Marine Biology 56, 49–57 (1980)

MARINE BIOLOGY

Predator-Prey Interactions Between the Larvacean *Oikopleura dioica* and Bacterioplankton in Enclosed Water Columns

K. R. King[1], J. T. Hollibaugh[2] and F. Azam[2]

[1] Department of Oceanography and Friday Harbor Laboratories, University of Washington; Seattle, Washington 98195, USA
[2] Institute of Marine Resources, A-018, Scripps Institution of Oceanography; La Jolla, California 92093, USA

Abstract

The larvacean *Oikopleura dioica* Fol was fed ^{3}H-labeled natural assemblages of marine bacterioplankton. Grazing rates ranged from < 1 to > 100 ml day^{-1} individual^{-1} and were highly dependent on larvacean body size. These rates were combined with estimates of abundance of *O. dioica* in large floating enclosures with semi-natural populations (Controlled Ecosystems Populations Experiment, CEPEX) to determine the impact of the larvacean on the bacterial populations and to estimate the amount of bacteria ingested by the larvaceans. Apparently, *O. dioica* has minimal influence on the population dynamics of the free-living bacteria, although bacteria may form a substantial portion of the larvacean's diet.

Introduction

Free-living bacteria, or bacterioplankton, have recently been shown to be far more abundant and metabolically active in epipelagic waters than bacteria associated with particles (e.g. Azam and Hodson, 1977; Watson *et al.*, 1977). The large microbial biomass and high metabolic rates suggest a high rate of bacterioplankton production. Although bacteria attached to particles may be readily consumed by herbivorous zooplankton (reviewed in Sorokin, 1978), similar planktonic sources of predation on free-living marine bacteria have rarely been demonstrated (Haas and Webb, 1979). Thus, the transfer of bacterial production to higher trophic levels and its importance for the pelagic realm cannot be easily determined.

In order to graze successfully on the small free-living bacteria (generally less than 1 µm in the largest dimension), bacteriovores must possess a very fine filtering apparatus. This restriction excludes not only raptorial copepods (e.g. the genera *Corycaeus, Euchaeta*); but also the nauplii and copepodites of suspension-feeding copepods (e.g. the genera *Acartia, Calanus, Pseudocalanus*; Nival and Nival, 1973, 1976; Corkett and McLaren, 1978; Fernández, 1979) as potential predators. The most likely bactivorous zooplankters are feeders with mucous filters (larvaceans, salps, and pteropods) and the ciliary-feeding microzooplankton (ciliates and some meroplankton). Salps and ciliates, however, retain particles greater than 3 to 5 µm more efficiently than smaller particles (Spittler, 1973; Harbison and McAlister, 1979). The larvaceans, on the other hand, may possibly have a strong influence on coastal bacterioplankton populations. The feeding filter of the larvacean *Oikopleura dioica* Fol is known to have a mesh size capable of retaining free-living bacteria (Flood, 1978). Larvaceans may also attain very high population densities (Seki, 1973; King, unpublished data). However, there are few published data of their grazing rates on algae (Paffenhöfer, 1976), and no published studies of their grazing rates on bacteria.

A further problem is that bacteriovore and bacterioplankton population dynamics have not been measured concurrently in a natural system. These data are needed to estimate the impact of grazing for both populations. The effects of advection and the differences in time scales that encompass prey growth, predator growth, and possible lag periods between the two make it difficult to determine the trophodynamics of pelagic communities. The CEEs (Controlled Experimental Ecosystem; 1300 m^3 plastic enclosures moored in Saanich Inlet, Canada; Menzel and Case, 1977) of CEPEX (Controlled Ecosystems Populations Experiment) maintain populations of bacterioplankton and their probable predators in conditions approximating those of the ambient water column but which eliminate advection. Thus, the CEEs offer a unique opportunity to study the trophodynamics of the free-living bacteria and their predators in an environment which can be revisited and is free from advective noise.

We report here estimates of the grazing rates of the larvacean *Oikopleura dioica* on radioactively labeled natural bacterioplankton assemblages obtained under

0025-3162/80/0056/0049/$01.80

laboratory conditions. We then use these rates and estimates of the population densities of *O. dioica* and bacteria in the CEEs to model the dynamics of trophic interactions of the bacterioplankton with a higher trophic level during a portion of a CEPEX experiment when the dominant bacteriovores (and herbivores) in the enclosures were larvaceans, ciliates, and nudibranch veligers. After determining that larvaceans feed on unattached bacteria, we assessed the quantitative importance of the bacterioplankton as food for the larvaceans; in addition, we studied the impact of predation by *O. dioica* on the population dynamics of bacterioplankton. Finally, we discuss the possible impact of other predators on the bacterioplankton.

Materials and Methods

Grazing Experiments

The rationale and technique for preparing the labeled bacteria for grazing experiments have been presented elsewhere (Hollibaugh *et al.*, 1980). To summarize briefly, a subsample of seawater was filtered through a 3 µm pore-size Nuclepore filter (NPF) to remove colonies, bacteria attached to particles, and predators. The filtrate was incubated with 0.1 to 1 µCi ml^{-1} methyl-^{3}H-thymidine for 2 to 20 h. About 2 h before the grazing experiment was to begin, the labeled bacteria were concentrated on a 0.2 µm pore-size NPF and washed 3 times with unlabeled HA (0.45 µm) Millipore-filtered (HAMF) seawater. The concentrated, labeled cells were next resuspended in about 50 ml of HAMF seawater and allowed to equilibrate for 1 h. The cells were then concentrated and washed as before. Finally, they were washed from the 0.2 µm NPF and filtered through a 1.0 µm NPF to remove any aggregates that might have formed during the concentration and washing steps.

