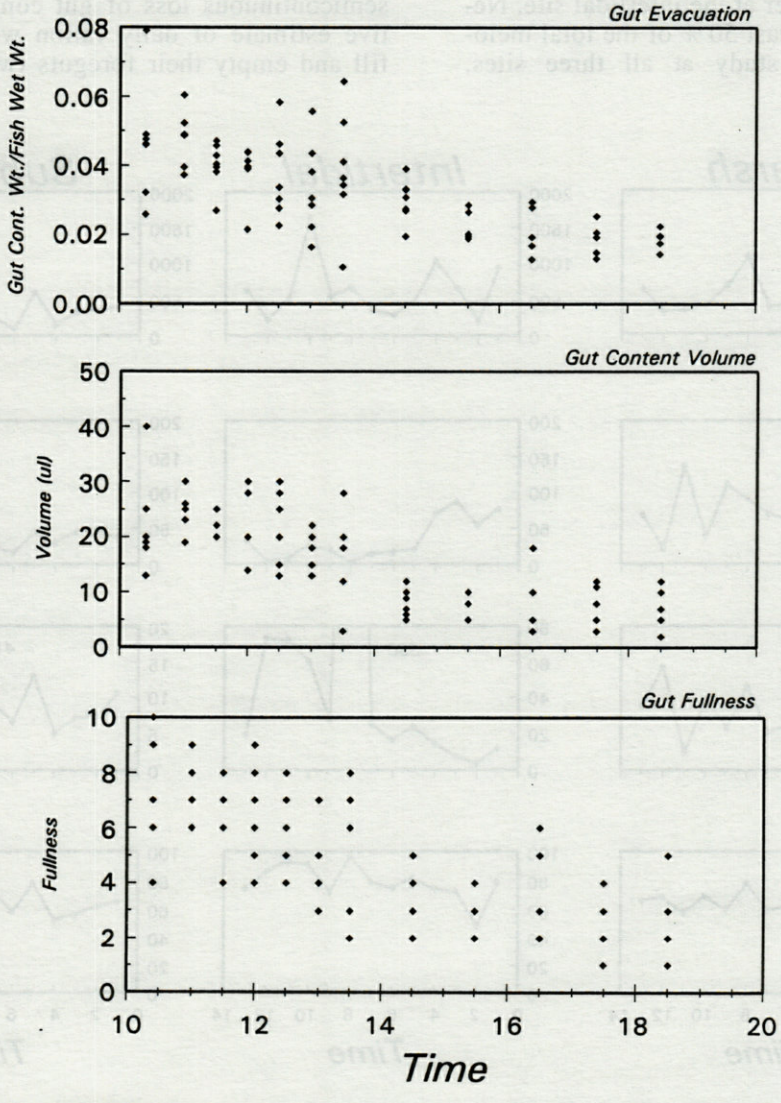

Fig. 5. – Wet weight of gut contents as a percentage of a spot's wet body weight (in grams) as a function of time during the gut evacuation study on 21 May 1986.

Although the slopes of the lines describing the loss rate of nematodes and copepods from the foreguts of fish were significantly different from zero (P = 0.001 and 0.005, respectively), their linear fit was poor, explaining only about 12% of the variance. A decelerating negative exponential curve would probably have fit these data better, but scatter was so high that this was not attempted.

Core Collections at Three Sites

Nematode abundance in the sediments was almost inversely related to tidal height at the marsh and intertidal sites, and was lowest at the subtidal site (Fig. 6). Copepods were also least abundant at the subtidal site. The ratio of nematodes to copepods (N/C) fluctuated dramatically over the 24-h sampling period at all three sites and was often considerably higher at the intertidal site. Nematodes comprised at least 50% of the total meiofauna throughout the study at all three sites, averaging 63, 81, and 72% at the marsh, intertidal and subtidal sites respectively (Fig. 6). The 3-site average proportions over the 24-h period were 71% nematodes and 12% copepods (including nauplii, Table III). Average abundances of total meiofauna (no • 10 cm^{-2}) were 1669 (marsh), 1450 (intertidal), and 848 (subtidal). Total mean copepod abundances at the same three sites, respectively, in 1986/1988 were 147/128, 57/40, and 49/20.

Calculations of Daily Ration

Archambault and Feller (1991) determined that juvenile spot gut fullness peaked twice per 24-h and emptied in about 6 h. This same bimodality of fullness was seen clearly only for foregut nematodes in 1986 (Fig. 1), but a similar emptying time was measured in 1986 (Fig. 5). Ignoring the semicontinuous loss of gut contents, a conservative estimate of daily ration would assume spot fill and empty their foreguts twice a day. Taking

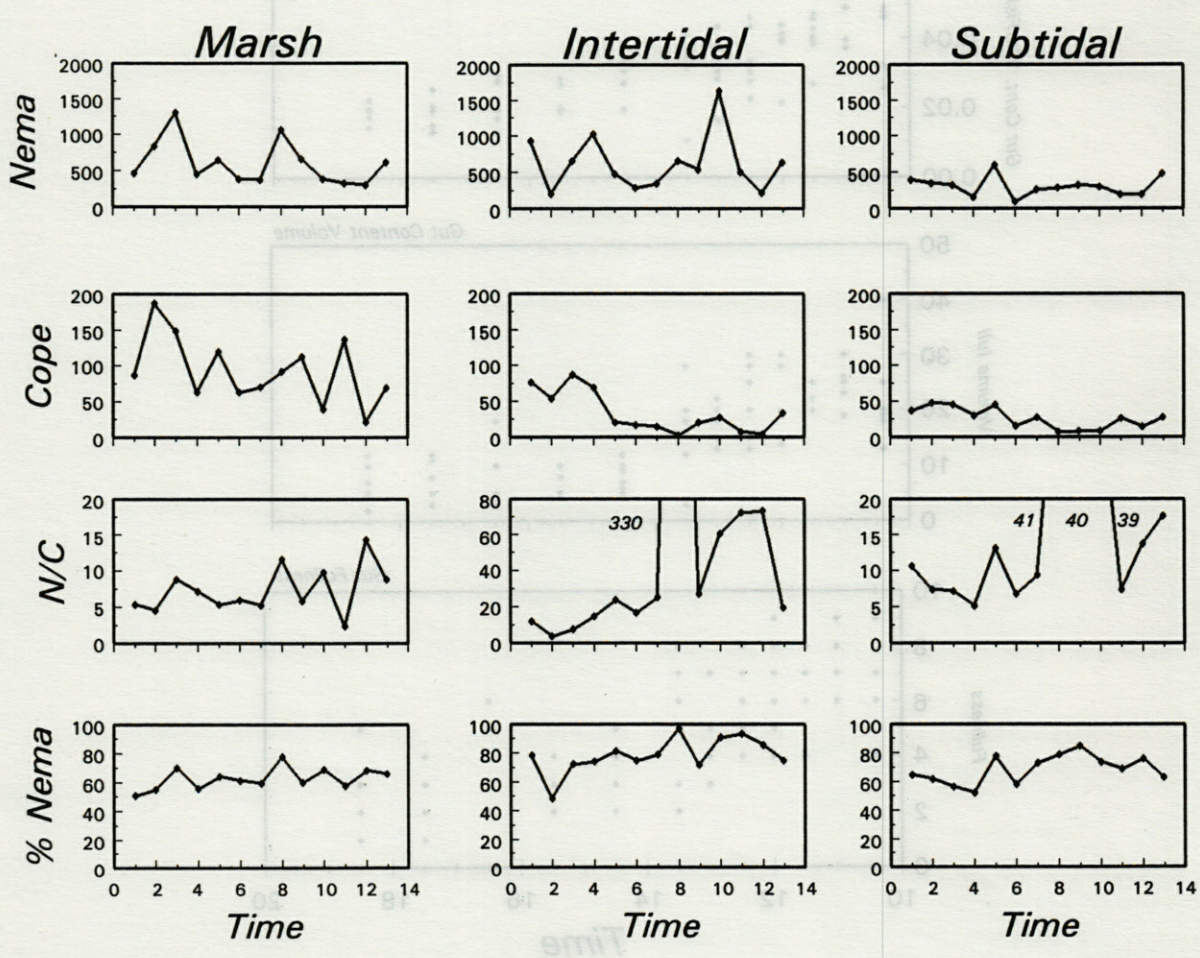

Fig. 6. – The abundance of meiofaunal nematodes and copepods (per core of area 5.6 cm^2), the nematode/copepod ratio (N/C), and the percentage of nematodes in the samples collected every 2 hours beginning on 22 May at 1000 hrs (= Time 1) and ending at 1000 hrs on 23 May 1986 (= Time 13).

gut evacuation into account, one might double this estimate because the feeding period (time during which prey abundance in the foregut was increasing) often lasted an equivalent amount of time. Thus a low estimate of 2 and a high estimate of about 4 gut fillings per day is appropriate.

Under the conditions above, the highest average number of prey in a presumably full foregut (Tables I, II) was multiplied by 2 and by 4 to estimate a low and high crude numerical daily ration each year (Table IV). A maximum ration was obtained by multiplying the maximum number observed in any one fish's foregut by 4.

DISCUSSION

The most important aspect of this study is not the crude estimates of daily ration provided but rather the clear demonstration that meiofauna, particularly nematodes, are ingested by juvenile spot in about the same proportion as they exist in the sediment. Our data provide strong evidence that juvenile spot utilize benthic prey non-selectively, despite their apparent selective ingestion of benthic copepods. The close correspondence between the proportion of prey in the foregut that are nematodes (Table III) and the nematode fraction of meiofauna taken in sediments where juvenile spot feed (Fig. 6) is strong evidence for non-selective incorporation of meiofaunal prey in the young spot's diet. Even though juvenile spot only bite to 2-4 mm in the sediment (Billheimer & Coull 1989), and there are proportionally more copepods in the upper 5 mm of sediments (Coull *et al.* 1989), nematodes still comprise a substantial portion of the diet (Fig. 4). We have no doubt that juvenile spot are selective about where they take bites from the sediment surface – they prefer to take live benthic copepods over dead ones and prefer both of these to nematodes (dead or alive, see Nelson and Coull, 1989). The prevalence of copepods reported in diet descriptions of spot (e.g., Roelofs 1954, Kobylinski and Sheridan 1979, Sheridan and Livingston (1979), Chestnut 1983, Martin *et al.* 1989, and others cited by Coull 1990) may be incorrect, perhaps resulting from analysis of fish collected long after they had actually fed or when only hindguts contained prey. Thus collections made at times between the late phase of low tide and the beginning of ebb tides in nearshore tidal creek systems are least likely to provide samples that reflect the true diet of juvenile spot.

Our estimates of the number of meiofaunal nematodes, copepods, and foraminiferans eaten by 27-33 mm spot are, of course, based on averages. There are undoubtedly spot of this size that ingest many more prey than we suggest is a reasonable maximum daily ration. Our experience with this fish convinces us that there is considerable variability in gut contents among individuals of the same size. Laboratory experiments with small schools of juvenile spot have revealed the presence of feeding dominance hierarchies (Scholz *et al.* 1991), so it is understandable why such dramatic fish-to-fish variability exists. Given the typical spatial heterogeneity of meiofaunal distributions (e.g., Fleeger and Decho 1987), fish that feed in different areas will obviously ingest very different numbers of prey. Therefore, our estimates would not have tight confidence limits. In fact, computation of such limits might be so misleading (and wide, see Andersen 1985; Bromley 1994) that we chose not to try and compute them.

The last point to make is the potential impact of spot on meiofaunal populations. First recall how many meiofauna were present in the sediment at our three sampling sites during high and ebbing tides when spot were feeding (Fig. 6). Seldom did the counts exceed 1000 nematodes or 100 copepods per core of area 5.6 cm^2, both numbers that fall readily within the range of daily ration estimates (Table IV). Thus a spot can probably attain its daily ration from the equivalent of a few 10 cm^2 plots of sediment in the saltmarsh. Since numerical standing stocks of these taxa 1) maintain themselves at fairly constant levels over intervals of time on the order of days to weeks and change dramatically only on the seasonal scale (Coull 1985; Eskin and Coull 1987), 2) replenish themselves via tidally-induced recolonization (Palmer 1988) and 3) reproduce so rapidly that mimicked predation of 90% made no impact on the population density (Woods & Coull 1992), the impact of spot predation on the abundance of meiofauna in North Inlet is not great. Juvenile spot abundances in North Inlet are not known with

	Daily Ration Estimates					
	1986			1988		
Prey Taxon	Low	High	Max	Low	High	Max
Copepods	144	288	392	108	216	484
Forams	60	120	580	30	60	120
Nematodes	988	1976	4000	322	644	2008

Table IV. – Low, high, and maximum estimates of the number of meiofaunal copepods, foraminifera, and nematodes in the daily ration of juvenile spot in North Inlet, South Carolina, in 1986 and 1988.

great precision, but estimates from seine collections (Allen *et al.* 1992), creek block netting (Bozeman and Dean 1980), and quantitative sampling in other estuaries (e.g., Hettler 1989; Kneib 1991; Baltz *et al.* 1993) indicate between 1 and 10 fish per m^2 are present during high tide at this time of year (schools of juvenile spot, however, may attain much higher densities). Therefore, by removing prey from only a few thousandths of a square meter each day, juvenile spot predation alone cannot be a major source of mortality for meiofauna.

ACKNOWLEDGEMENTS – We would like to thank many others who helped collect and analyze samples for this study, especially Brian Hentschel who performed most of the stomach contents analyses and Bettye Dudley who counted the core samples. Others who assisted in various ways include Maria Ellis, Lauren Billheimer, Amy and Bob Ferguson, Greg Zagursky, Debbie Scholz, and Lara Matthews. Funding was provided by NSF grant OCE 85-21345 (B.C. Coull and R.J. Feller, principal investigators). This is Contribution Number 1042 from the Belle W. Baruch Institute.

LITERATURE CITED

ALLEN D.M., S.K. SERVICE, M.V. OGBURN-MATTHEWS, 1992. Factors influencing the collection efficiency of estuarine fishes. *Trans. Am. Fish. Soc.* **121** : 234-244.

ANDERSEN K.P., 1985. Some reflections on the possibility of estimating predation and digestion parameters from stomach data. *Dana* **5** : 87-94.

ARCHAMBAULT J.A., R.J. FELLER, 1991. Diel variations in gut fullness of juvenile spot, *Leiostomus xanthurus* (Pisces). *Estuaries* **14** : 94-101.

BALTZ D.M., C. RAKOCINSKI and J.W. FLEEGER, 1993. Microhabitat use by marsh-edge fishes in a Louisiana estuary. *Env. Biol. Fish.* **36** : 109-126.

BILLHEIMER L.E. and B.C. COULL, 1988. Bioturbation and recolonization of meiobenthos into juvenile spot (Pisces) feeding pits. *Estuar. Coast Shelf Sci.* **27** : 335-340.

BOISCLAIR D. and F. MARCHAND, 1993. The guts to estimate fish daily ration. *Can. J. Fish. Aquat. Sci.* **50** : 1969-1975.

BOZEMAN E.L. Jr., and J.M. DEAN, 1980. The abundance of estuarine larval and juvenile fish in a South Carolina tidal creek. *Estuaries* **3** : 89-97.

BROMLEY P.J., 1994. The role of gastric evacuation experiments in quantifying the feeding rates of predatory fish. Reviews in Fish Biology and Fisheries **4** : 36-66.

CHESTNUT D.E., 1983. Feeding habits of juvenile spot *Leiostomus xanthurus* (Lacepede) in North Inlet Estuary, Georgetown, S.C. M.S. Thesis, Univ. of South Carolina, Columbia, SC, 68 pp.

COULL B.C., 1985. Long-term variability of estuarine meiobenthos : an 11-year study. *Mar. Ecol. Prog. Ser.* **24** : 205-218.

COULL B.C., 1990. Are members of the meiofauna food for higher trophic levels ? *Trans. Amer. Microsc. Soc.* **109** : 233-246.

COULL B.C. and R.J. FELLER, 1988. Site-to-site variability in abundance of meiobenthic copepods along a tidal gradient over 24 hours. *Hydrobiologia* **167/168** : 477-483.

COULL B.C., M.A. PALMER and P.E. MYERS, 1989. Controls on the vertical distribution of meiobenthos in mud : field and flume studies with juvenile fish. *Mar. Ecol. Prog. Ser.* **55** : 133-139.

CURRIN B.M., J.P. REED and J.M. MILLER, 1984. Growth, production, food consumption, and mortality of juvenile spot and croaker : a comparison of tidal and non-tidal nursery areas. *Estuaries* **7** : 451-459.

ELLIS M.J. and B.C. COULL, 1989. Fish predation on meiobenthos : field experiments with juvenile spot *Leiostomus xanthurus* Lacepede. *J. exp. mar. Biol. Ecol.* **130** : 19-32.

ESKIN R.A. and B.C. COULL, 1987. Seasonal and three-year variability of meiobenthic nematode populations at two estuarine sites. *Mar. Ecol. Prog. Ser.* **41** : 295-303.

