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Crustacean Biology.
FILTER-FEEDING SHRIMPS (ANOSTRACA) GRAZING ON BACTERIA

K. R. Dierckens, L. Beladjal, J. Vandenberghe, J. Swings, and J. Mertens

ABSTRACT

Streptocephalus torvicornis and Branchipus schaefferi, males and females separately, were fed Escherichia coli at densities ranging from $1 \times 10^6$ to $5.5 \times 10^7$ ml$^{-1}$. These shrimps are able to consume large quantities of E. coli. Branchipus schaefferi consumed $4 \times 10^8$ E. coli ind$^{-1}$ hr$^{-1}$ and S. torvicornis consumed $5 \times 10^8$ E. coli ind$^{-1}$ hr$^{-1}$ ($0.082$ mg hr$^{-1}$ ind$^{-1}$). Consumption leveled at an initial density of $4 \times 10^7$ E. coli ml$^{-1}$ for B. schaefferi, while for S. torvicornis consumption leveled at $4.5 \times 10^7$ E. coli ind$^{-1}$ hr$^{-1}$.

The functional response curves of these filter-feeding shrimps showed an unexpected pattern. For the males of both species, at $15 \times 10^6$ E. coli ml$^{-1}$ there was a small decrease in consumption, while the curves of the females showed a similar decrease at $20 \times 10^6$ E. coli ml$^{-1}$. This phenomenon is explained by assuming that the distance between the setae of the limbs, the movement rate of the thoracic appendages, or the inclination of the filtering apparatus is altered.

During 5-h experiments, both species do not feed at a constant rate.

Several attempts have been made to culture anostracans. Until recently, anostracans were mainly fed algae (Mitchell, 1991; Brendonck, 1993; Ali and Dumont, 1995) and agricultural waste products (Platon et al., 1987; Lavens et al., 1987; Ali and Brendonck, 1995). Ephemeral argilotropic (rich in clay particles) waterbodies usually contain low amounts of algae, because sunlight cannot penetrate deep into the water. This limits the availability of algae for fairy shrimps. It has been shown through field studies on Streptocephalus dichotomus Baird (see Bernice, 1971), laboratory experiments on S. proboscideus (Frauenfeld) (see Mertens et al., 1990; Ali, 1995) and on S. torvicornis (Waga) (see Dierckens et al., 1995) that Anostraca are able to feed on animal diets. We observed that temporary pools in Spain and Algeria, where fairy shrimps can be found, are frequently used as drinking places for goats and sheep. These enrich the water with their droppings while drinking. Since these pools are endorheic, a large amount of organic waste is swept into them during heavy rainfalls. It is therefore not surprising that bacteria blooms and that fairy shrimps feed upon these organisms in nature.

Studies on other filter-feeding zooplankton, such as Daphnia (see McMahon and Rigler, 1965; Lampert, 1974; Peterson et al., 1978), provided evidence that these animals substantially reduce bacterial populations.

Streptocephalus torvicornis and Branchipus schaefferi (Fisher) are found sympatri-}

ally in several places (Thiéry, 1991, 1996; Petrov and Cvetkovic, in press). In the present paper, these species are used to determine whether they are able to graze, and to what extent, on a bacterial population of Escherichia coli (Migula).

Using 2- and 5-h experiments, we measured the concentration at which both species consume the highest number of E. coli and defined the consumption rate for males and females separately.

MATERIALS AND METHODS

Streptocephalus torvicornis and B. schaefferi (origin: Used, Spain, and Boughzoul, Algeria) were successfully reared in the laboratory for over 4 years. Dried cysts, harvested from these laboratory cultures, were hatched to obtain the experimental animals. Nauplii were raised in EPA-medium and initially fed Scenedesmus acutus Meyen at a density of 200,000 cells ml$^{-1}$ at 20°C. Larvae were maintained in 40-l aquaria at a density of 20–50 individuals l$^{-1}$ and raised on a mixture of Scenedesmus, hay infusion, and E. coli at varying densities.

The animals were sexed and measured from the median axis of the eyes to the base of the cercopods, using a stereomicroscope fitted with a camera lucida and a digitizing plate (SummaSketch™ III Professional). Male B. schaefferi measured $1.24 \pm 0.15$ cm, females $1.26 \pm 0.15$ cm; male S. torvicornis measured $1.36 \pm 0.17$ cm and females $1.41 \pm 0.08$ cm.

Escherichia coli LMG 2092 was used throughout the experiments. It was placed on nutrient agar plates and kept at 25°C for 24 h. After harvesting, the culture was washed 2 times with sterile physiological (0.85% NaCl) water and centrifuged (MSE, Europa 24; 10,000 g). Then, E. coli were resuspended in 0.85% NaCl solution. The
concentration was roughly defined with a spectrophotometer (Philips, PU 8620) at 550 nm and more precisely by plating a diluted sample on Tergitol-7 (modified) agar (Oxoid) using a spiral plater (Spiral System Inc., Cincinnati, Ohio).

In the 2-h experiments, 8 concentrations of E. coli, between 1 and $42 \times 10^6$ ml$^{-1}$ were provided to B. schaefferi and 9 concentrations between 1 and $55 \times 10^6$ ml$^{-1}$ to S. torvicornis. The experiments were conducted in 200-ml beakers each containing 2 fairy shrimps. For each concentration there were 4 replicates and 4 controls. From each replicate, serial dilutions were made and plated twice on Tergitol-7 (modified) agar (Oxoid) using a spiral plater.