The seawater used in the grazing experiment was filtered through 10 µm Nitex screen to remove large particles and grazers. Unlabeled thymidine was added to this water (1 µM final concentration) 1 h prior to the grazing experiment. The labeled bacteria were added to the mixture just prior to the experiment. The seawater was then divided amongst the beakers as follows: one beaker with no *Oikopleura dioica* Fol for determination of final dissolved label pools (Bact); one with a control larvacean(s) (CA) and house(s) (CH); and experimental beakers with a larvacean(s) and house(s) (Experimental). About 1 h prior to the experiment, the larvaceans were placed in HAMF seawater which contained 1 µM unlabeled thymidine. For the experiment, 1 to 4 (usually 1) individuals *in* their houses were transferred to each of the experimental beakers; they usually remained in their houses for the duration of the experiment (15 min to 4 h depending on the size of the specimen). Control larvaceans were prevented from feeding by removing them from their houses and gently crimping their tails with fine forceps without killing them. The crippled larvacean could not build a new house.

At the end of the run, the larvaceans and their houses were rinsed in HAMF seawater, each larvacean's trunk length was measured, and the number of houses formed during the experiment was counted. Larvaceans and houses were placed in separate scintillation vials and dissolved in Protosol (New England Nuclear). Water samples were taken prior to and after the experiment and assayed for dissolved and particulate radioactivity. For the assay, the particulate matter was collected on an HA Millipore filter and placed in a scintillation vial and fluor was added. One 1 ml subsample from the filtrate was placed in a dry scintillation vial containing fluor; a second was placed in a scintillation vial and lyophilized to remove any labeled water that might have been produced by catabolism of the methyl group of thymidine. Fluor was added to the second vial after the solutes were redissolved in 1 ml of distilled water. All samples were radioassayed in a Beckman LS 100C liquid scintillation spectrometer using Aquasol II (New England Nuclear) fluor after storing overnight in the dark to allow the filters to clear and to prevent chemiluminescence due to Protosol in the cocktail. A correction for counting efficiency and quenching was made using the external-standard-ratios method and appropriately quenched standards. The self-absorption correction was determined with ^{3}H-glucose-labeled natural populations of bacteria. All radioassay data were converted to disintegrations per minute (DPM) for use in calculations.

Particles filtered by *Oikopleura dioica* are either ingested by the larvacean or adhere to the house. We differentiate between the two processes by referring to grazing rates (g, volume of water containing particles swept clear and ingested by the larvacean as ml individual^{-1} day^{-1}) and clearance rates (F, volume swept clear by larvacean and house as ml individual^{-1} day^{-1}). Thus:

$$I = g \cdot C$$

$$g = \frac{1}{t} \cdot -\ln\left[\frac{\mathrm{DPM(bact)} - [\mathrm{DPM(larvacean)} - \mathrm{DPM(CA)}]}{\mathrm{DPM(bact)}}\right] \cdot V$$

$$F = \frac{1}{t} \cdot -\ln\left[\frac{\mathrm{DPM(bact)} - [\mathrm{DPM(larvacean)} + \mathrm{DPM(house)} - \mathrm{DPM(CA)} - \mathrm{DPM(CH)}]}{\mathrm{DPM(bact)}}\right] \cdot V,$$

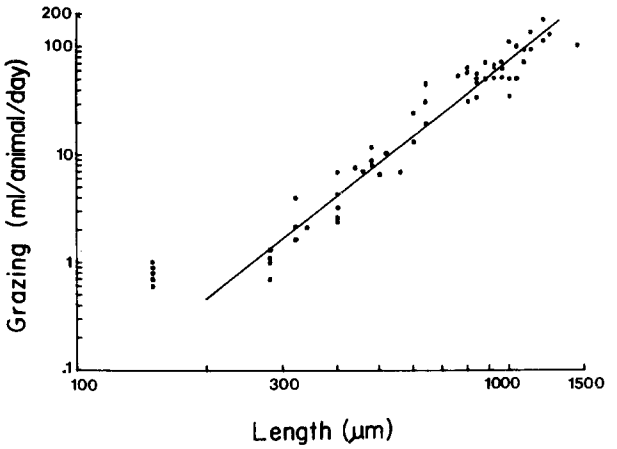

Fig. 1. *Oikopleura dioica.* Grazing rate (volume swept clear of particles which were ingested by larvacean) versus trunk length at 13.5 °C. Points at 160 μm are probably due to poor controls, and are not used in regression. Log y = 3.0828 (log x) − 7.4070 (r^2 = 0.937)

where I = ingestion (μg C larvacean^{-1} day^{-1}), C = bacteria concentration (μg C ml^{-1}), t = duration of experiment (day), ln = natural logarithm, DPM(bact) = initial bacterial DPM, DPM(larvacean) = DPM taken up by larvacean, DPM(CA) = DPM taken up by control larvacean, DPM(house) = DPM taken up by the house, DPM(CH) = DPM taken up by the control house, and V = volume of water in beaker (ml).

Field Collections

Bacteria and bacteriovore populations were collected from CEEs 78-2 and 78-3 of the Food Web I (FW I) CEPEX experiment (Grice *et al.*, in press). Bacteria were sampled by pump in the 0 to 4, 4 to 8, 8 to 12, and 12 to 16 m layers. The bacteria were enumerated and the percentage of the population in each of three size classes was determined by the acridine orange direct-count method (Hobbie *et al.*, 1977). Microbial carbon concentrations were estimated by multiplying numbers in a size class by the average volume of a cell in the size class times 125 fg C μm^{-3} (Watson *et al.*, 1977) (1 femtogram = 10^{-15} g) and summing over the three size classes. These estimates were then integrated over a 16 m depth interval.