FELLER R.J., B.C. COULL and B.T. HENTSCHEL, 1990. Meiobenthic copepods : tracers of where juvenile *Leiostomus xanthurus* (Pisces) feed ? *Can. J. Fish. Aquat. Sci.* **47** : 1913-1919.

FLEEGER J.W. and A.W. DECHO, 1987. Spatial variability of interstitital meiofauna : a review. *Stygologia* **3** : 35-54.

GEE J.M., 1989. An ecological and economic review of meiofauna as food for fish. *Zool. J. Linnean Soc.* **96** : 243-261.

HETTLER W.F., Jr., 1989. Nekton use of regularly-flooded saltmarsh cordgrass habitat in North Carolina, USA. *Mar. Ecol. Prog. Ser.* **56** : 111-118.

KNEIB R.T., 1991. Flume weir for quantitative collection of nekton from vegetated intertidal habitats. *Mar. Ecol. Prog. Ser.* **75** : 29-38.

KOBYLINSKI G.J. and P.F. SHERIDAN, 1979. Distribution, abundance, feeding and long-term fluctuations of spot, *Leiostomus xanthurus*, and croaker, *Micropogonias undulatus*, in Apalachicola Bay, Florida, 1972-1977. *Contrib. Mar. Sci.* **22** : 149-161.

MARTIN T.H., R.A. WRIGHT and L.B. CROWDER, 1989. Non-additive impact of blue crabs and spot on their prey assemblages. *Ecology* **70** : 1935-1942.

MULLEN A.J., 1986. The effect of the choice of evacuation model on the estimation of feeding rate. *Env. Biol. Fish.* **16** : 213-217.

NELSON A.L. and B.C. COULL, 1989. Selection of meiobenthic prey by juvenile spot (Pisces) : an experimental study. *Mar. Ecol. Prog. Ser.* **53** : 51-57.

PALMER M.A., 1988. Epibenthic predators and marine meiofauna : separating predation, disturbance, and hydrodynamic effects. *Ecology* **69** : 1251-1259.

PENNINGTON M., 1985. Estimating the average food consumption by fish in the field from stomach contents data. *Dana* **5** : 81-86.

PERSSON L., 1986. Patterns of food evacuation in fishes : a critical review. *Env. Biol. Fish.* **16** : 51-58.

ROELOFS E.W., 1954. Food studies of young sciaenid fishes, *Micropogon* and *Leiostomus*, from North Carolina. *Copeia* **2** : 151-153.

SCHOLZ D.S., L.L. MATTHEWS and R.J. FELLER, 1991. Detecting selective digestion of meiobenthic prey by juvenile spot *Leiostomus xanthurus* (Pisces) using immunoassays. *Mar. Ecol. Prog. Ser.* **72** : 59-67.

SHERIDAN P.F. and R.J. LIVINGSTON, 1979. Cyclic trophic relationships of fishes in an unpolluted, river-dominated estuary in north Florida. *In* Ecological Processes in Coastal and Marine Systems. R.J. Livingston (ed.), Plenum Press, New York, 143-161.

SMITH L.D. and B.C. COULL, 1987. Juvenile spot (Pisces) and grass shrimp predation on meiobenthos in muddy and sandy substrata. *J. exp. mar. Biol. Ecol.* **105** : 123-136.

WOODS R.E. and B.C. COULL, 1992. Life history responses of *Amphiascus tenuiremis* (Copepoda, Harpacticoida) to mimicked predation. *Mar. Ecol. Prog. Ser.* **79** : 225-234.

YETMAN K.T., 1979. The morphology of the buccopharyngeal filter of an estuarine fish, the spot, *Leiostomus xanthurus*. M. Sc. Thesis, University of South Carolina, Columbia, SC USA, 80 pp.

Reçu le 16 août 1994; received August 16, 1994
Accepté le 15 novembre 1994; accepted November 15, 1994

VIE MILIEU, 1995, 45 (1) : 61-73

PREDATION BY JUVENILE FISH ON HYPERBENTHIC MEIOFAUNA : A REVIEW WITH DATA ON POST-LARVAL *LEIOSTOMUS XANTHURUS*

***J.N. McCALL*[1] *and J.W. FLEEGER*[2]**

[1]*Department of Biology, Livingston University, Livingston, AL 35470 USA*
[2]*Department of Zoology, Louisiana State University, Baton Rouge, LA 70803 USA*

MEIOFAUNA
HYPERBENTHOS
FISH
PREDATION

ABSTRACT – In recent years, the significance of meiofauna, notably harpacticoid copepods, in the diet of many species of post-larval and juvenile fish has been increasingly recognized. In many cases, however, much uncertainty remains regarding the manner in which these meiofaunal prey are utilized. Often, ingested meiofauna differ from those present in the sediment at the level of major taxon, species or demographic composition. An investigation of the life-styles demonstrated by meiofauna can aid in understanding these patterns of apparent selectivity. Of particular importance may be the tendency of many meiofaunal organisms to emerge, actively or passively, from sediments during immersion. In a number of cases, ingested meiofauna bear closer resemblance to near-bottom water-column assemblages than to sediment-dwelling ones. An example is presented for post-larval spot (*Leiostomus xanthurus*) in coastal Louisiana. Copepod prey of four size classes of spot (16 mm, 16-26 mm, 26-30 mm, 30-35 mm standard length) were compared to assemblages collected by various methods. Collections included sediment cores, settlement traps that collect water-column animals settling to (or moving along) the sediment surface, emergence traps that sample organisms moving away from the sediment surface at heights of approximately 7 cm and phytal samples (plant surfaces). Principal components analysis demonstrated that, as spot grew in size, their prey changed from an assemblage most closely resembling that found in settlement traps to one closely resembling a sediment assemblage. Additional study is needed to better understand the dynamics of hyperbenthic meiofauna and the manner in which this prey resource is utilized by fish and other predators.

MÉIOFAUNE
HYPERBENTHOS
POISSONS
PRÉDATION

RÉSUMÉ – L'importance de la méiofaune, notamment des Copépodes Harpacticoïdes, dans le régime alimentaire de nombreuses espèces de Poissons juvéniles est de plus en plus reconnue. Cependant, il persiste la plupart du temps, beaucoup d'incertitude en ce qui concerne la manière dont ces proies méiobenthiques sont utilisées. La méiofaune ingérée diffère souvent de celle qui se trouve dans le sédiment aux niveaux des taxons majeurs, des espèces ou de la composition démographique. Des recherches à propos des modes de vies concernant la méiofaune peuvent permettre de comprendre ces types de sélection apparente. La tendance de nombreux organismes de la méiofaune à émerger des sédiments de façon active ou passive pendant l'émersion semble d'une importance particulière. Dans certains cas, la méiofaune ingérée montre une ressemblance plus grande avec la structure faunistique la colonne d'eau qu'avec celle du sédiment. Nous présentons l'exemple de stades post-larvaires *Leiostomus xanthurus* de la côte de Louisiane. Les Copépodes-proies de 4 classes de taille de *L. xanthurus* (16 mm, 16-26 mm, 26-30 mm, 30-35 mm de longueur) sont comparés aux communautés récoltées par diverses méthodes. Ces prélèvements comprennent des carottages, des pièges « d'installation » qui recueillent les organismes de la colonne d'eau qui se fixent ou nagent à la surface du sédiment, des pièges retenant ceux qui s'échappent du sédiment jusqu'à une hauteur de 7 cm et des récoltes des phytontes de surface. Une analyse en composante principale montre que, tandis que la taille de *L. xanthurus* croit, les proies passent de celles des pièges « d'installation » à celles des communautés intrasédimentaires. Une étude complémentaire permettra de mieux comprendre la nature de la méiofaune hyperbenthique et comment les ressources alimentaires dues aux proies sont utilisées par le Poisson et les autres prédateurs.

INTRODUCTION

Meiofauna have been recognized as a significant component of marine and estuarine ecosystems for over fifty years (Coull & Giere, 1988). Over the last twenty years, however, our view of their place in the trophic dynamics of marine and estuarine food webs has changed dramatically. Once considered to be a trophic sink (McIntyre, 1969; McIntyre & Murison, 1973) responsible primarily for recycling of organic matter within sediments, meiofauna are now recognized as an important pathway of energy transfer to selected juvenile fish and other epibenthic predators (Gee, 1989; Coull, 1990).

Although researchers have gained much insight into fish predation on meiofauna, a number of questions remain unanswered. Several questions deal with the manner in which the predatory behavior of meiofaunal-feeding fishes interacts with the behavior of their prey. One critical need in fish trophic ecology is synoptic studies of feeding habits and prey availability (Miller & Dunn, 1980). Determining prey availability, however, is not as straight-forward as measuring prey densities. Many components of prey preference, including encounter rates and capture success, are under the influence of both prey and predator behavior (Sih & Moore, 1990). Interactions between juvenile fish behavior and the behavior of their meiofaunal prey should affect their predator-prey relations in a number of ways, including the degree of selectivity, real or apparent, demonstrated by the predator and the specific meiofaunal assemblage utilized among the several distinct groups available.

The purpose of this review is to broadly address meiofaunal-fish behavioral interactions and to explore in detail one aspect of prey behavior. Emergence of meiofauna is just being recognized and is an important behavioral influence on predatory interactions between fishes and their prey. This review is structured to examine :
1) the manner in which meiofaunal prey are utilized by fish,
2) prey selectivity at the level of major taxa, species, and demographic group, and
3) the significance of the specific habitat utilized by the meiofaunal prey, with particular emphasis on emergent meiofauna.

The nature of emergence by meiofauna is discussed, and the behavior of meiofauna, regarding emergence, as well as the predator's response, forms the central theme of this review. In addition, the results of studies with post-larval spot (*Leiostomus xanthurus*) from coastal Louisiana are included to further illustrate the significance of these interactions.

HOW DO FISH FEED ON MEIOFAUNA

Now that the significance of meiofauna in the diets of juvenile fish can no longer be questioned, we must begin to address more sophisticated questions about the ways in which meiofaunal predators interact with their prey. This requires a consideration of the manner in which the predatory behavior of the meiofaunal feeder interacts with the morphology and behavior of the potential prey.

Hyatt (1979) examined data from a variety of aquatic ecosystems and found that in most cases, carnivores feeding on benthic invertebrates made up the greatest percentage of fish species. Within this broad class, however, a number of different feeding modes can be identified. Keenleyside (1979) identified feeding four categories in benthic-feeding carnivorous fishes :
1) picking at small prey,
2) picking up substrate and sorting prey,
3) disturbing substrate, then picking up prey, and,
4) grasping relatively large prey.

Meiofaunal-feeding fish likely demonstrate all of these modes of feeding. Certain grassbed fishes, such as the spotted dragonet (*Callionymus pauciradiatus*), are among those meiofaunal feeders that pick at small prey (Sogard, 1984). Several marine fishes, mostly in the families Gobiidae and Sciaenidae, feed by biting the sediment and sorting prey. Perhaps the most well-studied meiofaunal feeding fish is spot, *Leiostomus xanthurus*. At sizes > 30 mm SL, juvenile spot feed heavily on meiofauna by biting into soft sediments, manipulating the sediment within the mouth, extracting the contained organisms with gill rakers and pharyngeal teeth, and then expelling the sediment through the gill openings and the mouth (Billheimer & Coull, 1988). Spot continue to feed heavily on meiofauna to standard lengths > 100 mm (Stickney *et al.* 1975). At these sizes, their reliance on meiofauna is so strong that the composition of stomach contents may be used to identify the site of feeding (Feller *et al.*, 1990). Spot is somewhat unique, not only in its prolonged utilization of meiofaunal-sized prey but also its sediment-sieving mode of feeding (McCall & Fleeger, 1993). However, the Atlantic croaker (*Micropogonias undulatus*) feeds in a similar manner. In addition, there is evidence that some gobies take bites of sediment (Grossman, 1980; Carle & Hastings, 1982; Toepfer & Fleeger, 1994). Other studies have found that gobies prey on meiofauna (Hartney, 1989; Aarnio & Bonsdorff, 1993; Zander, 1990); however, the source of meiofaunal prey is uncertain in these studies.

While there is no documentation of fishes deliberately disturbing the substrate and preying

on suspended meiofauna, it is likely that benthic fish prey heavily on meiofauna from the near-bottom water, perhaps suspended by currents or present in the water column by active emergence. Juvenile salmon and flatfishes are perhaps best studied. Several species of salmon are known to feed on harpacticoid copepods (Sibert *et al.*, 1977; Sibert, 1979; Webb, 1991), and most evidence suggests that the source of their prey consists of emergent copepods from seagrass beds. Cordell (1986) found that harpacticoids common in the diet are also common in epibenthic sled samples and Webb (1991) found that harpacticoids in the diet of salmon are capable swimmers that are ingested by salmon swimming over grass beds. The group perhaps best known for preying on emerged meiofauna is the juvenile flatfish. Many species of flatfish feed using a lay-in-wait method and strike at prey above them (Stickney *et al.*, 1973). Juveniles of several flatfish feed heavily on harpacticoid species known to be active in the near-bottom water (McCall, 1992; Toepfer & Fleeger, 1994). Juvenile predation on meiofauna may begin soon after metamorphosis, at standard lengths (SL) < 10 mm (McCall, 1992). At such sizes, the meiofaunal prey, which are between 0.5 and 1.0 mm, constitute a relatively large prey item. Other species of fish may well feed in the near-bottom waters. Likely prospects include gobies, blennies and pipefish.

In short, meiofaunal-preying fish rely on the full gamut of feeding strategies available to benthic carnivorous fishes, and the particular strategy utilized by a given species or size class will doubtless influence the manner in which it interacts with meiofaunal prey.

PREY SELECTIVITY

The degree to which meiofaunal-feeding fish select one prey type over another and the causal factors underlying such selection are the subject of ongoing debate. Fish and meiofauna present an excellent opportunity for the investigation of selective feeding, since large numbers of individuals can be collected and processed to allow for proper statistical analysis of selection. Selectivity can be considered at three levels : major taxon, species and demographic group. Each level of selectivity carries with it specific questions about the manner in which behavioral mechanisms of both predator and prey impact on the interaction.

Major taxon selectivity

The question of whether juvenile teleosts select one component of the meiofauna over another has been extensively investigated, and has been discussed in detail in two recent reviews (Gee, 1989; Coull, 1990). This question typically focuses on two meiobenthic groups, harpacticoid copepods and nematodes. In general, harpacticoids are the most common meiofaunal prey of juvenile fish, even though they are typically outnumbered, by as much as two orders of magnitude, by nematodes in the sediments. At least three explanations have been suggested for this phenomenon.

1) Active selection of harpacticoids over nematodes may result from greater energetic content, high concentrations of essential fatty acids, or movement-related visibility differences (Coull, 1990).

2) Differential rates of digestion for nematodes and harpacticoids may lead to nematodes becoming rapidly indistinguishable in the digestive tract, resulting in prey counts which are biased toward harpacticoids (Scholz *et al.*, 1991).

3) Differential availability of harpacticoids and nematodes resulting from their differing vertical distribution within the sediment (Gee, 1989). Nematodes are typically distributed to a much greater depth in the sediment than harpacticoids, particularly in the muddy sediments in which fish predation on meiofauna is most significant.

To this list, we may now add a fourth potential explanation. Harpacticoids commonly emerge into near-bottom waters, while nematodes are underrepresented in this habitat (Armonies, 1988; Walters & Bell, 1986). Sun & Fleeger (1994) report that harpacticoids colonize through the water column while nematodes colonize on the sediment surface. This difference in behavior is potentially important to fish that do not bite sediments, but nevertheless ingest large numbers of harpacticoids.

Species selectivity

Relatively few studies have attempted to determine the species composition of harpacticoids preyed upon by juvenile fish. Although Feller *et al.* (1990) found that harpacticoids may be used to trace feeding in different habitats, most studies have found that the assemblage of copepods ingested by fish does not closely correspond to that found in meiobenthos in the area in which the fish were collected (Alheit and Scheibel 1982, Tito de Morais & Bodiou 1984, Gee 1987). This tendency seems to be substrate-related, with fish feeding in areas with muddy substrates preying on an assemblage more closely resembling that collected in sediment samples than fish feeding in sandy areas (Gee, 1987), perhaps reflecting the more surficial distribution of harpacticoids in muds. Species-specific selectivity of predators for one or several

harpacticoid species has been observed in a number of studies (Woodin, 1977; Gee, 1987). Many investigators, however, still do not make time-consuming species-level identifications. Several recent investigations (Keats *et al.*, 1993; Keats & Steele, 1993; Shaw & Jenkins, 1992; Sogard, 1992; Zander & Heymer, 1992) point out the importance of harpacticoids in the diet of various fishes, but because species-specific identifications were not made, it is not possible to determine the meiofaunal assemblage that served as prey.

