Factors affecting zooplankton feeding by the sea anemone *Aiptasia pallida*

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ABSTRACT: Effects of various treatments on prey capture, prey ingestion and ingestion time of individual *Artemia salina* nauplii by the sea anemone *Aiptasia pallida* Verrill were studied in the laboratory. Exposure to crude *Artemia* homogenate, 5×10^{-4} M reduced glutathione or 5×10^{-4} M proline significantly decreased the number of *Artemia* that were captured and ingested but had no significant effect on the ingestion time of individual *Artemia*. Multiple captures increased the total ingestion time but decreased ingestion time per prey item. Results suggest that, under these conditions, the prey capture phase of zooplankton feeding was somewhat distinct from the ingestion phase since chemical stimuli that significantly reduced prey capture had no significant effect on ingestion time.

INTRODUCTION

Zooplankton feeding by cnidarians represents a major interaction of the organism with the environment. For example, the importance of zooplankton feeding in determining population size and individual growth has been repeatedly demonstrated in *Hydra* (Lenhoff & Loomis, 1957; Muscatine, 1961; Slobodkin, 1964; Muscatine & Lenhoff, 1965; Clayton, 1984), siphonophores (Purcell, 1981a, 1982), and anthozoans (Muscatine, 1961; Minasian, 1976, 1979, 1982; Sebens, 1980, 1981; Clayton & Lasker, 1985).

Given the significance of zooplankton feeding for cnidarian growth and survival, much work has focused on the feeding process itself. The "feeding reflex" or "feeding response" refers to those behaviours that occur between prey capture and ingestion (Loomis, 1955; Lenhoff, 1961a) and has been thoroughly examined in many species. For example, factors that initiate the feeding response have been determined for *Hydra* (Loomis, 1955; Lenhoff, 1961a, b; Mariscal, 1971), hydroids (Lenhoff & Schneiderman, 1959; Fulton, 1963; Pardy & Lenhoff, 1968), siphonophores (Lenhoff & Schneiderman, 1959; Mackie & Boag, 1963), corals (Mariscal & Lenhoff, 1968; Lehman & Porter, 1973) and sea anemones (Lindstedt et al., 1968; Lindstedt, 1971; Reimer, 1971a, b, 1973; and many others). Many environmental parameters can affect the feeding response (Lenhoff, 1961a, b, 1965) but it is uncertain whether nematocyst discharge and zooplankton feeding can be controlled, in any manner, by the individual (Picken & Skaer, 1966; Mariscal, 1974).

Host control of nematocyst discharge and zooplankton feeding have been studied by examining individuals following the cessation of prey ingestion. For example, discharge

of stenotele nematocysts, which are used for prey capture, decreases in *Hydra* when there is food in the gastrovascular cavity (Burnett et al., 1960; Hand, 1961). Feeding also inhibits subsequent nematocyst activity by sea anemones (Sandberg et al., 1971; Mariscal, 1973). In addition, fed *Hydra* exhibit a rise in the chemical threshold necessary to initiate feeding (Koizumi & Maeda, 1981). It has been suggested that fed hydra may modify nematocyst action after stimulation of stretch receptors in the gastrovascular cavity (Burnett et al., 1960), following sensation of prey factors in the gastrovascular cavity (Smith et al., 1974), or from the accumulation of soluble factors in the environment (Ruch & Cook, 1984).

Studies of those factors controlling zooplankton feeding have not considered effects on a central feeding parameter, the number of prey actually ingested. Clearly, this aspect of feeding is important since it represents the culmination of the feeding process and is fundamental for individual growth and survival. In addition, there is evidence that prey digestion can alter subsequent zooplankton feeding (Burnett et al., 1960; Hand, 1961; Smith et al., 1974; Lasker et al., 1982). In this study I examined the effects of various treatments on prey capture, prey ingestion and ingestion time by the sea anemone *Aiptasia pallida*. Results suggest that, under these conditions, zooplankton capture and ingestion can be modified by the individual while ingestion time remains unaltered.

MATERIALS AND METHODS

Specimen maintenance

Aiptasia pallida used in all experiments were derived, by pedal laceration, from a single individual initially obtained from Carolina Biological Supply Company. All individuals were symbiotic and contained zooxanthellae. Anemones were maintained in artificial sea water (Dayno Sea Salts) at 23 ± 1 °C on a LD 14 : 10 photoperiod at a light intensity of 65 $\mu E \cdot m^{-2} \cdot sec^{-1}$. Individuals used in all experiments were starved for two days and were taken from cultures fed to repletion twice per week (Monday and Thursday) with freshly hatched (24–48 h) *Artemia salina* nauplii.

