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Bacteria not an Energetically Favorable Food Source for Larvae of *Artemia salina*

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Bacteria not an energetically favorable food source for *Artemia salina* larvae

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Abstract

Larvae of the crustacean *Artemia salina* are reported to differentially ingest food particles of different sizes. We exposed 2-to-5-day-old A. salina larvae to equal volumetric concentrations (bead volume per mL) of 6 µm-diameter and 0.5µm-diameter polystyrene beads. The clearance rate (volume cleared of particles per unit time) of each bead size was positively correlated with developmental stage of the larvae (r=0.598, p<0.0001 for 6-µm beads; r=0.610, p<0.0001 for 0.5-µm beads). The average clearance rate for all larvae exposed to 6-µm beads (3.88 ± 2.15 µl/hr, mean ± SD) was significantly and 69 times greater than that that of larvae exposed to 0.5-µm beads (0.0560 ± 0.0234 µl/hr, mean ± SD). These clearance rates suggest that large cells (6-µm particles) contribute significantly to fulfilling the energetic demands of the larvae (as calculated from published values of estimated metabolic rates) while bacteria-sized particles (0.5-µm diameter) do not.

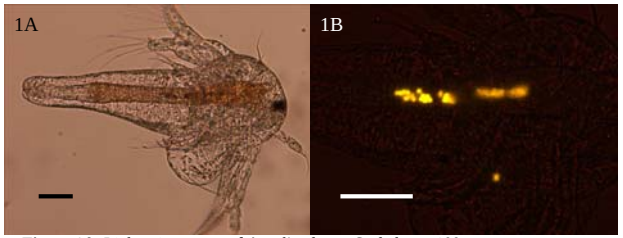

Figure 1A. Light microscopy of *A. salina* larva. Scale bar = 100 µm
Figure 1B. Fluorescence microscopy of *A. salina* larva after exposure to 6-µm polystyrene beads. Scale bar = 100 µm

Introduction

Herbivorous zooplankton such as *Artemia salina* (Crustacea; Anostraca) play an important role in marine food webs as an energy pathway between unicellular producers and carnivorous zooplankton (Evjemo et. al, 2000). Although algal cells are thought to be the primary food source for *A. salina*, there is evidence that bacteria may be a food source or at least a dietary supplement for these animals (Intriago and Jones, 1992).

Artemia ingest food particles of varying sizes at different rates, and particles of a size equivalent to that of algal cells (4-10 µm diameter) are captured at the highest rate (Makridis and Vadstein, 1999). *Artemia* are known to feed on alga such as *Dunaliella* in natural habitats (Evjemo et al., 2000), but little is known about the contribution of bacteria to the larval diet and nutritional demands. Intriago and Jones (1993) reported that *A. franciscana* larvae exhibited an increase in growth rate and biomass when a diet of the alga *Rhodomonas* was supplemented with the bacterium *Flexibacter* when compared to a diet of *Rhodomonas* alone. These results would indicate that bacteria may be a significant nutritional source for *Artemia*, or at least contribute nutritional material which algal cells do not. We have investigated the ability of *Artemia* larvae to capture bacteria-sized particles by comparing the ability of the larvae to ingest 6-µm diameter and 0.5-µm diameter polystyrene beads. The data collected were then used to estimate the potential energetic contribution of algae and bacteria as food for *Artemia* larvae.

Materials and Methods

Artemia salina larvae were raised in 15‰ filtered sea water (FSW) and fed Roti-Rich® daily. For each experiment, approximately 20 individuals were transferred to 10 mL of FSW containing polystyrene beads (6-µm or 0.5-µm diameter, Polysciences; Tables 1 & 2). After a 10 minute incubation, individuals were transferred to carbonated FSW to immobilize the specimens. The number of beads within the digestive system was observed using a Nikon E600 compound microscope equipped for fluorescence microscopy. The stage of development of each individual was determined following the designations given by Schrehardt (1987). All statistical analyses were completed using SPSS (version 17.0).

Bead Size	Actual Diameter (µm)	Final Concentration (beads/mL)	Bead Volume (µm³/mL)
6 µm	5.681	$5.0x10^3$	$4.8x10^5$
0.5 µm	0.513	$6.0x10^6$	$4.3x10^5$

Table 1. Final concentrations of 6-µm and 0.5-µm beads in experimental vials, if present.

Solution	Contents
A	0.5-µm beads
B	6-µm and 0.5- µm beads

Table 2. Experimental solutions used. Each experiment performed consisted of larvae from the same population being separated into one vial of each solution. Data were collected simultaneously.