Given the increasing number of studies that conclude that juvenile fish do not select meiofaunal prey in proportion to their sediment abundance, it should prove prudent to more closely examine the species of meiofauna that are ingested. Such studies are necessary to understand why a particular subset of the meiofaunal community is disproportionately significant in the feeding ecology of juvenile fish. This could be of particular importance in assessing the energetic value of meiofauna to juvenile fish, and could influence estimates of the value of a given habitat to developing juveniles.

Demographic group selectivity

The question of whether one demographic group might be more heavily preyed upon than others has remained largely unaddressed, although there are size, behavioral and morphological differences among males, females and copepodites of harpacticoid copepods that might well be expected to contribute to such differential predation.

McCall (1992) found that juvenile starry flounder ingested male *Microarthridion littorale* in proportions much higher than their representation in sediments and suggested that this phenomenon might be the result of an increased tendency of males to enter the water column. Hicks and Marshall (1985) found that the guts of deep-sea carnivorous bivalves contained almost exclusively male harpacticoids, and go on to suggest that selective predation on males might account for the typical dominance of female harpacticoids in the deep sea.

Selective predation on female calanoid copepods in freshwater ponds has been related to greater visibility of females, particularly those carrying eggs (Hairston *et al.*, 1983). Furthermore, Maly (1970) found that predation could alter the adult sex ratios of calanoid copepods in a manner which was influenced by predator hunting behavior and by differences in size and activity of the male and female prey.

MEIOFAUNAL LIFE-STYLES

Prey selection by fish feeding on meiofaunal organisms may be a function of the meiofaunal habitat as modified by prey behavior. Given the diverse nature of meiofaunal assemblages, quantitative studies characterizing these assemblages in a given area are necessary for an understanding of the feeding behavior of juvenile fish utilizing them. If emergent behavior of meiofauna is an important factor in predator-prey interactions, it is important that the pattern of this behavior be examined in greater detail.

Hicks and Coull (1983), in their review of harpacticoid copepod ecology describe a variety of modes of existence. Within the benthos, harpacticoids may be found living interstitially, epibenthically, or as infaunal burrowers, with the interstitial lifestyle limited primarily to sandy substrates and the burrowers found mainly in muddy sediments. Hicks and Coull also recognize phytal harpacticoids and a few species which are wholly planktonic. To their list should be added another mode of benthic existence, that of tube-dwelling as demonstrated by Chandler and Fleeger (1984) for *Pseudostenhelia wellsi*. In addition, it is now well-established that many harpacticoid species occupy a hyperbenthic or demersal habitat, emerging to spend some fraction of their life in the near-bottom waters within a few mm-cm of the sediment surface (Sibert, 1981).

Given the diversity of feeding strategies utilized by predatory fishes and the wide range of microhabitats occupied by harpacticoid copepods, it is appropriate to consider the manner in which the feeding behavior of juvenile fish interacts with habitat utilization of harpacticoid copepods to produce specific predator-prey relationships. A given assemblage of potential prey does not result solely from the behavior of the predatory fish, or from that of the meiofauna in the area, but rather from how these two behaviors interact to bring predator into contact with prey.

The significance of emergent meiofauna

Perhaps the least understood, albeit potentially important, assemblage of harpacticoids with regard to trophic interactions with juvenile fish is the emerged, sometimes called hyperbenthic or demersal, assemblage. Beyer (1958) introduced the term hyperbenthos in reference to plankton populations near the sediment-water interface, although we use this term to refer to meiofauna emerged into the near-bottom water. Sibert (1981) illustrates that the hyperbenthos is typically dominated by animals of two origins, downward moving planktonic species and upwardly mobile

surface-dwelling benthic species. The existence of a near-bottom meiofaunal assemblage has been documented in recent years, and it is now apparent that the traditional meiofauna, particularly harpacticoids, occur regularly in the water column (Jacoby & Greenwood, 1989; 1993, Metaxas & Scheibling, 1994). This may result from passive resuspension (Hagerman & Rieger, 1981; Palmer & Gust, 1985), active emergence (Armonies, 1988; Bell *et al.*, 1988; Walters & Bell, 1986; Alldredge & King, 1985; Armonies, 1989) or a combination of the two. The relative importance of the two mechanisms is related to the species under consideration and to the habitat (Palmer & Gust, 1985). Passive resuspension, like active emergence, involves behavioral aspects of harpacticoid ecology, since the habitat occupied by the organisms greatly influences their likelihood of being resuspended (Palmer, 1988b). There is much to learn about the hyperbenthos because sampling problems have slowed study.

For harpacticoid copepods, evidence suggests that interstitial species avoid suspension by moving deeper in the sediment during flow events (Foy & Thistle, 1991), however, mud-dwelling, epibenthic harpacticoids do not appear to avoid emergence through behavior (Palmer, 1984). Thus, the emergence, by whatever mechanism, of harpacticoids into the water column may provide some adaptive advantage with regard to reproduction (Hicks, 1988), feeding (Decho, 1986; Sibert, 1981) or avoidance of infaunal predators (Thayer, 1985). Emergence almost certainly, however, increases their susceptibility to predation by small fish feeding near the sediment surface. Very few species of fish actually bite into sediments in search of prey (spot is a notable exception). Most adult demersal predatory fish feed on individual prey which are in near-bottom waters. This is likely true of juvenile fish which utilize this habitat as well. If, in fact, much predatory behavior is focused on the near-bottom water, then it is reasonable to suppose that the most significant prey assemblage is the one which frequents this habitat. Unfortunately, this is perhaps the most poorly understood of all meiofaunal assemblages.

As information begins to accumulate on the hyperbenthic meiofauna, it is becoming clear that this fauna is often quite different from that in the sediments, both with regard to species composition and demographic status. Walters and Bell (1986) found that harpacticoid copepods numerically dominated the taxa which actively migrated in a subtidal creek bed. They found that from 13 to 67 % of all benthic harpacticoids migrated into the water column. Adult harpacticoids exhibited both diel and sampling date differences in migration. This difference may well be reflected in greatly different prey assemblages in juvenile fish than might be predicted based on a knowledge of the benthic meiofauna. McCall (1992) found that harpacticoids collected in settlement traps differed from those in adjacent sediments, and were more closely similar to those ingested by juvenile starry flounder.

Bell *et al.* (1988) present evidence that the adult sex ratios of abundant copepods collected in the water column may differ significantly from conspecifics on the substratum, with males typically much more abundant in the water column than on seagrass blades or in sediments. This is in keeping with the hypothesis that emergent behavior might be linked to a precopulatory association between adult males and juvenile females (Hicks, 1988). The potential impact that this differential utilization of the near-bottom habitat with its potentially greater risk of predation has remained largely unexamined.

EXPERIMENTAL RESULTS WITH POST-LARVAL SPOT

McCall (1992) showed that an approach that relates different potential prey assemblages to diet can be useful in more fully understanding the feeding behavior of a meiofaunal-feeding flatfish. Additional evidence comes from studies conducted on post-larval and juvenile spot (*Leiostomus xanthurus*) in a Louisiana estuarine complex. Spot's heavy and prolonged utilization of meiofauna (especially harpacticoid copepods, nematodes and small polychaetes) and amphipods as a food source is well documented (Smith & Coull, 1987; Marinelli & Coull, 1987; Stickney *et al.*, 1975; Palmer, 1988a; Sheridan & Livingston, 1979; Livingston, 1988). Much less is known, however, about early life-history (< 25 mm SL) feeding habits and ontogenetic shifts leading to meiofaunal feeding. Here, we focus on the feeding behavior of early post-larval spot (10-35 mm SL).

A total of 131 juvenile spot (from 12-35 mm SL) were seine-collected in winter and early spring of 1991 in Bay Champagne near the Louisiana Universities Marine Consortium facility at Port Fourchon, Louisiana. An assortment of meiofaunal samples were taken in conjunction with fish collections throughout the spring of 1991, but one sampling date (February 23, 1991) is discussed in detail because a broad size range of spot were collected, and weather conditions allowed collection of a complete array of meiofaunal samples. Collections were conducted by the methods of McCall (1992) and included : 1) vertically sectioned cores from haphazardly selected locations along a transect at the 0 m tide level to a substrate depth of 2 cm with a hand-held piston corer and extruded at 2-mm-thick intervals to a

depth of 1 cm, 2) emergence traps that specifically sample the near bottom assemblage, 3) settlement/bedform traps to sample organisms settling to or moving along the sediment surface and 4) phytal samples collected by clipping submerged algae, mangrove roots, etc., in the area. Meiofauna were identified to the major taxon level, and copepods were identified, where possible, to species and demographic status. Copepod (calanoid, cyclopoid and harpacticoid) prey of juvenile spot determined by gut content analysis of various size classes were compared to assemblages collected by various sampling techniques. Comparisons were conducted using principal components analysis of the correlation matrix of the species-centered mean abundances of copepod species in the various assemblages (Ludwig & Reynolds, 1988).

The habitat information given here promotes understanding of the life style of copepods while it provides clues as to the mode of feeding by spot. Additional interpretation of meiofaunal life styles comes from Sun and Fleeger (1994) who sampled meiofauna colonizing sediment depressions along the Louisiana coast.

The sediment meiofaunal assemblage was numerically dominated by nematodes, with harpacticoid copepods the second most abundant taxon (Table I). Nematode densities were relatively high (*ca.* 330cm^{-3} in the upper 2 mm of sediment). Nematodes were most abundant in the surface sediments (0-2 mm depth), but remained abundant to depths of at least 1 cm, as is typical in muddy sediments (Hicks & Coull 1983). Densities of both adult harpacticoids and copepodites were 14.9cm^{-3} and 5.3cm^{-3}, respectively, in the 0-2 mm stratum, somewhat low for soft-sediment

Table I. – Summary of four vertically-sectioned meiofaunal samples collected on February 23 at Bay Champagne site. Data are presented as densities in number per cm^3 ± 1 standard error for each 2 mm thick sediment stratum for total meiofauna and for major components. Total Meio. = total meiofauna, Adult Harp. = adult harpacticoid copepods, Harp. Copep. = harpacticoid copepodites.

Stratum (mm depth)	Total Meio.	Nematodes	Adult Harp.	Harp. Copep.
0-2	362 ± 82	332 ± 78	14.9 ± 0.4	5.3 ± 2.0
2-4	129 ± 26	121 ± 26	4.5 ± 0.9	1.0 ± 0.5
4-6	143 ± 15	140 ± 16	2.1 ± 1.7	0
6-8	137 ± 15	128 ± 16	1.5 ± 0.8	1.3 ± 0.6
8-10	104 ± 14	95 ± 14	1.0 ± 0.6	0.5 ± 0.4

intertidal habitats of this type (Fleeger, 1980; 1985). The most abundant harpacticoids in sediment samples were *Paronychocamptus wilsoni,* adult *Coullana* sp. (referred to as *Scottolana canadensis* in previous work from Louisiana) and *Enhydrosoma* sp. All were concentrated in the upper 2-4 mm of the sediments, and densities dropped rapidly with depth (Fig. 1).

Settlement/bedform traps collected relatively low numbers of copepods (< 10 per trap). The majority of those collected, however, were cyclopoids and harpacticoids, suggesting an epibenthic/hyperbenthic life style (Fig. 2a). The most abundant harpacticoid was *Paronychocamptus wilsoni*; *Mesochra mexicana* and *Harpacticus* sp. were also found, but in lower numbers. Emergence traps contained cyclopoids and calanoids, but were dominated by harpacticoids, of which the majority were again *Paronychocamptus wilsoni* and *Coul-*

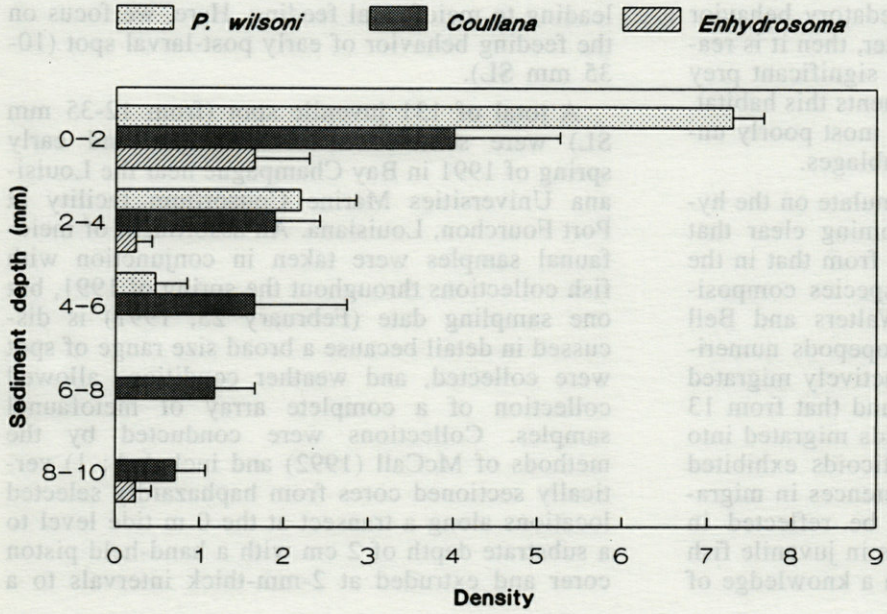

Fig. 1. – Vertical distribution of common harpacticoid species in sediment samples taken on February 23, 1991. Densities (no. cm^{-3} ± 1 SE) are given for each 2 mm thick sediment stratum. Each is based on four replicate samples.

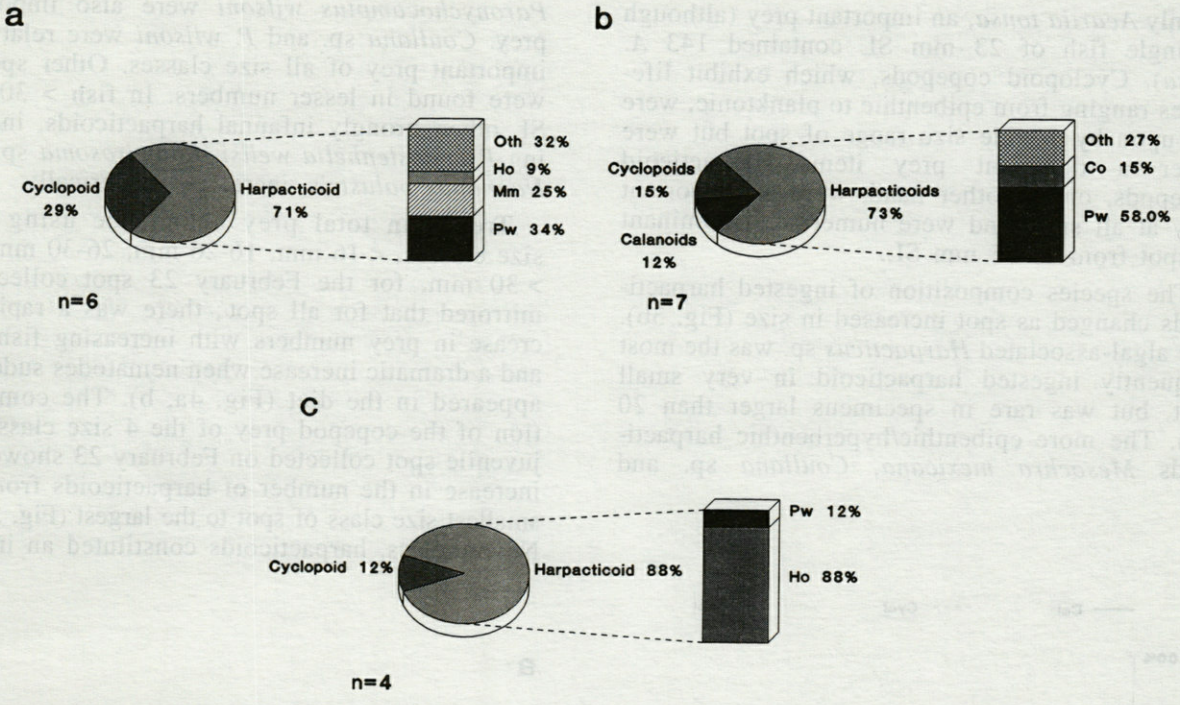

Fig. 2. – Composition of copepods collected in (a) settlement traps, (b) emergence traps and (c) algal samples on February 23, 1991. Pie diagram represents percentage by copepod order while bar chart represents species composition of harpacticoid copepods. Pw = *Paronychocamptus wilsoni*, Mm = *Mesochra mexicana*, Ho = *Harpacticus* sp., Co = *Coullana* sp., Oth = other harpacticoids.

lana sp. (Fig. 2b). *P. wilsoni* was represented in emergence trap samples by all demographic groups, while over 90 % of *Coullana* in these traps were juvenile copepodites. Other harpacticoids collected in appreciable numbers included *Harpacticus* sp., *Zausodes arenicola* and *Pseudobradya* sp. *Pseudobradya*, however, was collected only in one trap. Harpacticoids dominated phytal samples (Fig. 2c). *Harpacticus* sp. were dominant, making up some 80 % of the phytal harpacticoids, although *Paronychocamptus wilsoni* was also present in substantial numbers. Nematodes were common in settlement traps, reflecting the fact that these traps collect not only organisms that are actively emergent, but also those that are resuspended from surface sediments. Nematodes were rare, however, in emergence traps.