Feeding experiments

Individual anemones were placed in separate plastic cups with 40 ml sea water and allowed to attach and expand (45–75 min). Fifty extensively washed *Artemia salina* nauplii were then presented singly using a Pasteur pipette. Each nauplius was repeatedly released near the tentacles until the nauplius swam into a tentacle (Ruch & Cook, 1984). Impact of the nauplius with a tentacle is termed prey interception (Lasker et al., 1982). Following interception a nauplius was either: (1) captured and ingested; (2) captured but not ingested or; (3) not captured. Ingestion time of nauplii in the first category was arbitrarily defined as the time elapsed from first contact with a tentacle to closure of the mouth after ingestion. The second category includes nauplii that were killed and either remained on the tentacles or were subsequently released from the tentacles. Nauplii in the third category had to swim normally for at least thirty sec after interception to be considered not captured.

Effects of crude *Artemia* homogenate, 5×10^{-4}M reduced glutathione (GSH) and 5×10^{-4}M proline on zooplankton feeding were also examined. 3 ml of wetpacked *Artemia* (Slobodkin, 1964) were homogenized with a glass tissue grinder, filtered (0.45 μm), and the filtrate used as chemical stimulus. GSH and proline were selected as chemical stimulants since they are known to elicit the feeding response in a variety of cnidarians (c.f. Loomis, 1955; Fulton, 1963; Lenhoff, 1961a, b; Lindstedt, 1971; Lehman & Porter, 1973). Following a 15 min exposure to *Artemia* homogenate, 5×10^{-4} M GSH or 5×10^{-4} M proline feeding experiments were conducted as described previously.

The relationship between number of prey captured and total ingestion time for all prey items was examined by presenting 2–8 *Artemia* near the tentacles of an individual anemone and determining the number captured and total ingestion time for all *Artemia*. The number of nauplii present in a multiple capture was defined as the total number captured by an anemone within 5 seconds of the first prey capture.

RESULTS AND DISCUSSION

Number of prey ingested by individual *Aiptasia pallida* (Table 1) was significantly less for anemones exposed to either crude *Artemia* homogenate ($t_s = 12.06$; df = 8; p < 0.001), 5×10^{-4} M GSH ($t_s = 12.59$; df = 8; p < 0.001) or 5×10^{-4} M proline ($t_s = 13.39$; df = 8; p < 0.001). However, ingestion time (Table 2) for those *Artemia* consumed

Table 1. Effects of various treatments on the fate of the first 50 intercepted *Artemia salina* nauplii by *Aiptasia pallida* ($\overline{X} \pm$ s.d.). In all cases n = 5 anemones. Percent change column lists the percent difference of each treatment compared to the control for the number of nauplii captured and ingested

Treatment	No. captured and ingested	No. only captured	No. not captured	% change	p
Control	49.80 ± 0.45	0.20 ± 0.45	0.00 ± 0.00	–	–
Crude homogenate	25.06 ± 4.44	4.40 ± 2.07	20.54 ± 5.18	− 49.68	< 0.001
5×10^{-4} M GSH	23.19 ± 4.81	4.90 ± 2.42	21.91 ± 5.63	− 53.43	< 0.001
5×10^{-4} M proline	21.40 ± 4.72	5.60 ± 3.21	23.00 ± 4.47	− 57.03	< 0.001

Table 2. Effects of various treatments on ingestion time (in sec) of *Artemia salina* nauplii by *Aiptasia pallida* ($\overline{X} \pm$ s.d.). In all cases n = 5 anemones. Percent change column lists the percent difference of each treatment compared to the control

Treatment	Ingestion time	% change	p
Control	22.39 ± 1.47	–	–
Crude homogenate	21.98 ± 2.25	− 1.83	> 0.50
5×10^{-4} M GSH	23.86 ± 3.21	+ 6.57	> 0.05
5×10^{-4} M proline	24.04 ± 3.82	+ 7.37	> 0.05

by anemones was not significantly affected by exposure to *Artemia* homogenate (t_s = 0.34; df = 8; p > 0.50), 5×10^{-4} M GSH (t_s = 1.91; df = 8; p > 0.05) or 5×10^{-4} M proline (t_s = 2.04; df = 8; p > 0.05). There was a significant positive correlation between the number of prey captured and total ingestion time for anemones with multiple *Artemia* captures (Fig. 1; r = 0.92; p > 0.001).