Larvacean populations (principally *Oikopleura dioica*) were sampled daily with duplicate vertical tows (0 to 16 m) using a 35 cm diameter, 64 μm mesh net (6:1 open mesh-to-mouth area ratio). Samples were split with a Folsom splitter to approximately 100 to 150 *O. dioica* per subsample; the larvaceans were identified, enumerated, and their trunk length measured to the nearest 20 μm.

Carbon analysis of *Oikopleura dioica* was performed on larvaceans from cultures and the field. Individuals were measured (trunk length) and then placed on pre-ashed Whatman GFC filters, dried at 60 °C, and stored in a desiccator. Elemental analyses were done with a Carlo Erba CHN analyzer using the dry combustion method of Sharp (1974).

Model

Grazing rates of *Oikopleura dioica*, as measured in the laboratory, were coupled with estimates of *O. dioica* biomass to calculate grazing pressure in an exponential bacterial growth model. The model is:

$$N_1 = N_0 \, e^{(\mu - d)(t_1 - t_0)},$$

where N_1 = bacteria biomass (mgC m^{-3}) at time t_1, N_0 = bacteria biomass (mgC m^{-3}) at time t_0, μ = bacteria growth rate (day^{-1}), and d = grazing rate (day^{-1}). Grazing rate is the total population grazing rate:

$$d = \Sigma g_i \cdot L_i,$$

where g_i = larvacean size-specific grazing rate (m^3 individual^{-1} day^{-1}), and L_i = number of *O. dioica* in size class (number m^{-3}). The population numbers referred to here are daily averages. The model is computed on a daily time step (i.e., $t_1 - t_0$ = 1 day) for 22 days. The initial bacterial biomass (N_0 on Day 1) was the biomass observed in each CEE on Sampling Day 39 of FW I, as were larvacean population densities. The model output, bacterial biomass observed on each day of the 22-day run, was adjusted to fit the measured field data in each enclosure by altering the daily growth rate of the bacteria.

The amount of bacteria carbon ingested by the larvaceans was computed as:

$$I = \bar{N} \cdot d,$$

where I = daily ingestion (mg C day^{-1} m^{-3}), and $\bar{N}$ = average bacterial biomass (mg C m^{-3}). $\bar{N}$ is calculated as:

$$\bar{N} = N_0 \cdot \frac{(e^{(\mu - d)(t_1 - t_0)} - 1)}{(t_1 - t_0)(\mu - d)}.$$

Thus, our model does not predict the dynamics of the enclosed bacteria populations but assesses the impact of larvacean grazing on them and the importance of the bacteria as a food source of the larvacean.

Results

Grazing Experiments

The grazing rate of *Oikopleura dioica* on bacterioplankton is strongly dependent on the size of the larvacean (Fig. 1). For example, an individual of 300 μm trunk length is estimated to graze 1.7 ml day^{-1}, while a larvacean of 1000 μm trunk length is predicted to graze 69.4 ml

Table 1. *Oikopleura dioica.* Budget of label pools for a typical grazing experiment. All results are in disintegrations min^{-1}. HA: particulate matter retained on HA Millipore filter. na: not applicable

Beaker	Filtrate	HA	Larvacean	House	Total	Expected[a]	% difference
Bact	22473	268767	na	na	291240	283965	+2.56
Control	20947	266981	65	1155	289148		+1.83
1	22228	234882	15854	4160	277124		−2.41
2	25811	229815	16138	10708	282482		−0.52
3	24979	206933	29155	18889	279956		−1.41
4	26420	213380	22511	14227	276538		−2.62
5	27875	224220	15987	4041	272123		−4.17
6	30795	231099	15742	4593	282229		−0.61

[a]Initial disintegrations min^{-1}: direct (283965) = filtrate (15492) + HA (268473)

day^{-1}. Our data show little variation in grazing rate at various natural concentrations of bacteria, suggesting that grazing rates are independent of bacterial concentrations within the range in the CEEs. Furthermore, other work where *O. dioica* were grazing on nanophytoplankton (King, unpublished data) indicates that clearance rates equivalent to those found here were also independent of particle concentrations (in $\mu gC\ l^{-1}$) encompassing the concentrations of bacteria and nanoplankton in the enclosures.

A budget of the distribution of tritium in the various fractions in a grazing experiment is shown in Table 1. The negative difference between the expected DPM and the recovered DPM in beakers with feeding larvaceans is probably due to the method of transfer of larvaceans and houses. Care was taken to insure that animals were transferred in their houses. Water added during this process would slightly dilute the label; the average dilution seen in this experiment is equivalent to 0.5 ml of unlabeled water ($\sim 1\%$ of the experimental volume). The control does not show dilution because little water is transferred with the maimed larvacean and its house.

Loss of label from the bacteria into the water during a grazing rate measurement was generally less than 5% ($\bar{x} = 4.6\%, s_x = 3.3$) during the course of an experiment. Thus, there was little recycling of the label between the bacteria and water pools. Release of label in experimental beakers was no greater than the level of release in controls; half of the experimental beakers contained less dissolved label than the controls. This suggests that the thymidine label in the bacteria is not excreted by the larvacean but is incorporated into the animal or egested as fecal pellets. There was an indication that tritiated water was produced in the containers with feeding larvaceans at a slightly greater rate than in beakers with control individuals, which would suggest that a small portion of the labeled thymidine was being oxidized to volatile dissolved organics or water through the demethylation of thymidine. These data, however, are inconclusive due to variability between and within experiments.

Fecal pellets generally remain inside the house until the larvacean abandons the house. The average accumulation of labeled particulate matter (bacteria and fecal pellets) in houses was 12.5% (s_x = 14.2) of the total label collected by the larvacean and its house. The low accumulation of tritium in the houses indicates that egestion was a small fraction, 10% or less, of ingestion. We suggest that the radiotracer method we employed to determine the grazing rates of larvaceans on bacteria is a good estimate of the actual rate of ingestion. This is supported by the similarity between such rates and the clearance rates of larvaceans grazing on small flagellates, as determined by cell counts (King, unpublished data).