Of the spot examined, 106 contained prey. Over all size classes studied, post-larval and juvenile spot diets were dominated numerically by copepods and nematodes (Table II). Mean number of prey showed a general increase up to ca 30 mm SL, but was highly variable. At approximately 26 mm SL, juvenile spot began to take large numbers of nematodes in addition to copepods. This feeding shift was quite sudden and likely represents the initiation of sediment biting. At less than 25 mm SL, the strongly infaunal nematodes made up less than 1 % of ingested prey of spot, but comprised 35-73 % of the prey items in 25-40 mm SL spot. Developing spot also underwent a change in the types of copepods consumed at the ordinal level (Fig. 3a). Only in the smallest fish examined (< 15 mm SL) were planktonic calanoid copepods,

Size Class	n	Mean # prey	% Nema.	% Cal.	% Cycl.	% Harp.	% Oth.
12-15	28	5.3 ± 1.0	0.0	49.0	14.1	17.4	18.8
15-18	20	7.4 ± 1.9	0.1	4.1	2.7	66.0	27.2
18-21	10	18.2 ± 8.4	0.0	8.2	1.6	75.3	14.8
21-25	9	45.4 ± 17.1	0.0	30.0	0.0	66.3	3.7
25-28	6	6.8 ± 3.0	73.5	0.0	0.0	11.8	23.5
28-32	17	80.8 ± 44.0	65.6	0.1	0.1	11.6	1.5
32-35	10	41.3 ± 11.5	35.2	0.1	1.8	53.5	9.2
35-40	6	19.8 ± 3.2	51.9	0.0	0.0	34.2	13.9

Table II. – Summary of spot feeding data. Results are presented by size class (mm SL). Indicated are size classes, number of fish containing prey examined in each size class (n), mean and one standard error of number of prey within size classes, and percentage of prey of each size class made up of nematodes, calanoid copepods, cyclopoid copepods, harpacticoid copepods and other prey. Fish with empty stomachs are excluded.

mainly *Acartia tonsa*, an important prey (although a single fish of 23 mm SL contained 143 *A. tonsa*). Cyclopoid copepods, which exhibit lifestyles ranging from epibenthic to planktonic, were fed upon by a wide size range of spot but were never a dominant prey item. Harpacticoid copepods, on the other hand, were an important prey at all sizes and were numerically dominant in spot from 15-25 mm SL.

The species composition of ingested harpacticoids changed as spot increased in size (Fig. 3b). The algal-associated *Harpacticus* sp. was the most frequently ingested harpacticoid in very small spot, but was rare in specimens larger than 20 mm. The more epibenthic/hyperbenthic harpacticoids *Mesochra mexicana*, *Coullana* sp. and *Paronychocamptus wilsoni* were also important prey. *Coullana* sp. and *P. wilsoni* were relatively important prey of all size classes. Other species were found in lesser numbers. In fish > 30 mm SL other strongly infaunal harpacticoids, including *Pseudostenhelia wellsi*, *Enhydrosoma* sp. and *Nannopus palustris*, increased numerically.

Trends in total prey abundance using four size classes, < 16 mm, 16-26 mm, 26-30 mm and > 30 mm, for the February 23 spot collections mirrored that for all spot; there was a rapid increase in prey numbers with increasing fish size and a dramatic increase when nematodes suddenly appeared in the diet (Fig. 4a, b). The composition of the copepod prey of the 4 size classes of juvenile spot collected on February 23 showed an increase in the number of harpacticoids from the smallest size class of spot to the largest (Fig. 5a-d). Nevertheless, harpacticoids constituted an impor-

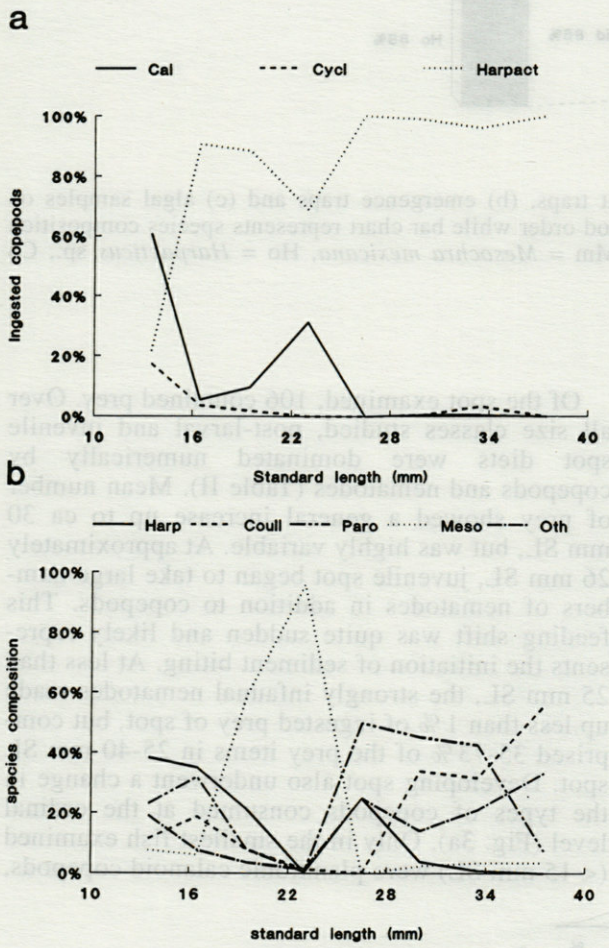

Fig. 3. – Copepod prey of 106 *Leiostomus xanthurus* in Bay Champagne, Louisiana in spring of 1991. (a) Percentage of copepod prey of various size classes represented by different orders of copepods. Cal = calanoids, Cyc = cyclopoids, Harpact = harpacticoids. (b) Percentage of ingested harpacticoids belonging to various species. Harp = *Harpacticus* sp., Coul = *Coullana* sp., Paro = *Paronychocamptus wilsoni*, Meso = *Mesochra mexicana*, Oth = other species of harpacticoids. Sample size within size class is indicated in Table II.

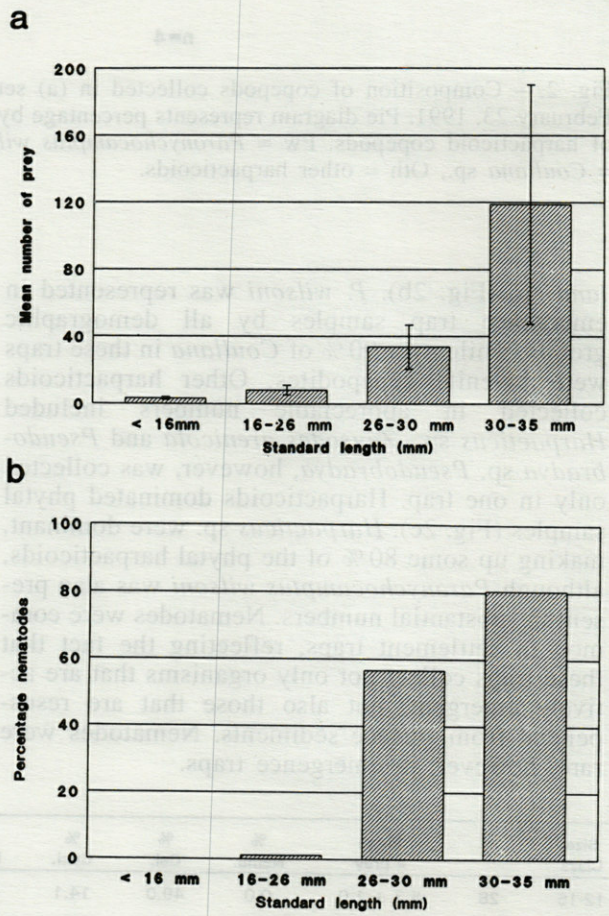

Fig. 4. – Prey contents of four size classes of spot collected on February 23, 1991. (a) Mean (and 1 SE) number of prey by size class. (b) Percentage of total prey (pooled) comprised by nematodes in each size class. Number of fish examined in each size class are as follows : < 16 mm – n=10, 16-26 mm – n=10, 26-30 mm – n=11, > 30 mm – n=11.

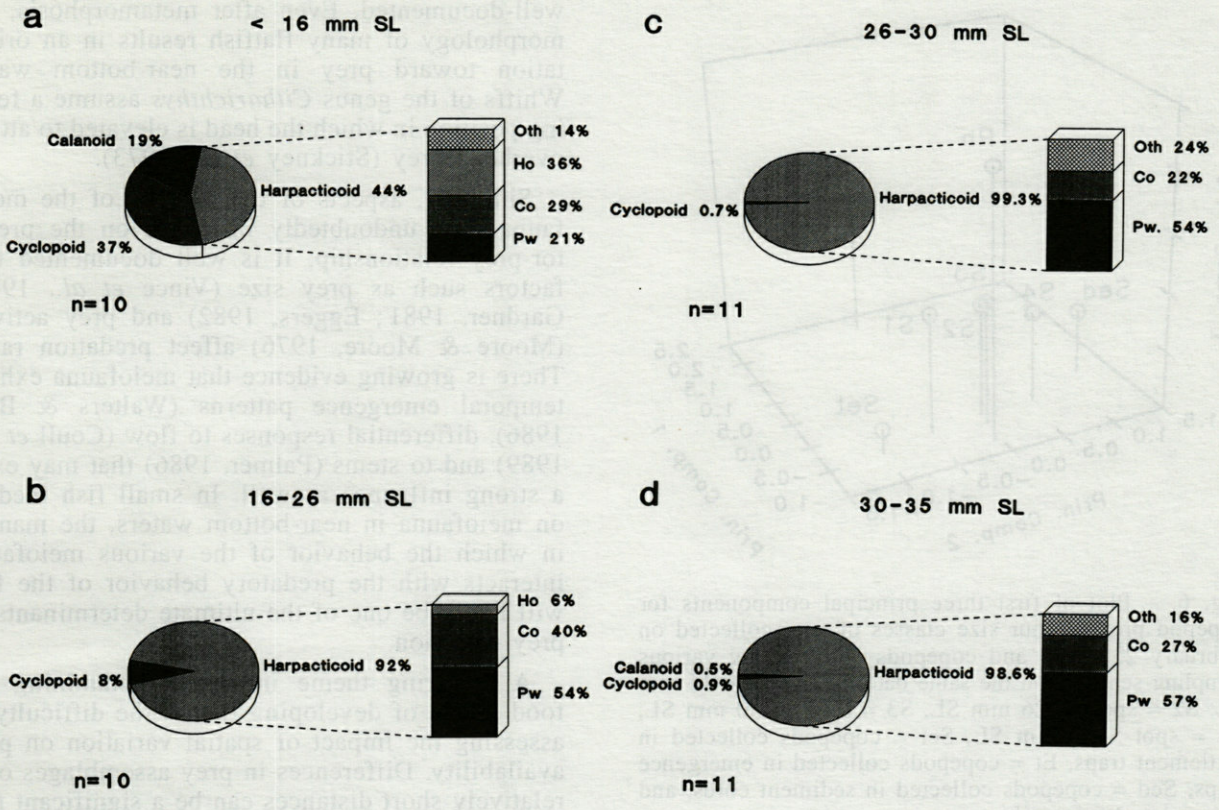

Fig. 5. – Composition of copepod prey in stomach contents of spot of four size classes collected on February 23, 1991. (a) Spot < 16 mm SL, (b) spot 16-26 mm SL, (c) spot 26-30 mm SL and (d) spot > 30 mm SL. Pie diagram represents percentage by copepod order while bar chart depicts species composition of harpacticoid copepods. Ho = *Harpacticus* sp., Co = *Coullana* sp., Pw = *Paronychocamptus wilsoni*, Oth = other species of harpacticoids.

tant prey resource in even the smallest fish. There was a decline in the importance of the epiphytic or hyperbenthic *Harpacticus* sp. as the fish grew larger. The widespread *Paronychocamptus wilsoni* and *Coullana* sp. were found in all size classes of spot.

It should be noted that, although nematodes make up over 80% of the prey of the largest size class of spot examined, they are still underrepresented in the diet relative to their sediment densities. In sediments, nematodes outnumber harpacticoid copepods by an order of magnitude or more, even in the uppermost 2 mm stratum. Thus, juvenile spot do ingest harpacticoids in greater numbers than would be predicted by their sediment abundance. This could be attributable to active selection or to mechanical selection for the more complexly shaped harpacticoids within spot's bucco-pharyngeal filtering apparatus (Nelson & Coull, 1989). Another possibility is that the larger spot, in addition to feeding on sediment-dwelling nematodes and harpacticoids, also ingest harpacticoids in the near-bottom waters, thus biasing their gut contents towards harpacticoids.

A plot of the first 3 principal components yielded by PCA of the copepod-species-centered data (Fig. 6) indicated that the prey assemblage of the smallest size class of spot did not closely resemble the sediment assemblage but grouped more closely with settlement-trap (near-bottom) assemblages. The prey ingested by intermediate size classes was less similar to the settlement-trap assemblage, moving closer to the sediment assemblage in principal component space. Prey of the largest spot examined were found to be most similar to the sediment copepods.

In summary, post-larval spot displayed a significant ontogenetic dietary shift from hyperbenthic/epiphytic species of copepods to sediment-dwelling meiofaunal prey. The diet of very early post-larval spot (< 16 mm) includes cyclopoid copepods, which inhabit a number of habitats, and planktonic copepods. The mouth of spot of this size moves from a somewhat terminal to a decidedly subterminal position (Yetman, 1979), and spot of this size probably shift from a diet of planktonic to hyperbenthic prey. Harpacticoid copepods that dominate the diet of these fish from 16-30 mm are ones known to exhibit epiphytic or hyperbenthic lifestyles, likely reflecting epibenthic feeding in these fish. The increased proportion of sediment-dwelling harpacticoids and the

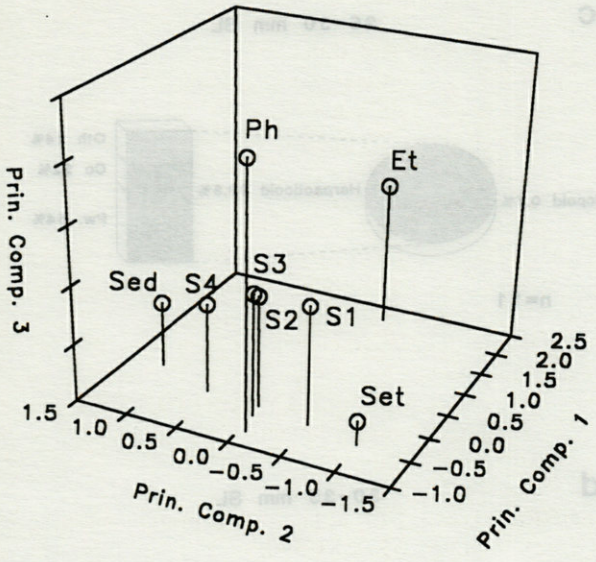

Fig. 6. – Plot of first three principal components for copepod prey of four size classes of spot collected on February 23, 1991 and copepods collected by various sampling schemes on the same date. S1 = spot < 16 mm SL, S2 = spot 16-26 mm SL, S3 = spot 26-30 mm SL, S4 = spot > 30 mm SL, Set = copepods collected in settlement traps, Et = copepods collected in emergence traps, Sed = copepods collected in sediment cores, and Ph = phytal copepods.

scarcity of calanoid and cyclopoids in the diet of larger spot (> 30 mm), coinciding with a dramatic increase in the number of nematodes in the diet, are certainly attributable to a shift in feeding location from the near-bottom water to the benthos.

FUTURE WORK

Many of the fish that rely heavily on a meiofaunal diet do so at a time when they are undergoing dramatic changes in both morphology and behavior. For example, the mouth of spot moves from a somewhat terminal to a decidedly subterminal position during its early post-larval development (Yetman, 1979). The change in prey utilization by juvenile spot from a near-bottom or hyperbenthic assemblage to a sediment-dwelling one likely results from ontogenetic changes in mouth morphology and behavior in combination with behavioral aspects of meiofaunal ecology and temporal changes in assemblage structure. This is probably the case in many predator-prey interactions between juvenile fish and meiofauna. Morphological changes probably exert a strong influence on the ability of fish to utilize various prey assemblages. The dramatic morphological changes undergone by developing flatfish are well-documented. Even after metamorphosis, the morphology of many flatfish results in an orientation toward prey in the near-bottom water. Whiffs of the genus *Citharichthys* assume a feeding position in which the head is elevated to attack overhead prey (Stickney *et al.*, 1973).