Results

Clearance rate (the volume of fluid particles per unit time, CR = IR x [bead]) was positively correlated with developmental stage for both 6-µm beads (r=0.598, p<0.0001) and 0.5-µm beads (r=0.610, p<0.0001). The larvae showed a strong preference for clearing 6-µm beads from solution at a rate that was an average of 69 times greater than the clearance rate of 0.5-µm beads (3.88 ± 2.15 µl/hr for 6-µm beads, 0.0560 ± 0.0234 µl/hr for 0.5-µm beads, average of all stages; Figure 2). In addition, when exposed to 0.5-µm beads and 6-µm beads together, the larvae captured significantly fewer 0.5-µm beads from the solution (0.0816 ± 0.0246 µl/hr without 6-µm beads, 0.0622 ± 0.0229 µl/hr with 6-µm beads for stage 5 larvae; Figure 3).

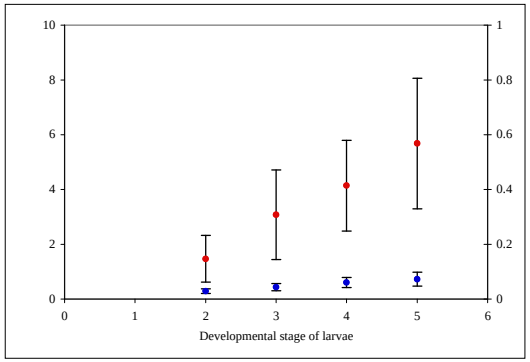

Figure 2. Average observed clearance rates of 6-µm beads (red dots; left axis) and 5-µm beads (blue dots; right axis) by larvae of developmental stages 2-5.

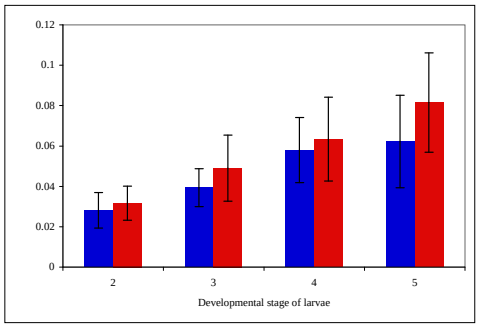

Figure 3. Comparison of clearance rates of 0.5-µm beads in experiments with (blue) and without (red) 6-µm beads.

Discussion

Our results confirm earlier work (e.g. Makridis and Vadstein, 1999) that *Artemia* larvae preferentially ingest larger, algal-sized particles compared to submicron-sized particles. At all stages of development tested, larvae ingested 6-µm particles at a significantly higher rate than 0.5-µm particles although the bead concentrations used gave an equivalent encounter rate. A more unexpected result is that the presence of 6-µm particles in solution significantly reduces the clearance of 0.5-µm particles. This suggests that the ingestion of larger, algal-sized particles by the larvae somehow reduces the ingestion of submicron-sized particles at the same rate as when the larger particles are absent. This behavior warrants further investigation.

Combining our data on particle capture rates with data from Gnaiger (1983), Lee and Furman (1987), and Szela and Marsh (2005), we calculated the energetic contribution of algal cells (6-µm particles) or bacteria (0.5-µm particles) and used our observed clearance rates to estimate the percent contribution of each to the metabolic demands of an Artemia larva:

$$E_{IN} = CR \times 1\ mL/1000\ \mu l \times [bead] \times V_{bead} \times m_{bio}/V_{bio} \times 1\ pmol/12\ pg\ C \times H_C$$

Particle diameter (µm)	Average CR (µl/hr)	Energy contribution (nJ/hr)	Percent of metabolic demand
6	3.88	$2.79x10^4$	55.4%
0.5	0.056	$3.56x10^2$	0.706%

Table 3. Estimated energy contribution of 6-µm particles and 0.5-µm particles based on the above equation. Biovolume to biomass ratio (m_{bio}/V_{bio}) was defined as 0.38 pg C/µm³ (Lee and Furman, 1987). Enthalpic equivalence of carbon (H_C) was defined as 473 nJ/pg C for 100% carbohydrate composition (Gnaiger, 1983). Metabolic demand was defined as $5.06x10^4$ nJ/hr/larva (Szela and Marsh, 2005).

Our analysis indicates that ingestion of algal-sized (6-µm diameter) particles at our observed average clearance rates contributes over half of the energy consumed by the larvae per unit time, while ingestion of bacterium-sized (0.5-µm diameter) particles at our observed clearance rates contributes less than one percent of the energy consumed. This leads us to conclude that bacteria are not a reasonable or energetically favorable food source for *Artemia* larvae.

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