Field Populations

Bacterioplankton populations varied greatly in number (range, 0.8 to 4.2 x 10^6 cells ml^{-1}) during Days 39–60 of the FW I experiment. The variation in numbers resulted in an approximately tenfold range of bacterioplankton biomass (Figs. 2, 4). Although different phytoplankton populations were maintained in the two CEEs during this period, the free-living bacteria maintained strikingly similar populations in both, with a large peak in abundance at Day 44 followed by a rapid decline in numbers and cell size. The bacterial population outbursts in FW I were typically preceded by intense phytoplankton blooms.

Zooplankton populations in the enclosures were also somewhat similar during this period. Herbivorous copepods had declined in abundance in the CEEs to the point that *Oikopleura dioica,* ciliates, and nudibranch veligers were the numerically dominant herbivorous zooplankters. The greatest peaks of zooplankton abundance during this period were observed in CEE 78-3 with a flagellate-dominated community. The maximum concentration of *O. dioica* in CEE 78-3 was 13390 m^{-3} (Fig. 2B), compared to a maximum of 6420 m^{-3} attained in the diatom-dominated CEE 78-2 at this time (Fig. 2A). The larvaceans appeared to respond to the increased bacterioplankton populations. Larvacean populations attained their maximum abundance within 10 days of the time of maximal bacterial biomass. The generation time of *O. dioica* in the CEEs was 8 to 12 days, and the populations typically double in number daily during periods of population increase (King, unpublished data).

Numbers of *Oikopleura dioica* were converted to biomass (carbon) by the length-weight relationship shown in Fig. 3. The resulting biomass data (Fig. 4) reinforce

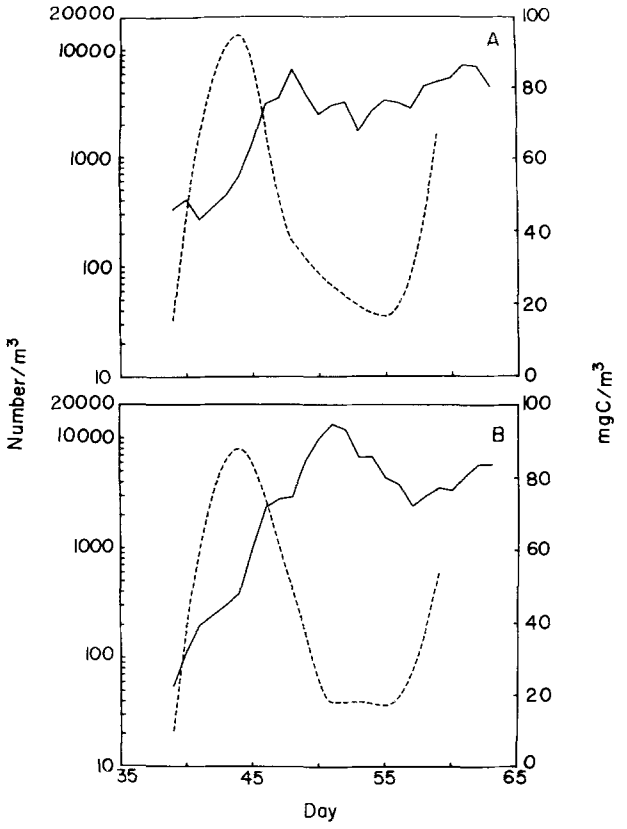

Fig. 2. *Oikopleura dioica.* Abundance of larvaceans (continuous line, left ordinate) and biomass of bacterioplankton (dashed line, right ordinate) during Days 39–64 of Food Web I (FW I) CEPEX experiment. (A) Controlled Experimental Ecosystem, CEE, 78–2; (B) CEE 78–3

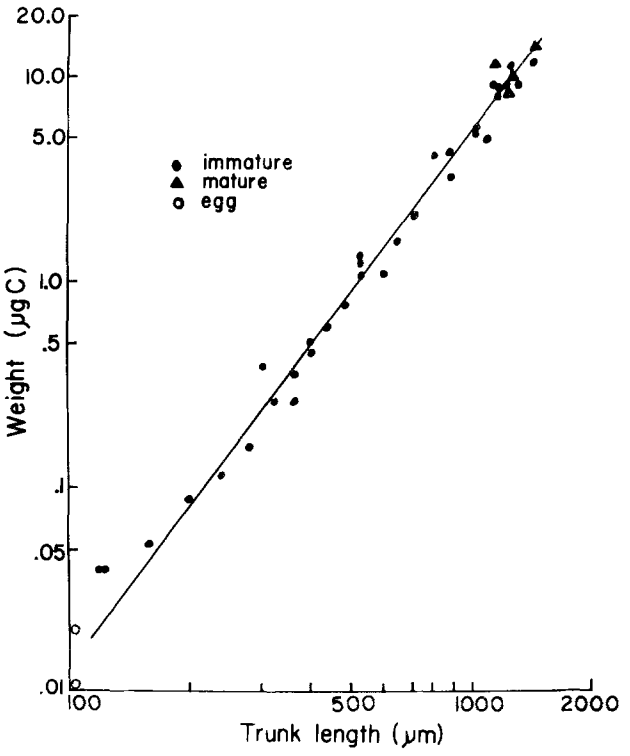

Fig. 3. *Oikopleura dioica.* Weight versus trunk length. Regression does not include egg weights. Log y = 2.6270 (log x) − 7.1348 (r^2 = 0.987)

Table 2. Bacterioplankton growth rates (doublings day^{-1}) for Controlled Experimental Ecosystems (CEEs) 78-2 and 78-3 from the model. See text for explanation