Similarly, aspects of the biology of the meiofauna prey undoubtedly influence on the predator-prey relationship. It is well documented that factors such as prey size (Vince *et al.*, 1976; Gardner, 1981; Eggers, 1982) and prey activity (Moore & Moore, 1976) affect predation rates. There is growing evidence that meiofauna exhibit temporal emergence patterns (Walters & Bell, 1986), differential responses to flow (Coull *et al.*, 1989) and to stems (Palmer, 1986) that may exert a strong influence as well. In small fish feeding on meiofauna in near-bottom waters, the manner in which the behavior of the various meiofauna interacts with the predatory behavior of the fish will likely be one of the ultimate determinants of prey selection.

A recurring theme in studies examining the food habits of developing fish is the difficulty in assessing the impact of spatial variation on prey availability. Differences in prey assemblages over relatively short distances can be a significant factor in determining the food of sediment-feeding fishes such as spot (Feller *et al.*, 1990). There is a need for studies employing broad-spatial sampling of fish and prey assemblages to address the relative role of ontogenetic shifts and spatial variation in dietary composition. Spatial variation of the hyperbenthos has been little studied, but it seems likely that broadly homogenous locations such as muddy salt-marsh bottoms might be little influenced by microhabitat differences, suggesting that apparent changes in diet associated with hyperbenthos are due to ontogeny rather than spatial variation.

There is a growing body of evidence that prey resources in the near-bottom water are critical to demersal juvenile fish. A variety of fish, including juvenile spot (this study), salmonids (Sibert, 1979; Cordell, 1986; Webb, 1991), starry flounder (McCall, 1992) and tonguefish (Toepfer & Fleeger, 1994), have been shown to rely heavily on such a prey assemblage. This study indicates that early post-larval spot rely on prey that more closely resemble hyperbenthic assemblages than sediment-dwelling ones. Certainly, additional unstudied fish species also use hyperbenthic assemblages. Previous work (*e.g.* Sogard, 1984) has reported harpacticoids only to major taxon, making it difficult to determine the overall importance of the hyperbenthos. Researchers have made significant progress in determining the effect of such factors as flow. More studies investigating these and other effects as well as more effective ways of sampling the near-bottom meio-

fauna are needed to more fully understand the role of the hyperbenthos in the trophic ecology of fishes.

ACKNOWLEDGEMENTS – A number of people made significant contributions to this work. Among them are K. Carman, D. Baltz, B. Moser, E. Weidner, J. Siebenaller, and W. Stickle for improving earlier drafts. R. Bouchard assisted in the production of figures. Special thanks must go to J. Gruner for his efforts in the processing of samples. C. Toepfer, D. Milsted, S. Carr and B. Sun assisted in the collection of samples. The manuscript was also improved through the comments of an anonymous reviewer. Support from a LUMCON Foundation Grant to JNM is appreciated.

BIBLIOGRAPHY

AARNIO and BONSDORFF, 1993. Seasonal variation in abundance and diet of the sand goby *Pomatoschistus minutus* (Pallas) in a northern Baltic archipelago. *Ophelia* **37** : 19-30.

ALHEIT J. and W. SCHEIBEL, 1982. Benthic harpacticoids as a food source for fish. *Mar. Biol.* **70** : 141-147.

ALLDREDGE A.L. and J.M. KING, 1985. The distance demersal zooplankton migrate above the benthos : implications for predation. *Mar. Biol.* **84** : 253-260.

ARMONIES W., 1988. Active emergence of meiofauna from intertidal sediment. *Mar. Ecol. Prog. Ser.* **43** : 151-159.

ARMONIES W., 1989. Meiofaunal emergence from intertidal sediment measured in the field – Significant contribution to nocturnal planktonic biomass in shallow waters. *Helgolander Meeresuntersuchungen* **43** : 29-44.

BELL S.S., G.R.F. HICKS and K. WALTERS, 1988. Active swimming in meiobenthic copepods of seagrass beds : geographic comparisons of abundances and reproductive characteristics. *Mar. Biol.* **98** : 351-358.

BEYER F., 1958. A new bottom-living trachymedusa from the Oslo fjord. *Nytt. Mag. Zool.* **6** : 121-143.

BILLHEIMER L.E. and B.C. COULL, 1988. Bioturbation and recolonization of meiobenthos in juvenile spot (Pisces) feeding pits. *Estuar. Cstl Shelf Sci.* **27** : 335-340.

CARLE K.J. and P.A. HASTINGS, 1982. Selection of meiofaunal prey by the darter goby, *Gobionellus boleosoma* (Gobiidae). *Estuaries* **5** : 316-318.

CHANDLER G.T. and J.W. FLEEGER, 1984. Tube-building by a marine meiobenthic harpacticoid copepod.. *Mar. Biol.* **82** : 15-19.

CORDELL J.R., 1986. Structure and dynamics of an epibenthic harpacticoid assemblage and the role of predation by juvenile salmon. M.S. thesis, University of Washington, Seattle, WA, USA.

COULL B.C., M.A. PALMER and P.E. MYERS, 1989. Controls on the vertical distribution of meiobenthos in mud : field and flume studies with juvenile fish. *Mar. Ecol. Prog. Ser.* **55** : 133-139.

COULL B.C., 1990. Are members of the meiofauna food for higher trophic levels ? *Trans. Am. Microsc. Soc.* **109** : 233-246.

COULL B.C. and O. GIERE, 1988. The history of meiofaunal research. *In* : Introduction to the Study of Meiofauna. Edited by R.P. Higgins and H. Thiel. Smithsonian Institution Press, Washington, 14-17.

DECHO A.W., 1986. Water-cover influence on diatom ingestion rates by meiobenthic copepods. *Mar. Ecol. Prog. Ser.* **33** : 139-146.

DE MORAIS L.TITO and J.Y. BODIOU, 1984. Predation on meiofauna by juvenile fish in a western Mediterranean flatfish nursery ground. *Mar. Biol.* **82** : 209-215.

EGGERS D.M., 1982. Planktivore preference by prey size. *Ecology* **63** : 381-390.

FELLER R.J., B.C. COULL and B.T. HENTSCHEL, 1990. Meiobenthic copepods : tracers of where juvenile *Leiostomus xanthurus* (Pisces) feed ? *Can. J. Fish. Aq. Sci.* **47** : 1913-1919.

FLEEGER J.W., 1980. Community structure of an estuarine meiobenthic copepod assemblage. *Estuar Coast. Mar. Sci.* **10** : 107-118.

FLEEGER J.W., 1985. Meiofauna densities and copepod community composition in a Louisiana, USA estuary. *Trans. Am. Microsc. Soc.* **104** : 321-332.

FOY M.S. and D. THISTLE, 1991. On the vertical distribution of a benthic harpacticoid copepod-field, laboratory, and flume results. *J. Exp. Mar. Biol. Ecol.* **153** : 153-163.

GARDNER M.B., 1981. Mechanisms of size selectivity by a planktivorous fish : a test of hypotheses. *Ecology* **62** : 571-578.

GEE J.M., 1987. Impact of epibenthic predation on estuarine intertidal harpacticoid copepod populations. *Mar. Biol.* **96** : 497-510.

GEE J.M., 1989. An ecological and economic review of meiofauna as food for fish. *Zool. J. Linn. Soc.* **96** : 243-261.

GROSSMAN G.D., 1980. Ecological aspects of ontogenetic shifts in prey size utilization in the bay goby (Pisces : Gobiidae). *Oecologia* **47** : 233-238.

HAGERMAN G.M. Jr. and R.M. RIEGER, 1981. Dispersal of benthic meiofauna by wave and current action in Bogue Sound, North Carolina, USA. *Mar. Ecol. Prog. Ser.* **2** : 245-270.

HAIRSTON N.G.,Jr., W.E. WALTON and K.T. LI, 1983. The causes and consequences of sex-specific mortality in a freshwater copepod. *Limnol. Oceanogr.* **28** : 935-947.

HARTNEY K.B., 1989. The foraging ecology of two sympatric gobiid fishes : importance of behavior in prey type selection. *Environ. Biol. Fish.* **26** : 105-118.

HICKS G.R.F., 1988. Evolutionary implications of swimming behavior in meiobenthic copepods. *Hydrobiologia* **167/168** : 497-504.

HICKS G.R.F. and B.C. COULL, 1983. The ecology of marine meiobenthic harpacticoid copepods. *Oceanogr. Mar. Biol. Ann. Rev.* **21** : 67-175.

HICKS G.R.F. and B.A. MARSHALL, 1985. Sex-selective predation of deep-sea, meiobenthic copepods by pectinacean bivalves and its influence on copepod sex ratios. *N. Z. J. Mar. Freshwat. Res.* **19** : 227-231.

HYATT K.D., 1979. Feeding strategy. *In* : Fish Physiology. Edited by W.S. Hoar, vol. 8, Academic Press, New York, 71-119.

KEATS D.W. and D.H. STEELE, 1993. Food of 0-Group ocean pout (*Macrozoarces americanus* (Schneider) in eastern Newfoundland – the importance of harpacticoid copepods. *J. Fish. Biol.* **42** : 145-148.

KEATS D.W., D.H. STEELE, J.M. GREEN and G.M. MARTEL, 1993. Diet and population size structure of the Arctic shanny, *Stichaeus punctatus* (Pisces, Stichaeidae), at sites in eastern Newfoundland and the eastern Arctic. *Environ. Biol. Fish.* **37** : 173-180.

KEENLEYSIDE M.H.A., 1979. Diversity and Adaptation in Fish Behaviour. Springer-Verlag, New York, 208 p.

JACOBY C.A. and J.G. GREENWOOD, 1989. Emergent zooplankton in Moreton Bay, Queensland, Australia : seasonal, lunar, and diel patterns in emergence and distribution with respect to substrata. *Mar. Ecol. Prog. Ser.* **51** : 131-154.

JACOBY C.A. and J.G. GREENWOOD, 1993. Spatial, temporal, behavioral patterns in emergence of zooplankton in the lagoon of Heron Reef, Great Barrier Reef, Australia. *Mar. Biol.* **97** : 309-328.

LIVINGSTON R.J., 1988. Inadequacy of species-level designations for ecological studies of coastal migratory fishes. *Environ. Biol. Fish.* **22** : 225-234.

LUDWIG J.A. and J.F. REYNOLDS. 1988. Statistical Ecology : A Primer on Methods and Computing. John Wiley and Sons, New York.

MALY E.J., 1970. The influence of predation on the adult sex ratios of two copepod species. *Limnol. Oceanogr.* **15** : 566-573.

MARINELLI R.A. and B.C. COULL., 1987. Structural complexity and juvenile fish predation on meiobenthos : an experimental approach. *J. Exp. Mar. Biol. Ecol.* **108** : 67-81.

MCCALL J.N., 1992. Source of harpacticoid copepods in the diet of juvenile starry flounder. *Mar. Ecol. Prog. Ser.* **86** : 41-50.

MCCALL J.N. and J.W. FLEEGER, 1993. Recognition and utilization of meiofaunal aggregations by juvenile spot. *J. Exp. Mar. Biol. Ecol.* **174** : 121-134.

MCINTYRE A.D., 1969. Ecology of marine meiobenthos. *Biol. Rev. Cambridge Phil. Soc.* **44** : 245-290.

MCINTYRE A.D. and D.J. MURISON, 1973. The meiofauna of a flatfish nursery ground. *J. Mar. Biol. Ass. U. K.* **53** : 93-118.

METAXAS A. and R.E. SCHEIBLING, 1994. Spatial and Temporal Variability of Tidepool Hyperbenthos on a Rocky Shore in Nova-Scotia, Canada. *Mar. Ecol. Prog. Ser.* **108** : 175-184.

MILLER J.M. and M.L. DUNN, 1980. Feeding strategies and patterns of movement in juvenile estuarine fishes. *In* : Estuarine Perspectives. Edited by V.S. Kennedy, Academic Press, New York, 437-448.

MOORE J.W. and J.A. MOORE, 1976. The basis of food selection in flounders, *Platichthys flesus* (L.), in the Severn estuary. *J. Fish Biol.* **9** : 375-390.

NELSON A.L. and B.C COULL, 1987. Selection of meiobenthic prey by juvenile spot (Pisces) : an experimental study. *Mar. Ecol. Prog. Ser.* **53** : 51-57.

PALMER M.A. 1984. Invertebrate drift : behavioral experiments with intertidal meiobenthos. *Mar. Behav. Physiol.* **10** : 235-253.

PALMER M.A., 1986. Hydrodynamics and structure : interactive effects on meiofauna dispersal. *J. Exp. Mar. Biol. Ecol.* **104** : 53-68.

PALMER M.A., 1988a. Epibenthic predators and marine meiofauna : separating predation, disturbance, and hydrodynamic effects. *Ecology* **69** : 1251-1259.

PALMER M.A., 1988b. Dispersal of marine meiofauna : a review and conceptual model explaining passive transport and active emergence with implications for recruitment. *Mar. Ecol. Prog. Ser.* **48** : 81-91.

PALMER M.A. and G. GUST, 1985. Dispersal of meiofauna in a turbulent tidal creek. *J. Mar. Res.* **43** : 179-210.

SCHOLZ D.S., L.L. MATTHEWS and R.J. FELLER, 1991. Detecting selective digestion of meiobenthic prey by juvenile spot (*Leiostomus xanthurus* (Pisces) using immunoassays. *Mar. Ecol. Prog. Ser.* **72** : 59-67.

SHAW M. and G.P. JENKINS, 1992. Spatial variation in feeding, prey distribution and food limitation of juvenile flounder (*Rhombosolea tapirina* Gunther). *J. Exp. Mar. Biol. Ecol.* **165** : 1-21.

SHERIDAN P.F. and R.J. LIVINGSTON, 1979, Cyclic trophic relationships of fishes in an unpolluted river-dominated estuary in north Florida. *In* : Ecological Processes in Coastal and Marine Systems, Edited by R.J. Livingston, Plenum Press, New York, 143-161.

SIBERT J.R., 1979. Detritus and juvenile salmon production in the Nanaimo estuary : II. Meiofauna available as food to juvenile chum salmon (*Oncorhynchus keta*). *J. Fish. Res. Bd. Can.* **36** : 497-503.

SIBERT J.R., 1981. Intertidal hyperbenthic populations in the Nanaimo Estuary. *Mar. Biol.* **64** : 259-265.

SIBERT J., T.J. BROWN, M.C. HEALEY and B.A. KASK, 1977. Detritus-based food webs : exploitation by juvenile chum salmon (*Oncorhynchus keta*). *J. Fish. Res. Bd. Can.* **36** : 497-503.

SIH A. and R.D. MOORE, 1991. Interacting effects of predator and prey behavior in determining diets. *In* : Behavioural Mechanisms of Food Selection. Edited by R.N. Hughes, Springer-Verlag, New York, 771-776.

SMITH L.D. and B.C. COULL, 1987. Juvenile spot (Pisces) and grass shrimp predation on meiobenthos in muddy and sandy substrates. *J. Exp. Mar. Biol. Ecol.* **105** : 123-136.

SOGARD S.M., 1984. Utilization of meiofauna as a food source by a grassbed fish, the spotted dragonet *Callionymus pauciradiatus*. *Mar. Ecol. Prog. Ser.* **17** : 183-191.

SOGARD S.M., 1992. Variability in growth rates of juvenile fishes in different estuarine habitats. *Mar. Ecol. Prog. Ser.* **85** : 35-53.

STICKNEY R.R., D.B. WHITE and D. MILLER, 1973. Observations of fin use in relation to feeding and resting behavior in flatfishes (Pleuronectiformes). *Copeia* **1973** : 154-156.

STICKNEY R.R., G.L. TAYLOR and D.B. WHITE, 1975. Food habits of five species of young southeastern United States estuarine Sciaenidae. *Chesapeake Sci.* **16** : 104-114.

SUN B. and J.W. FLEEGER, 1994. Field experiments of the mechanism of colonization of meiofauna : functional group effects. *Mar. Ecol. Prog. Ser.* **110** : 167-175.

THAYER C.W., 1985. Brachiopods versus mussels : competition, predation, and palatability. *Science* **228** : 1527-1528.

TOEPFER C.S. and J.W. FLEEGER, 1994. Diet of juvenile *Citharichythys spilopterus*, *Symphurus plagiusa* and *Gobionellus boleosoma*. *Bull. Mar. Sci.* In press.

WALTERS K. and S.S. BELL, 1986. Diel patterns of active vertical migration in seagrass meiofauna. *Mar. Ecol. Prog. Ser.* **34** : 95-103.

WEBB D.G., 1991. Effects of predation by juvenile Pacific salmon on marine harpacticoid copepods. 1. Comparisons of copepod mortality with patterns of salmon consumption. *Mar. Ecol. Prog. Ser.* **72** : 25-36.

WOODIN S.A., 1977. Algal « gardening » behavior by nereid polychaetes : effects on soft-bottom community structure. *Mar. Biol.* **44** : 39-42.

VINCE S., I. VALIELA, N. BACKUS and J.M. TEAL. 1976. Predation by the salt marsh killifish *Fundulus heteroclitus* (L.) in relation to prey size and habitat structure : consequences for prey distribution and abundance. *J. Exp. Mar. Biol. Ecol.* **23** : 255-266.