For the 5-h experiments, a concentration of $4 \times 10^7$ ml$^{-1}$ for B. schaefferi and $4.5 \times 10^7$ ml$^{-1}$ for S. torvicornis was used. This concentration was chosen according to the results of the 2-h experiments. Every hour, 2 samples (5 and 10 ml) were taken and plated immediately, in the manner mentioned above. After 24-h incubation at 25°C, the CFUs (colony forming units) were counted on a spiral-plater colony viewer (Spiral System Inc., Cincinnati, Ohio).

All experiments were conducted at 20°C under continuous, diffuse, fluorescent light. In order to avoid stress, due to pH and salinity shocks, stock medium filtered through a 0.45-μm mesh (Gelman) was used.

Braun (1980) mentioned that densities exceeding 20 Artemia 1$^{-1}$ have a decreasing effect on the feeding rate. The same trend was observed by Brendonck (1993) using S. proboscideus. In order to avoid this effect, we maintained concentrations at 10 individuals 1$^{-1}$.

The dry weight of E. coli was determined as follows: after washing and resuspending the harvested bacteria 3
Both species showed density-dependent food consumption (Fig. 1). All functional response curves exhibited a small decrease at an initial concentration of 1.5 or 2.0 × 10⁷ E. coli ml⁻¹ for males and females, respectively. A one-way ANOVA indicated that these decreases are significantly lower than the two concentrations on either side.

At concentrations exceeding 4.2 × 10⁷ ml⁻¹, standard deviations became too large, because of too high dilutions, to make use of these data for response curves of B. schaefferi (Fig. 1a, b). The curves plateaued at an initial density of E. coli of 4.4 × 10⁷ ml⁻¹ for S. torvicornis (Fig. 1c, d). Higher concentration of E. coli did not significantly increase the ingestion (Kruskal-Wallis; P > 0.05).

Both species were able to ingest large numbers of E. coli, mean 4.0 × 10⁸ hr⁻¹ anostracan⁻¹ (0.066 mg dry weight) or 5.0 × 10⁸ hr⁻¹ anostracan⁻¹ (0.083 mg dry weight) for B. schaefferi and S. torvicornis, respectively. It was found that S. torvicornis is able to cope with higher initial concentrations. The dry weight of a concentration of an E. coli of 1.00 × 10⁹ ml⁻¹ is 0.165 mg. Figure 2 shows that fairy shrimps do not feed at a constant rate during the 5-h experiments. All graphs showed a decrease at the second hour. Figure 3 gives the cumulative consumption of E. coli after a 5-h period.
Fig. 3. Cumulative consumption of E. coli during a 5-h period for Branchipus schaefferi and Streptocephalus torvicornis, males (M) and females (F) separately (mean ± SD of four replicates).

**DISCUSSION**

Douillet (1987) showed by feeding dried diets under axenic and xenic conditions that none of the diets under the axenic condition met the nutritional requirements of Artemia. Here, it is shown that S. torvicornis and B. schaefferi are able to filter and ingest E. coli.

The 2-h experiments revealed original functional response curves. Each curve has a point with relatively low food intake. As the species involved use their thoracic appendages for filtering the medium, this might be explained by an alteration of their inclination, changing the distance between the setae or changing the beat rate of the filtering structures. Lowndes (1933) published an extensive study on the feeding mechanism of Chirocephalus diaphanus Prévost. He noticed that this fairy shrimp is able to alter the direction in which the thoracic appendages vibrate. The muscles in these appendages were also described. Three of the five distinct series of muscles control the fringing setae. From these observations, it is very likely that fairy shrimps are able to alter the distance between their setae.

Barlow and Sleigh (1980) reported that the beat frequency of the thoracic appendages in Artemia ranges from 2−4 Hz. It is possible that fairy shrimps are able to slow down the beat rate of the filter apparatus as they encounter higher concentrations of food, thus spending less energy to collect the same amount of food. It is also well known that, when the food concentration gets too high, it is accumulated immediately behind the labrum. The ball of food particles is caught in the setae of the first pair of thoracic appendages and is then removed (Reeve, 1963). By beating at a lower rate, particles can be removed more efficiently from the surrounding water. A slower movement creates a smaller current, pushing particles away from the sieving structures. This may explain the higher food uptake. The plateau can be explained by assuming that the mouthparts are not capable of processing more material in a unit of time.

From the five-hour experiments, it is apparent that the fairy shrimps, although their thoracic appendages keep moving, do not feed continuously (Fig. 2). If they encounter too much food and continue grazing, the passage time through the gut would be too low.

Both species are able to feed on E. coli; substantial amounts of these bacteria are cleared from the medium. Streptocephalus torvicornis ingests up to $5.0 \times 10^8$ (=0.083 mg dry weight) E. coli per anostracan per hour, while B. schaefferi ingests $4.0 \times 10^8$ (=0.066 mg dry weight) per anostracan per hour. Since the waterbodies where the anostracans are found are often used as drinking places for cattle, bacteria may constitute an important part of their diet.

Although fairy shrimps continuously move their thoracic appendages, they do not filter constantly. This suggests that the filtering apparatus is not a rigid sieve, but a flexible, adjustable apparatus.

**LITERATURE CITED**


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