Day	CEE 78–2			CEE 78–3		
	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
39	.53	.54	.54	.63	.63	.63
40	.53	.54	.54	.63	.63	.63
41	.53	.54	.54	.63	.63	.63
42	.52	.54	.54	.62	.63	.63
43	.52	.54	.54	.62	.63	.63
44	−.30	−.29	−.28	−.24	−.23	−.23
45	−.31	−.29	−.28	−.24	−.23	−.23
46	−.34	−.29	−.28	−.25	−.23	−.23
47	−.37	−.29	−.27	−.37	−.32	−.31
48	−.20	−.15	−.14	−.40	−.32	−.31
49	−.18	−.15	−.15	−.40	−.32	−.31
50	−.18	−.15	−.15	−.39	−.32	−.31
51	−.18	−.15	−.15	−.04	.00	.01
52	−.13	−.10	−.10	−.02	.00	.00
53	−.13	−.10	−.10	−.02	.00	.00
54	−.13	−.10	−.10	−.02	.00	.00
55	.43	.49	.50	.47	.49	.49
56	.43	.49	.50	.46	.49	.49
57	.45	.49	.49	.47	.49	.49
58	.46	.49	.49	.46	.49	.49
59	.45	.49	.49	.46	.49	.49
60	.45	.49	.49	.46	.49	.49

the apparent coupling between bacteria and larvacean abundance. There is a lag time between the appearance of peak microbial biomass and the appearance of two of the three larvacean irruptions. The decline in bacterial biomass would seem to be related to the increase in the larvacean populations. Our mathematical model will test this inference.

Model

Estimates of daily bacterioplankton growth rates from the model are given in Table 2. Run 1 shows the bacterial growth rate necessary to attain the concentrations observed in the CEEs if grazing mortality (d) is assumed to be zero. Model Run 2 uses the grazing rate for *Oikopleura dioica* (Fig. 1) to compute d. The difference between Runs 1 and 2 assesses the impact of consumption of bacteria by the larvaceans in the enclosures. Run 3 multiplies the grazing rate by 1.2 in the computation of d to account for adherence of bacteria to the house and egestion; in essence, the clearance rate F is used to calculate d. This run determines the effect of removal of free-living bacteria from the water column via ingestion by larvaceans and incorporation into their houses.

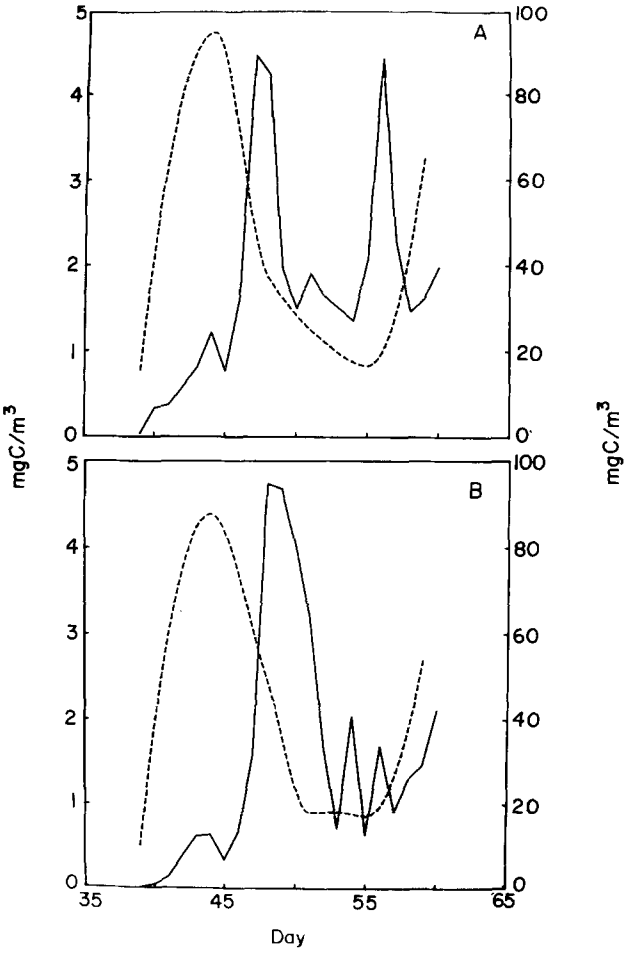

Fig. 4. *Oikopleura dioica.* Biomass of larvaceans (continuous line, left ordinate) and bacterioplankton (dashed line, right ordinate) during Days 39–64 of FW I. (A) CEE 78–2; (B) CEE 78-3

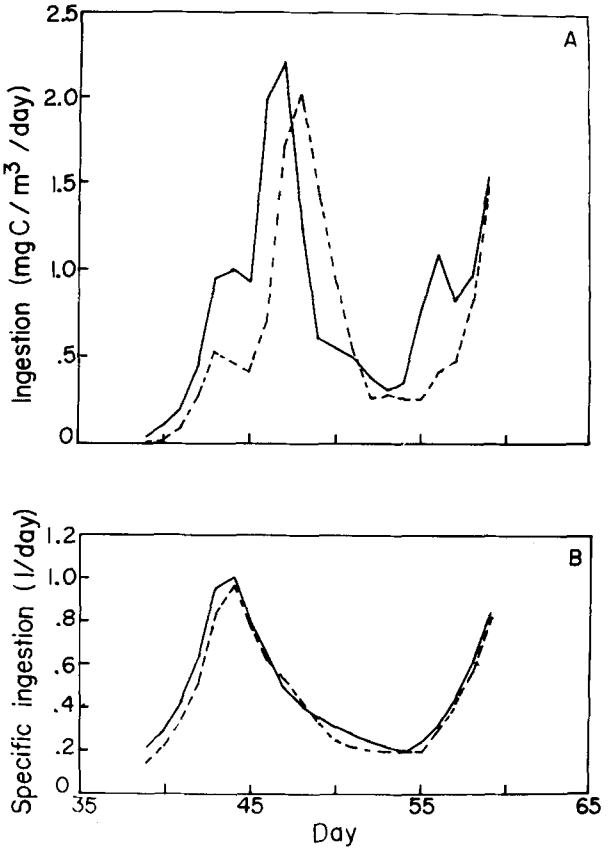

Fig. 5. *Oikopleura dioica.* (A) Ingestion of bacterioplankton by larvacean populations in CEEs 78-2 (continuous line) and 78-3 (dashed line) of FW I as calculated by the mathematical model. (B) Weight-specific consumption by larvacean population