YETMAN K.T, 1979. The morphology of the buccopharyngeal filter of an estuarine fish, *Leiostomus xanthurus*. M.S. thesis, University of South Carolina, Columbia, SC.

ZANDER C.D. and A. HEYMER, 1992. Feeding habits of *Gobius auratus* and other benthic small-sized fish from the French Mediterranean coast under regard of some alternating parameters. *Zool. Anz.* **228** : 220-228.

Reçu le 16 août 1994 ; received August 16, 1994
Accepté le 21 novembre 1994 ; accepted November 21, 1994

VIE MILIEU, 1995, 45 (1) : 75-83

FEEDING RATE CYCLE OF THE EPIBENTHIC HARPACTICOID COPEPOD *HARPACTICUS FLEXUS*: LABORATORY EXPERIMENTS USING FECAL PELLET COUNTS

L.P. SOUZA-SANTOS, J. CASTEL, P.J.P. dos SANTOS

Laboratoire d'Océanographie Biologique, Université de Bordeaux I, 2 rue du Professeur Jolyet, Arcachon 33120, France

MEIOBENTHOS,
HARPACTICOID COPEPOD
HARPACTICUS FLEXUS
TIDAL CYCLE
FEEDING RATE
FECAL PELLETS

ABSTRACT – The defecation rate of *Harpacticus flexus* Brady & Robertson was used as an index of feeding rate in laboratory experiments. A tidal cycle of defecation rate was observed in constant laboratory conditions with the highest values of defecation/feeding rates occurring during the expected low and high slack tides. The light regime (24h dark and 12h light/dark) did not alter this cycle, although the light may represent a zeitgeber. It is suggested that the adaptive significance of this cycle is to avoid animal passive erosion due to the high speed currents during the ebb and flood tides at the sampling site by restricting their feeding time to periods with low speed currents. The gut content of copepods, expressed both in number of fecal pellets and in pigment-content, did not change during the tidal cycle in the laboratory, thus changes in the gut passage time were the principal responsible for the feeding rate changes.

MEIOBENTHOS
COPÉPODE HARPACTICOIDE
HARPACTICUS FLEXUS
CYCLE DE MARÉE
TAUX DE BROUTAGE
PELOTES FÉCALES

RÉSUMÉ – Le taux de défécation de *Harpacticus flexus* Brady & Robertson a été utilisé comme un index du taux de broutage dans des expériences de laboratoire. Un cycle de marée du taux de défécation a été observé avec les plus forts taux de défécation/broutage aux heures correspondant aux étales de la marée (BM and HM). Le régime de lumière (24h obscurité, 12h lumière/12h obscurité) ne change pas le cycle, mais la lumière peut constituer un synchronisateur. Il est suggéré que l'importance adaptative du cycle est d'éviter aux animaux d'être érodés passivement par les forts courants de la marée descendante et montante au point de prélèvement, restraignant leur temps de broutage aux moments de faibles courants. Le contenu intestinal des Copépodes, exprimé en nombre de pelotes fécales et en contenu de pigment du tractus digestif, ne change pas pendant le cycle de marée en conditions de laboratoire, les variations du temps de passage sont donc principalement responsables du changement du taux de broutage.

INTRODUCTION

Diel feeding cycle is a common feature of the biology of planktonic copepods and although it is often associated with diel migration, the two behaviours appear to be controlled independently (Durbin *et al.*, 1990). Reports of diel cycles of meiobenthic copepods are scarce. Some studies showed that the abundance of meiobenthos in sediments (Coull and Feller, 1988; Armonies, 1989; 1991; Hicks, 1992) and in water column (Palmer and Gust, 1985; Walters, 1988; 1991; Armonies, 1989; 1991) can change during the tidal or diel cycle. Passive erosion by tidal currents (Palmer and Gust, 1985; Hicks, 1992) but also active emergence, mainly of copepods, during the night high water (Armonies, 1988b; 1989; 1991; Coull and Feller, 1988; Walters, 1988) are the main responsible for these changes. Differences in habitat, water flow intensity, aboveground structure and taxonomic composition of the community are important factors determining what kind of mechanism is playing the major role on the presence of meiobenthos in the water column (Palmer, 1988).

It is expected that changes in habitat (sediment or water) and in swimming activity of copepods during tidal or diel cycles affect the copepod feeding rates. There is no report on the diel variation of feeding rates of meiobenthic copepods in the field or in laboratory perhaps due to the methodo-

logical difficulties in estimating feeding rates both in laboratory and *in situ* experiments, related to the small size of the animals and to the necessity of working with sediment. Tidal variations of feeding rates of meiobenthic copepods were only investigated by Decho (1986; 1988). These studies showed that harpacticoid meiobenthic copepods do not feed at constant rates during a tidal cycle, both in laboratory and *in situ* experiments, particularly due to the water-cover influence. The changes found were species-specific, mainly due to the different exploitation of feeding resources by copepods.

The defecation rate, estimated by counting the fecal pellets produced in time, can be used as an index of the feeding rate, since these two parameters are normally well correlated (Gaudy, 1974; Gamble, 1978; Huntley *et al.*, 1983; Pagano and Gaudy, 1986). The advantage of using the defecation rate to estimate the feeding rate of meiobenthic copepods is that the animals can eat the natural microfilm of sediments and that it can be measured with a simple methodology in the laboratory.

Harpacticus flexus Brady & Robertson (Harpacticoida : Copepoda) is an epibenthic copepod that inhabits the lower level of the intertidal region of fine sand beaches (Hicks and Coull, 1983). Armonies (1989; 1991) proposed the denomination of semiplanktonic to this species, because the animals rest in superficial sediment layers at low tide and swim in the water column at high tide, reaching high densities in the water column at night in a North Sea tidal beach.

This study was designed to investigate if *Harpacticus flexus*, a common species of a temperate mesotidal sandy beach, presents feeding rate changes during the tidal and/or diel cycle, by means of controlled laboratory experiments.

METHODS

Copepods used in the experiments were collected from the lower level of the intertidal sandflat located in front of the Biological Station of Arcachon, France (44°40′N, 1°10′W). Tidal sea level change at the sampling area is semidiurnal with a spring range of about 4.5 m and a neap range of about 3 m. The sediment was collected during the low water of spring tides and transported to the laboratory where the sediment was washed with sea-water and copepods picked up under a stereo-microscope. Before the beginning of the experiments, the adult copepods were maintained on natural sand washed with sea-water to eliminate any other animals but owning a microfilm of bacteria and diatoms.

Diel variation of defecation rate

The first experiment investigated the diel variation of the defecation rate of *Harpacticus flexus* and the influence of the photoperiod on this variation. Copepods were collected on 27/11/91 (sediment temperature of 10°C) and were maintained in the laboratory at constant conditions and at 15°C during one week for acclimation to the two photoperiods tested : 24 h dark and 12 h light/dark. At the beginning of the experiment five copepods from each photoperiod were put individually in recipients with a few sand grains (treated in the same way as the stock culture sand and owning the natural microfilm) and sea-water. Then, at each 2 h interval the fecal pellets produced by copepods were picked up and counted under a stereo-microscope, during 24h. The 2 h interval seemed to be reasonable since the gut passage time of meiobenthic copepods was seen to vary from 30 to 90 minutes (Decho, 1988).

Tidal variation of defecation rate

The next three experiments were designed to investigate the tidal variation of the defecation rates. In these experiments the animals were maintained at a photoperiod of 12 h light/dark but in constant conditions of temperature and immersion.

For the second experiment, copepods were collected on 20/01/92 (sediment temperature of 6°C) and maintained one day at 6°C. The fecal pellets produced by five copepods were counted using the same method as in the previous experiment but at each 1 h interval during 12 h.

For the third experiment, copepods were collected on 23/01/92 (sediment temperature of 8°C) and maintained one day at 8°C. In this experiment seven copepods were used and, differently from the previous experiments, at each 1 h interval copepods were transferred into a new recipient, during 18 h. The fecal pellets were picked up and counted in the old recipients. This methodological change was aimed to avoid the stress that may represent the sorting of the pellets at each 1 h interval.

For the fourth experiment, copepods were collected on 11/03/93 (sediment temperature of 17°C) and maintained one day at 20°C. In this experiment 10 copepods were used and, at each 1h interval during 12 h, the copepods were transferred into new recipients. The fecal pellets were fixed with formalin in old recipients and picked up and counted later in counting plates. In this experiment the mean volume of the fecal pellets collected each hour was estimated. For this purpose, 30 pellets taken at each experimental time were measured using camera lucida drawings under a

microscope and the volume estimated by a cylindrical model of the fecal pellet. The evaluation of changes in the pellet volume is important because great variations of this volume can alter the expected relation between number of fecal pellets produced in time (defecation rate) and feeding rates.

Tidal variation of gut content

Fecal Pellet Content

During the third experiment of defecation rate, each hour five copepods from the maintenance culture were put individually in 0.7 µm filtered sea-water. Three hours later the number of fecal pellets produced was counted.

Chlorophyll-a Gut Content

During the third experiment of defecation rate, at each 3 h intervals, copepods from the maintenance culture were picked and immediately frozen. A few days later, these copepods were carefully sorted at minimum light intensity and washed both in distilled water and filtered sea-water. Three replicates of 15 adult copepods were then macerated in 5 ml of 90 % acetone. Extraction was made for 24 h at 4°C. The extraction tubes were centrifuged to discard the copepod carapaces and the supernatant used for measuring the fluorescence before and after acidification with a Turner Model 112 Fluorometer, according to Neveux (1983). Pigment concentrations were calculated using the equations of Lorenzen (1967). Results are given in equivalents of chl-a (µg chl-a + 1.51 µg pheopigment) (Bautista *et al.*, 1988).

Three groups of adult copepods were dried (24 h at 60°C) and weighed on a Mettler ME22 microbalance (± 0.1 µg), for estimation of the feeding rates in relation to the animal carbon weight, considered 40 % of the animal dry weight (Giere, 1993).

Tidal variation of the current speed at the sampling site

The current speeds at the sampling site were measured during two spring tidal cycles on 19/02/92 and 12/03/93 (at the same day of the fourth experiment of defecation rate) during typical anticyclonic weather conditions. Current velocities were measured using a flowmeter (minimum sensitivity of 5cm/sec) put in the center of an arc, maintained in a vertical position 50 cm under the water surface.

Statistical analysis

The non-parametric tests of Kruskal-Wallis and Friedman were used to test for significant differences between means of the measured variables through experimental times. Spearman Rank Correlation was used for testing the correlation between defecation rate, current speed and tidal condition. To test the influence of the tidal condition the scores 0 to 3 were attributed to the periods of slack tides to mid-tides, respectively. The level of probability was 0.05.

RESULTS

Diel variation of defecation rate

The defecation rate of *H. flexus* in the laboratory changed significantly during a diel cycle in both photoperiods tested (12 h light/dark – Friedman statistics = 27.6, p = 0.006 and 24 h dark – Friedman statistics = 22.8, p = 0.03). In the 12h light/dark photoperiod (Fig. 1a) three peaks of defecation rate can be observed : 6, 12 and 20 h from the beginning of the experiment, both in the light and in the dark period. In the 24 h dark photoperiod (Fig. 1b) four peaks were observed, though less clear, and occurred 2, 12, 18 and 24 h from the beginning of the experiment.

Tidal variation of defecation rate

During the three tidal cycles tested in the laboratory the defecation rates of *H. flexus* changed significantly (second experiment – Friedman statistics = 32, p = 0.001 ; third experiment – Friedman statistics = 36.6, p = 0.004 ; fourth experiment – Friedman statistics = 97.9, p = 0.000).

In the second experiment (Fig. 2a) three peaks from the four peaks of defecation rate occurred near the moment of the expected slack tides of the sampling day (one day before). These peaks were nearly of the same intensity although the decrease of defecation rate at the mid-tide was greater during the ebb than during the flow.

The peaks of defecation rate during the third experiment (Fig. 2b) occurred once more near the expected slack tides of the sampling day, although the last two ones were very small in intensity. The intensity of the decreases was quite similar to each other in this experiment.

The fourth experiment presented three peaks of defecation rate (Fig. 2c), two of these were near the expected slack tides and the other one was at the mid-tide of the sampling day. Here, once

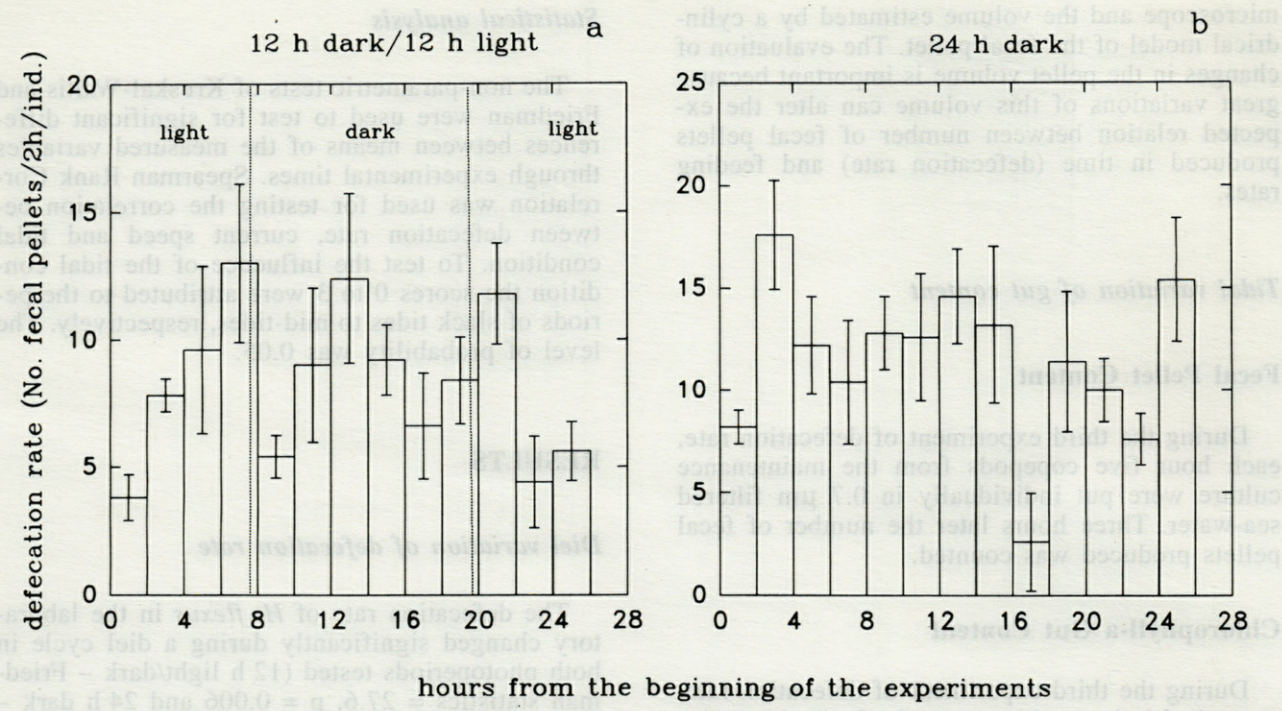

Fig. 1. – *Harpacticus flexus*. Diel variation of the defecation rates expressed in number of fecal pellets. $2h^{-1}$. $individual^{-1}$ (first experiment, 15°C). a, photoperiod of 12 h light /dark; b, photoperiod of 24h dark. The vertical bars represent standard errors.

more, the decrease occurring at the mid-tide of the ebb was greater than those of the flood tide.

The Spearman rank correlations between the standardized defecation rate and the tidal conditions (scores 0 to 3 corresponding to hours from or to slack tides) were significant and negative for the second experiment ($r_s = -0.77$, $n = 13$, $p = 0.007$) and for all experiments together ($r_s = -0.35$, $n = 43$, $p = 0.022$).

The volume of the fecal pellets changed significantly (Kruskal-Wallis statistics = 26.7, $p = 0.005$) during the fourth experiment. Figure 2d shows that the volume increased during the initial hours and did not change after anymore. The corrected defecation rate (mean volume x number of fecal pellets produced) (Fig. 2d) did not change very much from the previous rate (Fig. 2c), since the volume increase occurred when the number of fecal pellets produced was very small.

Tidal variation of gut content

Fecal Pellet Content

The number of pellets inside copepods in the constant laboratory conditions did not change significantly (Friedman statistics = 13.3, $p = 0.350$) during a tidal cycle (Fig. 3a). A mean of 1.5 pellets at 8°C was found.

Chlorophyll-a Gut Content

The chlorophyll-a gut content of the copepods in the constant conditions of laboratory did not change significantly (Kruskal-Wallis statistics = 2.73, $p = 0.603$) during a tidal cycle (Fig. 3b). A mean of 0.223 ng chlorophyll-a equivalent per adult copepod at 8°C was found. The mean dry weight of adult copepods was 2.2 µg (SE = 0.45).