Since bacterial biomass in the model is forced to specified levels, an increase in grazing pressure is responded to through increased bacterial growth. It is readily apparent that larvaceans have little impact on the bacterioplankton. The range of bacterial growth rates needed to overcome the effects of ingestion by *Oikopleura dioica* and adherence to its houses was 0.01 to 0.10 doublings day^{-1} in CEE 78-2 and 0.00 to 0.09 doublings day^{-1} in CEE 78-3. The CEE populations of larvaceans, although generally more numerous than those found in other field studies (Seki, 1973; Alldredge, 1976), have so few large individuals with high clearance rates that the total grazing pressure is low. Thus, what appears to be a classic Lotka-Volterra predator-prey relationship between an efficient predator, *O. dioica,* and its prey, bacteria (Figs. 2, 4), is perhaps fortuitous. Grazing by these larvaceans cannot cause or contribute greatly to the decline in bacterial biomass after Day 44.

On the other hand, bacteria are a major source of food for the larvaceans (Fig. 5). The population of *Oikopleura dioica* consumed up to 2.2 mgC m^{-3} day^{-1} in CEE 78-2 (Day 47) and 2.0 mgC m^{-3} day^{-1} in CEE 78-3 (Day 48). The maximum ingestion resulted in 5.2 and 5.5% of the bacterial biomass being consumed, respectively. The larvacean population consumed bacterioplankton biomass equivalent to 100% of its population biomass on Day 44 in both enclosures. The larvaceans, on the average, ingested an amount of bacteria equivalent to about half the biomass of the larvacean population.

Discussion and Conclusions

As far as we know, this is the first report of clearance rates for any zooplankter eating free-living bacterioplankton. Sorokin and colleagues (reviewed in Sorokin, 1978) measured the ingestion of ^{14}C-labeled bacteria (both free-living and attached) in terms of daily ration by various zooplankton species, including *Oikopleura dioica,* but did not report the concentration of prey items which is needed to calculate rates of feeding.

They concluded that *O. dioica* may derive 25 to 50% of its daily ration from the aggregate and free-living bacteria, assuming that the daily ration is equivalent to 80% of the larvacean's weight. However, *O. dioica* doubles its weight each day in culture at 13 °C (Paffenhöfer, 1976; King, unpublished data). In addition, the larvaceans also produce 3 to 10 houses day^{-1} (Lohmann, 1899; Paffenhöfer, 1973; King, unpublished data), each of which may constitute 20 to 40% of the animal's body carbon (Alldredge, 1976). Thus, the daily ration of a growing larvacean must be substantially greater than 80% of its weight. For instance, an individual of 500 µm trunk length contains 0.90 µg C and may produce 5 houses at 0.22 µg C (minimally?) while doubling its body weight. This represents a ration of 2 µg C and does not even include respiratory or excretory losses. At a bacterioplankton concentration of 30 µg Cl^{-1}, this same individual will ingest about 0.25 µg C of bacterioplankton which represents only 12% of the minimal daily ration. Clearly, bacterioplankton is not "the staple diet of Appendicularia in nature" (Pavlova *et al.*, 1971). Field populations may, in some instances, ingest 100% of their weight of bacterioplankton (Fig. 5) which may represent 25 to 50% of their daily ration.

The grazing and clearance rates of *Oikopleura dioica* on bacteria found in this study do not agree well with the clearance rates of *O. dioica* on nanophytoplankton observed by Paffenhöfer (1976). He found rates more than twice as high as our estimates at the same temperature. One could hypothesize that larvaceans filter bacterioplankton less efficiently than they filter slightly larger phytoplankton. However, new experiments similar in design to Paffenhöfer's, i.e., measuring particle removal (King, unpublished data), give clearance rates equivalent to those found herein and clearly do not support that hypothesis. Also, a preliminary grazing experiment utilizing differentially labeled phytoplankton and bacteria (cf. Gophen *et al.*, 1974; Lampert, 1974) demonstrated larvaceans grazing as efficiently on bacterioplankton as on flagellates (King and Hollibaugh, unpublished data). The differences between the rates observed by Paffenhöfer (1976) and our estimates may lie in the uncertainty in determining the number of larvaceans in his experimental grazing containers. He used, at times, the geometric mean of larvacean numbers before and after a specified period for computation of clearance rates (Paffenhöfer, personal communication), while we always employed a specified number of individuals. An exponential decrease in numbers of individuals in a culture may not be a proper assumption as it may not be in field populations (Fager, 1973). On the other hand, there may be real differences in grazing rates between *O. dioica* in the North Sea, Paffenhöfer's study area, and *O. dioica* in the fjords of the Northeast Pacific Ocean.

Laboratory estimates of bacterial growth in CEPEX enclosures and in neritic waters indicate that the bacterioplankton undergoes 1 to 3 divisions day^{-1} (μ = 0.63 to 2.08; J. A. Fuhrman and F. Azam, in preparation). If we account for bacterial mortality due to grazing by larvaceans (d = 0.10 to 0.15 at most), then a substantial amount of bacterial growth remains to be apportioned to the community. Assume, for the moment, that ciliates, veligers, and other microzooplankters remove bacteria and small flagellates with the same efficiency. Then, at most, ciliates could clear 20% (Heinbokel and Beers, 1979) and the other microzooplankters, excluding larvaceans, could clear about 25% of the water column (125,000 zooplankters m^{-3} at 2 ml day^{-1} $animal^{-1}$; Strathmann, 1971; Corkett and McLaren, 1978). Thus, the zooplankton could consume all the bacterioplankton production only if the bacteria were growing their slowest (one doubling day^{-1}) and all zooplankton were efficiently grazing at their highest capabilities (60 to 70% of the water column per day). Considering the assumptions involved, one can only conclude that this is most likely a gross overestimate of the impact of the zooplankton on the dynamics of bacterioplankton.

It has recently been suggested that the phagotrophic flagellates are major predators of the free-living bacteria (Haas and Webb, 1979). At the flagellate concentrations found in the CEEs (about 1000 ml^{-1}), they would need a clearance rate of 0.5 µl $cell^{-1}$ day^{-1} to equal the above maximal impact of the zooplankton. The necessary data on clearance rates are not available; however, if one assumes a growth rate of one doubling per day and calculates the respiration rate from Hemmingsen (1960), a 5 µm diameter flagellate would consume at least twice its body weight per day. Thus, the flagellate cells each could clear 0.5 to 1 µl day^{-1}, or more, and definitely be the principal consumers of the bacterioplankton.

On the other hand, the discrepancy between bacterial growth rates in the laboratory, and projected mortality due to predation may not exist in the natural environment. One could hypothesize that, although predation pressure is low but somewhat constant, bacterial population dynamics are rapidly influenced by the input of organics. If the bacteria are rapidly assimilating all the available food supply, then in periods of low supply one might expect cells to lyse (Postgate and Hunter, 1963) or become dormant (Novitsky and Morita, 1977; Stevenson, 1978; Wright, 1978). The observed rapid decline in bacteria biomass (Figs. 2, 4) might be explained by cell lysis and, most likely, by constant predation and no growth during periods of very low food supply. Low population growth rates would be maintained if only 10 to 20% of the bacterioplankton were assimilating food (Meyer-Reil, 1978). If this were the case, then the projected mortality due to zooplankton grazing could be a major factor in the dynamics of free-living bacteria populations.

As a result of the observations and inferences, a simplified summary of a food web based on bacterioplankton is presented in Fig. 6. At this time, we cannot say whether supply of dissolved organic matter (DOM) or predation by phagotrophs controls the dynamics of pelagic bacteria. In contrast to freshwater environments where zooplankton may be important consumers of bacteria (e.g. Gophen *et al.*, 1974; Peterson *et al.*, 1978), we

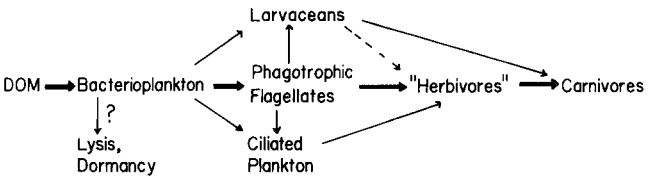

Fig. 6. Simplified heterotrophic food web; thickness of arrows denotes relative importance of pathways. Dashed line from larvaceans to "herbivores" (large particle grazers) represents discarded houses. For clarity, feedback loops are not presented (e.g. loss of DOM from higher levels to DOM pool)

would suggest that the small colorless flagellates are the primary predators of marine bacterioplankton. These phagotrophic flagellates, in turn, will be consumed by microphagous zooplankton. This creates another trophic level with its concomitant loss of energy between the recyclers of DOM, the bacteria, and the highest trophic levels. Larvaceans circumvent these energy losses by efficiently filtering both bacteria and flagellates. The larvaceans then transfer bacterioplankton carbon via their bodies to juvenile fish (Shelbourne, 1962; Manzer, 1969) and to grazers of large particles via their houses (Alldredge, 1972, 1976).

Acknowledgements. We thank J. Fuhrman for assistance in sampling and counting bacteria. We are grateful to our CEPEX colleagues, particularly J. Barwell-Clarke and F. Whitney, for their gracious help. F. Whitney and B. Dilke furnished materials that were otherwise not available. One of us (K.R.K.) thanks Dr. A. O. D. Willows, Director of the Friday Harbor Laboratories, for alloting space for completion of this study. Dr. K. Banse kindly reviewed a draft of the manuscript. This is Contribution No. 1117 from the Department of Oceanography of the University of Washington and is a contribution from the Scripps Institution of Oceanography. The research was supported by International Decade of Ocean Exploration Grants NSF OCE77-26400 to F. Azam and NSF OCE77-27224 to K. Banse.

Literature Cited

Alldredge, A. L.: Abandoned larvacean houses: a unique food source in the pelagic environment. Science, N.Y. *177*, 885–887 (1972)

Alldredge, A. L.: Discarded appendicularian houses as sources of food, surface habitats, and particulate organic matter in planktonic environments. Limnol. Oceanogr. *21*, 14–23 (1976)

Azam, F. and R. E. Hodson: Size distribution and activity of marine microheterotrophs. Limnol. Oceanogr. *22*, 492–501 (1977)

Corkett, C. J. and I. A. McLaren: The biology of *Pseudocalanus*. Adv. mar. Biol. *15*, 1–231 (1978)

Fager, E. W.: Estimation of mortality coefficients from field samples of zooplankton. Limnol. Oceanogr. *18*, 297–301 (1973)

Fernández, F.: Nutrition studies in the nauplius larva of *Calanus pacificus* (Copepoda: Calanoida). Mar. Biol. *53*, 131–147 (1979)

Flood, P. R.: Filter characteristics of appendicularian food catching nets. Experientia *34*, 173–175 (1978)