Tidal variation of the current speed at the sampling site

The results of the current speed variation during two spring tidal cycles at the sampling site are presented in Figure 4. Three peaks of current velocity can be observed in both cycles, a greatest one at the mid-tide of the ebb tide and the two others at the mid-tide of the flood tide. As expected, the current speed was minimum during the slack periods.

The Spearman rank correlation between current speeds and tidal conditions was not significant ($r_s = 0.36$, $n = 24$, $p = 0.088$), unless the mid-tides of the flood tide were discarded ($r_s = 0.68$, $n = 21$, $p = 0.002$).

The Spearman rank correlation between standardized defecation rate of the three tidal cycle ex-

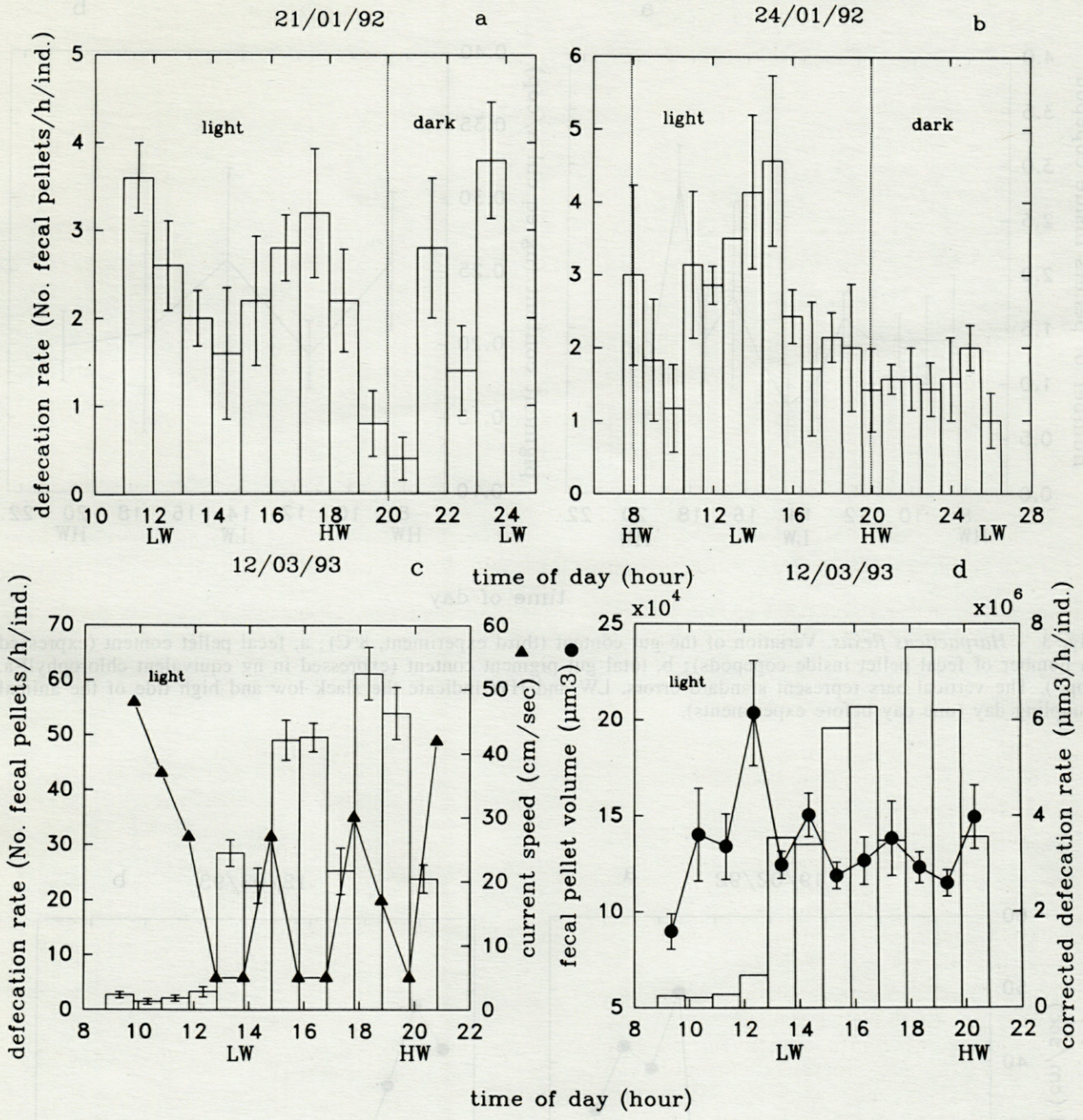

Fig. 2. – *Harpacticus flexus*. Variation of the defecation rates, expressed in number of fecal pellets.h^{-1}. individual^{-1} during three tidal period experiments. a, second experiment made on 21/01/92 at 6°C; b, third experiment made on 24/01/92 at 8°C; c, fourth experiment made on 12/03/93 at 20°C and also showing the variation of the current speeds during the tidal cycle measured on the same day; d, variation of the volume of fecal pellets and the corrected defecation rate from the fourth experiment. The vertical bars represent standard errors. LW and HW indicate the slack low and high tide of the animal sampling day (one day before experiments).

periments and the mean current speed for the two investigated tidal cycle was significant and negative (r_s = – 0.33, n = 43, p = 0.030). Testing each experiment individually, only for the fourth experiment, when the current speed and the defecation rate were measured during the same day, there was a significant correlation between defecation rate and current speed (r_s = – 0.59, n = 12, p = 0.049) (Fig. 2c).

The correlations between defecation rate and tidal condition were stronger if the mid-tides of the flood tide were discarded (total results – r_s = – 0.39, n = 40, p = 0.015 and fourth experiment – r_s = – 0.60, n = 11, p = 0.06).

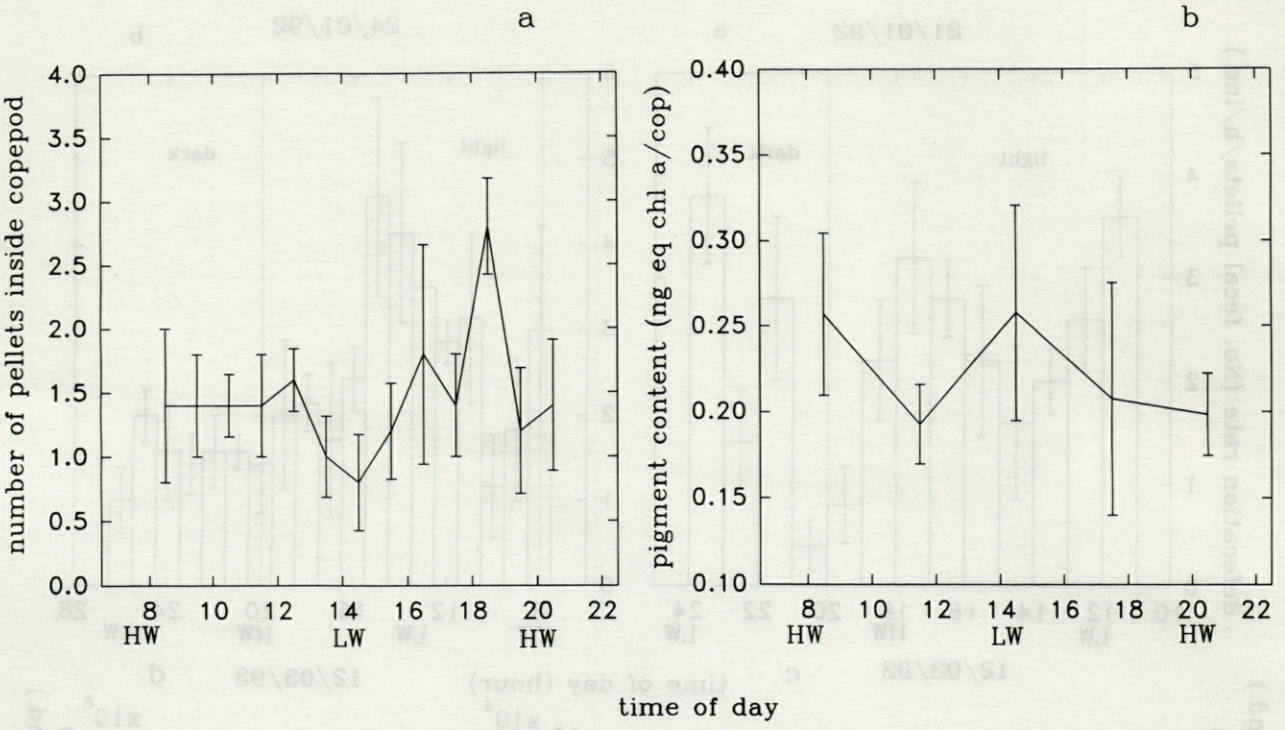

Fig. 3. – *Harpacticus flexus*. Variation of the gut content (third experiment, 8°C); a, fecal pellet content (expressed in number of fecal pellet inside copepods); b, total gut pigment content (expressed in ng equivalent chlorophyll-a. cop^{-1}). The vertical bars represent standard errors. LW and HW indicate the slack low and high tide of the animal sampling day (one day before experiments).

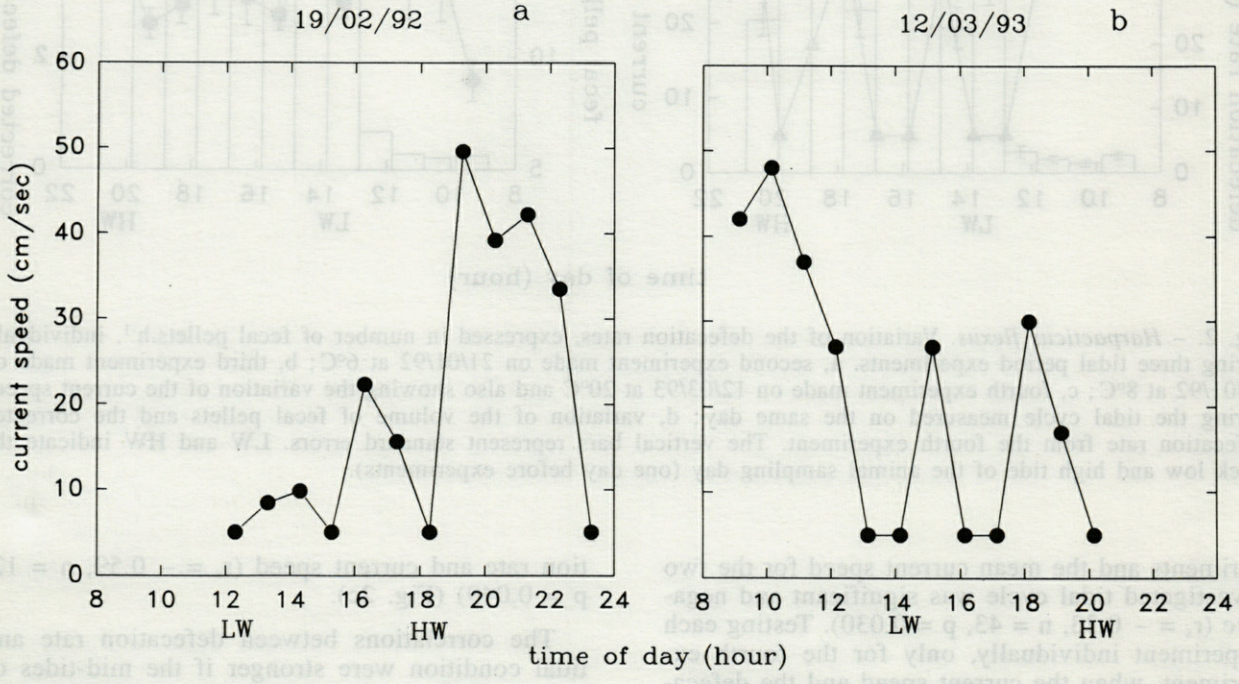

Fig. 4. – Variation of the current speeds at the sampling site measured during two tidal cycles : a, on 19/02/92 and b, on 12/03/93. LW and HW indicate the slack low and high tides.

DISCUSSION

The good linear correlation between feeding and defecation rates found in several works (Gaudy, 1974; Gamble, 1978; Huntley *et al.*, 1983; Pagano and Gaudy, 1986) is quite expected if the bioenergetic equation is looked as : **(1– A) I = F**; where **I** is the ingestion rate, **A** is the percentage of assimilation and **F** is the defecation rate. Only changes on the percentage of assimilation can alter this relation. Although it is recognized that the percentage of assimilation can be strongly affected by the nature of the food item, it seems to be largely independent on the ingestion rates (Conover, 1966; Tande and Slagstad, 1985; Pagano and Gaudy, 1986). Thus we considered **A** nearly like a constant for a same diet and used the defecation rate changes as estimates of the feeding rate changes during the diel cycle.

The results of the diel cycle experiment (Fig. 1) indicated that *Harpacticus flexus* presents a feeding cycle with peaks nearly every 6 hour in the constant laboratory conditions, a good evidence for an endogenous control. Although the cycles were quite similar in the two tested photoperiods, the presence of a light period seemed to be important in the regulation of this cycle (a possible zeitgeber), since in the 12 h light/dark photoperiod the peaks were clearer than in the 24 h dark photoperiod. A tidal cycle was thus suggested and more experiments were designed to clarify this one, since the long period of acclimation in constant laboratory conditions (one week) could cause large shifts of the peaks and the 2 h intervals could prevent a good evaluation of this 6 h cycle.

The results of the tidal cycle experiments (Fig. 2) suggested that the highest feeding rates occur during the slack tides and sometimes during the mid-tide. The significant correlation between defecation rate and tidal conditions (hours from or to slack tide) for the second experiment and for all tidal experiments together confirm this hypothesis. Although this cycle seems to be endogenous it must be adjusted by environmental factors (zeitgebers), because in the constant laboratory conditions the animals seem to answer to the tidal cycle of the sampling day. The drift of the peak of animal activity in relation to the controlling factors is a normal characteristic of endogenous free-running cycles (Brown, 1973). The measurement of the fecal pellet volume was not essential for the estimation of the defecation rates in this experiment since the volume changed only when the defecation rate was small (Fig. 2d).

Decho (1986; 1988) proposed that the water-cover influence and the specific feeding mode can explain the tidal differences in the feeding rates of three harpacticoid copepods. *Scottolana canadensis* feeds on planktonic diatoms from its burrows mainly on early low water when the sediment is still very wet but also during high water. *Microarthridion littorale* feeds on both planktonic and benthic diatoms during all tidal stages but mainly during early low water, perhaps due to the flocculent characteristic of sediments with high ambient food supply and without the high water disturbance of predation and hydrodynamics. *Paranychocamptus huntsmani* feeds only during high water and thus requires the water-cover to feed. These studies however did not investigate the possible variation of feeding rates during the different conditions of flood, slack and ebb high tides. The feeding mode of *Harpacticus flexus* has not been the subject of a detailed study to investigate if it feeds on benthic, planktonic or both types of diatoms. We could observe that in the laboratory conditions the individuals of this species browse the sand grain between periods of high swimming activity in the water. We can not discard the possibility of feeding on planktonic diatoms, though the small volume of natural seawater in the flasks (10 ml) could render difficult the exploitation of this resource. In the constant conditions of the laboratory *H. flexus* feeds during all the tidal cycle but mainly during the slack tides. This result, associated to the similar high defecation/feeding rates observed in both high and low slack tide conditions prevents the water-cover influence to explain this cycle.

The gut content of planktonic copepods normally varies positively with the feeding rate and Durbin *et al.* (1990) suggested that the reduction of the feeding rates may be a strategy to avoid visual predators during the day. In the case of *H. flexus*, however, the gut content, expressed in number of pellets inside copepod or in gut pigments, did not change during the tidal cycle (Fig. 3), discarding this kind of interpretation. Mackas and Bohrer (1976) proposed a model for the relation between gut content and feeding rates : **I = G / T**, where **I** is the ingestion rate, **G** is the gut content in pigment and **T** is the gut clearance time. Considering the previous result, that the gut content did not change during the experiment, changes in the gut passage time should be the main responsible for tidal changes in the feeding rates. This result is in accordance with several works showing that the gut passage time changes with the feeding rates (Baars and Oosterhuis, 1984; Dagg and Walser, 1987) and suggests that considering the gut passage time like a constant that changes only with temperature (Kiorboe *et al.*, 1982; Bautista and Harris, 1992) can lead to erroneous conclusions about the feeding rates. Decho (1988) also showed that the gut passage time of meiobenthic copepods varies with tidal conditions in the field.