Gophen, M., B. Z. Cavari and T. Berman: Zooplankton feeding on differentially labelled algae and bacteria. Nature, Lond. *247*, 393–394 (1974)

Grice, G. D., R. P. Harris, M. R. Reeve, J. F. Heinbokel and C. O. Davis: Large-scale enclosed water-column ecosystems: an overview of Foodweb I, the final CEPEX experiment. J. mar. biol. Ass. U. K. (In press)

Haas, L. W. and K. L. Webb: Nutritional mode of several non-pigmented microflagellates from the York River estuary, Virginia. J. exp. mar. Biol. Ecol. *39*, 125–134 (1979)

Harbison, G. R. and V. L. McAlister: The filter-feeding rates and particle retention efficiencies of three species of *Cyclosalpa* (Tunicata, Thaliacea). Limnol. Oceanogr. *24*, 875–892 (1979)

Heinbokel, J. F. and J. R. Beers: Studies on the functional role of tintinnids in the Southern California Bight. III. Grazing impact of natural assemblages. Mar. Biol. *52*, 23–32 (1979)

Hemmingsen, A. M.: Energy metabolism as related to body size and respiratory surfaces, and its evolution. Rep. Steno meml Hosp. *9*, 1–110 (1960)

Hobbie, J. E., R. J. Daley and S. Jasper: Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. envirl Microbiol. *33*, 1225–1228 (1977)

Hollibaugh, J. T., J. A. Fuhrman and F. Azam: A technique to radioactively label natural assemblages of bacterioplankton for use in trophic studies. Limnol. Oceanogr. *25*, 172–181 (1980)

Lampert, W.: A method for determining food selection by zooplankton. Limnol. Oceanogr. *19*, 995–998 (1974)

Lohmann, H.: Das Gehäuse der Appendicularien, sein Bau, seine Funktion und seine Entstehung. Schr. naturw. Ver. Schles.-Holst. *11*, 347–406 (1899)

Manzer, J. I.: Stomach contents of juvenile Pacific salmon in Chatham Sound and adjacent waters. J. Fish. Res. Bd Can. *26*, 2219–2223 (1969)

Menzel, D. W. and J. Case: Concept and design: controlled ecosystem pollution experiment. Bull. mar. Sci. *27*, 1–7 (1977)

Meyer-Reil, L.-A.: Autoradiography and epifluorescence microscopy combined for the determination of number and spectrum of actively metabolizing bacteria in natural waters. Appl. envirl Microbiol. *36*, 506–512 (1978)

Nival, P. et S. Nival: Efficacité de filtration des copépodes planctoniques. Annls Inst. oceanogr., Paris (N.S.) *49*, 135–144 (1973)

Nival, P. et S. Nival: Particle retention efficiencies of an herbivorous copepod, *Acartia clausi* (adult and copepodite stages); effects on grazing. Limnol. Oceanogr. *21*, 24–38 (1976)

Novitsky, J. A. and R. Y. Morita: Survival of a psychrophilic marine vibrio under long term nutrient starvation. Appl. envirl Microbiol. *33*, 635–641 (1977)

Paffenhöfer, G.-A.: The cultivation of an appendicularian through numerous generations. Mar. Biol. *22*, 183–185 (1973)

Paffenhöfer, G.-A.: On the biology of Appendicularia of the southern North Sea. Proc. 10th Eur. Symp. mar. Biol. *2*, 437–455 (1976). (Ed. by G. Persoone and E. Jaspers. Wetteren, Belgium: Universa Press)

Pavlova, E. V., T. S. Petipa and Yu. I. Sorokin: Role of bacterioplankton in the nutrition of marine planktonic animals. *In*: Life activity of pelagic communities in the tropical regions of the ocean, pp 142–151. Ed. by M. E. Vinogradov. Moscow: Nauka 1971. [Transl. from Russian by Israel Program for Scientific Translations, 1973]

Peterson, B. J., J. E. Hobbie and J. F. Haney: *Daphnia* grazing on natural bacteria. Limnol. Oceanogr. *23*, 1039–1044 (1978)

Postgate, J. R. and J. R. Hunter: The survival of starved bacteria. J. appl. Bact. *20*, 295–300 (1963)

Seki, H.: Red tide of *Oikopleura* in Saanich Inlet. La Mer (Bull. Soc. franco-jap. Océanogr.) *11*, 153–158 (1973)

Sharp, J. H.: Improved analysis for "particulate" organic carbon and nitrogen from seawater. Limnol. Oceanogr. *19*, 984–989 (1974)

Shelbourne, J. E.: A predator-prey size relationship for plaice larvae feeding on *Oikopleura*. J. mar. biol. Ass. U.K. *42*, 243–252 (1962)

Sorokin, Yu. I.: Decomposition of organic matter and nutrient regeneration. *In*: Marine ecology, Vol. 4. pp 501–616. Ed. by O. Kinne. Chichester: Wiley 1978

Spittler, P.: Feeding experiments with tintinnids. Oikos (Suppl.) *15*, 128–132 (1973)

Stevenson, L. H.: A case for bacterial dormancy in aquatic systems. Microb. Ecol. *4*, 127–133 (1978)

Strathmann, R. R.: The feeding behavior of planktotrophic echinoderm larvae: mechanisms, regulation, and rates of suspension feeding, J. exp. mar. Biol. Ecol. *6*, 109–160 (1971)

Watson, S. W., T. J. Novitsky, H. L. Quinby and F. W. Valois: Determination of bacterial number and biomass in the marine environment. Appl. envirl Microbiol. *33*, 940–946 (1977)

Wright, R. T.: Measurement and significance of specific activity in the heterotrophic bacteria of natural waters. Appl. envirl Microbiol. *36*, 297–305 (1978)

Date of final manuscript acceptance: February 1, 1980.
Communicated by N. D. Holland, La Jolla