In our experiments the gut passage time of *H. flexus*, estimated by the relation between the production rate of pellets and the pellets inside copepod, varied from 20 to 77 min at 8°C (data from the third experiment). This variation is very similar to those found for meiobenthic copepods using the azo-carmine dye in the field at 13°C (Decho, 1988). Using the model proposed by Mackas and Bohrer (1976), the gut pigment and the gut passages times measured during the third experiment (8°C), the adult dry weight of *H. flexus* (2.2 µg), the ingestion rates can be estimated as 0.17 ng chl-a equivalent pigment. µg dry weight^{-1}. h^{-1} or 4.13 ng chl-a equivalent pigment. µg dry weight^{-1}. day^{-1}. These values are very similar to those estimated for meiobenthic copepods in the field (Decho, 1988). Considering the value of 40 for the C/chl-a relation of the benthic diatoms (de Jonge, 1980), a daily ingestion rate of 41 % body carbon was estimated.

The current speed variations during the two studied tidal cycles of the sampling site were quite similar to each other (Fig. 4) and presented two interesting characteristics : the highest values of current speed occurred during the ebb tide and a decrease of current speed occurred at the mid-tide, particularly during the flood. These two non-expected features of the tidal current at the sampling point can be explained since in Arcachon Bay the principal flood tide channel is different from the principal ebb tide channel, which makes the dynamics of the tidal water very complex and particular for each point in the Bay (Bouchet, 1968 ; Gassiat, 1989). It can also explain the non-significant correlation between current speed and tidal conditions, unless the flood mid-tides were discarded.

The significant negative correlation between the current speed and the defecation rate for all experiments together and for the fourth experiment alone lead us to suggest that the adaptive significance of the endogenous tidal feeding cycle of *H. flexus* is to avoid the period of high current speed. Armonies (1988a) showed, in laboratory experiments, that current speeds greater than 1 cm/sec can affect the active emergence of harpacticoids from the sediment. In the field, the negative influence of strong current speed (50 to 80 cm/sec) of storm days on the active emergence of *H. flexus* during high tides at night was described by Armonies (1989). In Arcachon Bay the current velocities measured during the ebb tide were almost as high as the values of the strong currents mentioned above. It is expected therefore that *H. flexus* emergence in Arcachon Bay follows a different pattern as compared to its emergence in the North Sea. Palmer and Gust (1985) showed that in a tidal creek, with peaks of current speed in the order of 25 cm/sec, the meiofauna abundance in the water columm is governed by passive erosion/suspension and is positively correlated with the friction velocity, that is a function of the current speed. This effect can, by another side, be modified by meiofauna behaviour. Copepods, for example, are more susceptible to erosion by current than nematodes probably because they have a greater activity at the sediment surface (Palmer, 1984).

We suggest therefore that the decrease of feeding activity of *Harpacticus flexus* during periods of high current speed is a behaviour strategy to reduce the risk of passive suspension of animals by erosion (which can expose them to a high predation). This hypothesis is in accordance with the results of Palmer (1984) showing that in the slack low and high tides (no flow) the activity of meiofauna at the sediment surface is the greatest, and that when flow increases the activity at the sediment surface decreases. The results of this work demonstrate that hydrodynamics significantly influence the feeding behaviour of *Harpacticus flexus* and suggest that future estimations of the feeding rates for meiofauna must consider a possible daily variation of these ones both in the field and in the laboratory.

ACKONWLEDGMENTS – We would like to thank Xabier Irigoien for his help during the experimental work. LPSS and PJPS acknowledge a CAPES postgraduate research studentship (Brazil). This study was partly supported by a CEC Environment programme (CLEAN contract EV5V – CT92 – 0080).

REFERENCES

ARMONIES W., 1988a. Hydrodynamic factors affecting behaviour of intertidal meiobenthos. *Ophelia* **28** : 183-193.

ARMONIES W., 1988b. Active emergence of meiofauna from intertidal sediment. *Mar. Ecol. Prog. Ser.* **43** : 151-159.

ARMONIES W., 1989. Meiofaunal emergence from intertidal sediment measured in the field : significant contribution to nocturnal planktonic biomass in shallow waters. *Helgolander Meeresunters* **43** : 29-43.

ARMONIES W., 1991. Transport of benthos with tidal waters : another source of indertemination. *In* Modelling the benthos – COST 647 workshop Yerseke 20-22 March 1991, 13-25.

BAARS M. A. and S. S. OOSTERHUIS, 1984. Diurnal feeding rhythms in North Sea copepods measured by gut fluorescence, digestive enzyme activity and grazing on labelled food. *Neth. J. Sea Res.* **18** : 97-119.

BAUTISTA B. and R. P. HARRIS, 1992. Copepod gut contents, ingestion rates and grazing impact on phytoplankton in relation to size structure of zooplankton and phytoplankton during a spring bloom. *Mar. Ecol. Prog. Ser.* **82 :** 41-52.

BAUTISTA B., V. RODRIGUEZ and F. JIMENEZ, 1988. Short term feeding rates of *Acartia grani* in natural conditions : diurnal variation. *J. Plankton Res.* **10** : 907-920.

BOUCHET J-M., 1968. Etude océanographique des chenaux du Bassin d' Arcachon. Doct. Thesis, Univ. Bordeaux : 306 p.

BROWN F. A. Jr., 1973. Biological Rhythms. *In* Comparative Animal Physiology vol 1 Environmental Physiology. Edited by C. L. Prosser. Third Edition. W. B. Saunders Company Philadelphia, 429-456.

CONOVER R.J., 1966. Factors affecting the assimilation of organic matter by zooplankton and the question of superfluous feeding. *Limnol. Oceanogr.* **11** : 346-354.

COULL B. C. and R. J. FELLER, 1988. Site-to-site variability in abundance of meiobenthic copepods along a tidal gradient over 24 hours. *Hydrobiologia* **167/168** : 477-483.

DAGG M. J. and W.E. WALSER Jr., 1987. Ingestion, gut passage, and egestion by the copepod *Neocalanus plumchrus* in the laboratory and in the subartic Pacific Ocean. *Limnol. Oceanogr.* **32** : 178-188.

JONGE V. N. de, 1980. Fluctuations in the organic carbon to chlorophyll-a ratios for estuarine benthic diatom populations. *Mar. Ecol. Prog. Ser.* **2** : 345-353.

DECHO A. W., 1986. Water-cover influences on diatom ingestion rates by meiobenthic copepods. *Mar. Ecol. Prog. Ser.* **33** : 139-146.

DECHO A. W., 1988. How do harpacticoid grazing rates differ over a tidal cycle? Field verification using chlorophyll-pigment analyses. *Mar. Ecol. Prog. Ser.* **45** : 263-270.

DURBIN A. G., E. G. DURBIN and E. WLODARCZYK, 1990. Diel feeding behaviour in the marine copepod *Acartia tonsa* in relation to food availability. *Mar. Ecol. Prog. Ser.* **68** : 23-45.

GAUDY R., 1974. Feeding four species of pelagic copepods under experimental conditions. *Mar. Biol.* **25** : 125-141.

GAMBLE J. C., 1978. Copepod grazing during a declining spring phytoplankton bloom in the Northern North Sea. *Mar. Biol.* **49** : 303-315.

GASSIAT L., 1989. Hydrodynamique et évolution sédimentaire d'un système lagune – flèche littorale. Le Bassin d'Arcachon et la flèche du Cap Ferret. Doct. Thesis, Univ Bordeaux I : 228 p.

GIERE O., 1993. Meiobenthology. The microscopic fauna in aquatic sediments. Springer-Verlag, Berlin, 328 p.

HICKS G. R. F., 1992. Tidal and diel fluctuations in abundance of meiobenthic copepods on an intertidal estuarine sandbank. *Mar. Ecol. Prog. Ser.* **87** : 15-21.

HICKS G. R. F. and B. C. COULL, 1983. The ecology of marine meiobenthic harpacticoid copepods. *Oceanogr. mar. Biol. Ann. Rev.* **21** : 67-175.

HUNTLEY M. E., K.-G. BARTHEL and J. L. STAR, 1983. Particle rejection by *Calanus pacificus* : discrimination between similarly sized particles. *Mar. Biol.* **74** : 151-160.

LORENZEN C. J., 1967. Determination of chlorophyll and pheopigments : spectophotometric equations. *Limnol. Oceanogr.* **12** : 343-346.

KIORBOE T., F. MOHLENBERG and H. NICOLAJSEN, 1982. Ingestion rate and gut clearance in the planktonic copepod *Centropages hamatus* (Lilljeborg) in relation to food concentration and temperature. *Ophelia* **21** : 181-194.

MACKAS D. and R. BOHRER, 1976. Fluorescence analysis of zooplankton gut contents and an investigation of diel feeding patterns. *J. exp. mar. Biol. Ecol.* **25** : 77-85.

NEVEUX J., 1983. Dosage de la chlorophylle *a* et des phéopigments par fluorométrie. *In* Manuel des analyses chimiques en milieu marin. CNEXO ed. 11, 193-203.

PAGANO M. and R. GAUDY, 1986. Biologie d'un copépode des mares temporaires du littoral méditerranéen français : *Eurytemora velox.* I. Nutrition. *Mar. Biol.* **90** : 551-564.

PALMER M. A., 1984. Invertebrate drift : behavioral experiments with intertidal meiobenthos. *Mar. Behav. Physiol.* **10** : 235-253.

PALMER M. A., 1988. Dispersal of marine meiofauna : a review and conceptual model explaining passive transport and active emergence with implications for recruitment. *Mar. Ecol. Prog. Ser.* **48** : 81-91.

PALMER M. A and G. GUST, 1985. Dispersal of meiofauna in a turbulent tidal creek. *J. mar. Res.* **43** : 179-210.

TANDE K. S. and D. SLAGSTAD, 1983. Assimilation efficiency in herbivorous aquatic organisms – The potential of the ratio method using 14C and biogenic silica as markers. *Limnol. Oceanogr.* **30** : 1093-1099.

WALTERS K., 1988. Diel vertical migration of sediment-associated meiofauna in subtropical sand and seagrass habitats. *J. Exp. Mar. Biol. Ecol.* **117** : 169-186.

WALTERS K., 1991. Influence of abundance, behavior, species composition, and ontogenetic stage on active emergence of meiobenthic copepods in subtropical habitats. *Mar. Biol.* **108** : 207-215.

Reçu le 3 octobre 1994; received October 3, 1994
Accepté le 8 novembre 1994; accepted November 8, 1994

Directeur gérant de la publication : A. GUILLE

Printed in France *Dépôt légal : 1er trimestre 1995* *Commission paritaire n° 22217*

Imprimerie Louis-Jean, 05002 GAP cedex — Dépôt légal 198 *— Mars 1995*

INSTRUCTIONS AUX AUTEURS

TEXTE

Les manuscrits, dactylographiés en double interligne sur le recto seulement des feuilles numérotées (ne pas excéder 20 pages) sont présentés en trois jeux complets, sous leur forme définitive.

Le titre du manuscrit doit être le plus court possible; il est suivi du prénom et du nom de l'auteur (ou de chacun des auteurs) ainsi que de l'adresse (ou des adresses) du Laboratoire dans lequel a été effectué le travail.

Chaque manuscrit comportera :
— un résumé en français de 15 lignes maximum figurant en début d'article, suivi de sa traduction en anglais,
— des mots clés français et anglais (6 au maximum) permettant un traitement rapide par les revues analytiques,
— un titre abrégé pour haut de page (60 signes et espaces au plus),
— la traduction anglaise du titre de l'article,
— une liste hors-texte des légendes des illustrations et leur traduction en anglais,
— une liste hors-texte des légendes des tableaux numérotés en chiffres romains et traduites en anglais.

Les noms scientifiques (genres, espèces, sous-espèces) figurent en italiques ou soulignés d'un seul trait.

Les références bibliographiques des auteurs cités dans le texte sont regroupées à la fin du manuscrit dans l'ordre alphabétique des noms d'auteurs; elles doivent être conformes aux modèles suivants :

GRIFFITHS C.L. and J.A. KING, 1979. Some relationships between size, food availability and energy balance in the Ribbed Mussel *Aulacomya ater. Mar. Biol.* **51** (2) : 141-150.

EAGLE R.A. and P.A. HARDIMAN, 1977. Some observations on the relative Abundance of species in a benthic community. *In* Biology of Benthic Organisms. Edited by B.F. Keegan, P.O. Ceidigh and P.J.S. Boaden, Pergamon Press, Oxford-New York, 197-208.

Le titre des périodiques doit être abrégé d'après les règles internationales (World list of Scientific Periodicals).

Les notes infrapaginales et les remerciements seront aussi brefs que possible.

ILLUSTRATIONS

Les figures au trait doivent être exécutées à l'encre de chine sur papier calque assez fort, bristol, carte à gratter, papier millimétré bleu. Il est exigé des lettres et chiffres «transfert» par caractères autocollants pour le lettrage et la numérotation, dont la taille tient compte de la réduction à supporter. Les figures sont regroupées au maximum en planches dont la justification pleine page est 17 × 24,35 cm une fois réduites (penser à retrancher la légende de ces dimensions); largeur d'une colonne : 8,1 cm. Méthode des «rectangles homologues» pour la réduction : tracer les diagonales d'un rectangle de 17/24,35 cm, les prolonger; tout rectangle admettant ces prolongements comme diagonales correspondra à la justification après réduction. Indiquer le numéro d'ordre des figures en chiffres arabes et le nom de l'auteur au crayon bleu au recto ou au dos. Ne pas inscrire de légende sur les illustrations.

Regrouper les similis (photographies, lavis...) en planches. Employer une échelle graphique qui sera réduite avec la figure, et un lettrage par transfert. Tableaux et planches similis sont numérotés de I à N en chiffres romains. Limiter le nombre des tableaux et ne retenir que ceux qui sont indispensables à la compréhension du travail.

La revue publie gratuitement 2 planches au trait pleine page et 1 seule planche en simili; les illustrations supplémentaires ou en couleurs sont facturées aux auteurs.

ÉPREUVES ET TIRÉS À PART

Un jeu d'épreuves accompagné du manuscrit est envoyé à l'auteur qui doit retourner l'ensemble après correction et indication de la place des illustrations dans le texte, dans un délai maximum de 15 jours.

Les tirages à part, facturés directement par l'imprimeur, doivent être commandés dès réception de l'imprimé spécial joint aux épreuves.

INSTRUCTIONS TO AUTHORS

TEXT

The manuscript should by typed double-spaced on one side of white paper, format A4, pages numbered (max, 20 pp), and sent in definitive form, in triplicate, to the editor.

The title of the paper should be as concise as possible; it is followed by the first and second name of the author(s) and address of the laboratory at which the work was done. Each manuscript should include :
— a french summary of 15 lines max., followed by the english translation,
— key words in french and english (6 max.) allowing treatment by abstracting journals,
— short version of title (60 signs and spaces max.),
— english translation of the full title,
— a separate list of figure legends in english and french,
— a separate list of numbered tables with their legends in english and scientific names (genera, species, sub-species) should be written in italics or underlined once.

References of papers cited in the text should be listed at the end of the manuscript in alphabetical order, according to the following models :

GRIFFITHS C.L. and J.A. KING, 1979. Some relationships between size, food availability and energy balance in the Ribbed Mussel *Aulacomya ater. Mar. Biol.*, **51** (2) : 141-150.

EAGLE R.A. and P.A. HARDIMAN, 1977. Some observations on the relative abundance of species in a benthic community. In Biology of Benthic Organisms. Edited by B.F. Keegan, P.O. Ceidigh and P.J.S. Boaden, Pergamon Press, Oxford-New York, 197-208.

The title of a journal must be abbreviated according to the rules set by the World list of Scientific Periodicals.

Footnotes and acknowledgments should be as brief as possible.

ILLUSTRATIONS

Line drawings should be prepared with India ink on heavy tracing paper, bristol board, white drawing-cardboard or graph paper (blue grid). Letters and numbers on figures should be large enough to be easily readable after reduction; use letter transfer equipment. As far as possible, figures should be grouped in plates of 17 × 24.35 cm final size (generally the explanations are included in this frame); the width of a single column is 8.1 cm. Prepare figures using the "homologous rectangles" rule; indicate the number of the figure and the author's name with blue pencil on the back of the figure. Do not write explanations on the figure.

Photographs and half-tone drawings must always be grouped as plates. Scale bars are reduced with the figures. Number plates and tables in roman numbers on the back. Limit total number of tables to an absolute minimum of items indispensable for clear presentation.

The journal publishes free of charge two plates with line drawings and one plate of half-tone figures per article. Additional illustrations and colour plates are charged to the author(s).

GALLEY-PROOFS AND REPRINTS

One set of galley-proofs is sent with the manuscript to the author(s); both proofs and manuscript must be returned to the editor with indications on indispensable corrections and insertion of figures within two weeks.

Reprints can be ordered by using the form enclosed with the galley-proofs.

VIE ET MILIEU, 1995, 45 (1)

Life & Environment

SOMMAIRE – *SUMMARY*

Analysé dans / *indexed in :* Animal Behaviourial Abstracts, Aquatic Sciences and Fisheries Abstracts, Biological Abstracts, Bowker international Serials Data base, Current awareness in Biological Sciences, Excerpta Medica, Marine Science Contents Tables, Pascal (INIST-CNRS), Zoological Record.