Zooplankton feeding by cnidarians can be divided into a series of discrete actions (Forest, 1962; Lenhoff, 1974; Lasker et al., 1982). The first step is the impact, or interception, of a prey item with a tentacle. Following interception, the prey item may, or may not, be captured as a result of nematocyst discharge. Failure to capture prey may due to nematocyst inactivation or previous nematocyst depletion. In these experiments, a 15 min treatment with crude *Artemia* homogenate, 5×10^{-4} M GSH or 5×10^{-4} M proline decreased prey capture and ingestion by 49.68, 59.46 and 57.03 % respectively (Table 1). Decreased zooplankton feeding may have been due to the presence of fewer functional nematocysts in treated anemones since *Artemia* homogenate is known to elicit appreciable nematocyst discharge (Pantin, 1942; Burnett et al., 1963; Ruch & Cook, 1984). Reduction in number of prey captured and ingested following exposure to
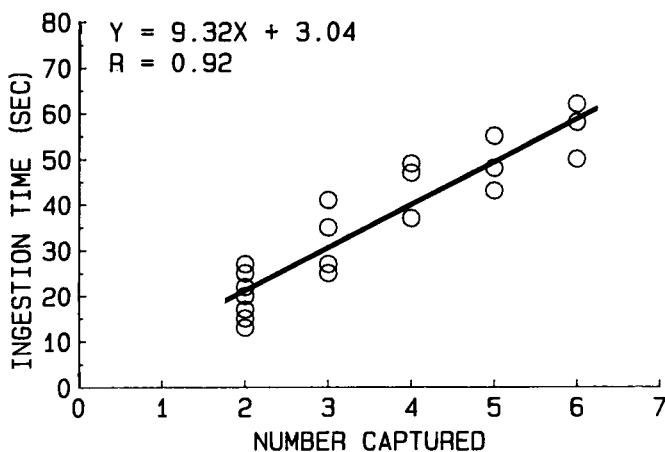

Fig. 1. Total ingestion time (Y) as a function of the number of prey captured (X)

Artemia homogenate suggests that zooplankton feeding is, in some manner, determined by the chemical stimulation of intercepted prey. However, chemical stimulation alone did not totally extinguish zooplankton feeding (Table 1), indicating that feeding is not only controlled by chemical stimulation (Lasker et al., 1982).

Although chemical stimulation reduced zooplankton capture and ingestion (Table 1), it had no effect on the ingestion time of *Artemia* (Table 2). This suggests that the prey ingestion phase of zooplankton feeding is distinct from the prey capture phase (Lenhoff, 1974; Lasker et al., 1982) since one (zooplankton capture) was reduced by chemical stimulation while the other (ingestion time) was not. Lindstedt (1971) has shown a similar response in *Anthopleura elegantissima*, where tentacle bending toward the mouth and prey ingestion following contact with the mouth are controlled by different chemical activators.

Examination of those factors which initiate the specific phases of zooplankton feeding may play a role in understanding the mechanisms which control zooplankton feeding. For example, prey ingestion is clearly related to prey capture (Lasker et al., 1982; Clayton, 1984). However, if the ingestion phase and capture phase are activated by different factors then zooplankton feeding may be controlled primarily by prey capture since the number of prey captured can be reduced by chemical treatments similar to those that occur during zooplankton feeding (Lasker et al., 1982; Ruch & Cook, 1984; Table 1) without altering the time required for ingestion (Table 2).

Prey capture partially determines the number of prey ingested (Lasker et al., 1982; Clayton, 1984). In *Hydra viridis* the number of prey ingested increases as the number captured increases while the length of the feeding response is unaltered (Lasker et al., 1982). Thus more prey are ingested during the same time period of the feeding response by an increase in ingestion rate and a decrease in the ingestion time for each prey item (Lasker et al., 1982). In these experiments, ingestion time was positively correlated with the number of prey in a multiple capture (Fig. 1). However, the total ingestion time for multiple captures could not be used to predict the ingestion time for a single capture. For example, the mean ingestion time for a single capture was 22.39 sec (Table 2), which is considerably longer than the calculated ingestion time for a single capture of 12.36 sec using the linear regression equation of $Y = 9.32 X + 3.04$ (Y = ingestion time; X = number captured; from Fig. 1). However, mean ingestion time for a single capture is very similar to the calculated ingestion time for two prey items (21.68 sec), suggesting that one or two *Artemia* require approximately the same amount of time for ingestion. In contrast, the calculated ingestion time for six prey items (from Fig. 1) is 58.96 sec, which is markedly less than the sum of the ingestion times for six separate prey items (6×22.39 sec = 134.34 sec). Thus the ingestion phase, which was unaffected by chemical stimulation (Table 1), was modified by multiple captures. During a feeding bout, when multiple captures occur, the ingestion rate (i.e. number of prey ingested/sec) apparently increases (c.f. Lasker et al., 1982), since the ingestion time for more than two prey would be less than the sum of the individual ingestion times for that many separate prey items.

This characteristic of zooplankton feeding could permit benthic cnidarians to ingest more prey, in a set time interval, when prey capture is high. Prey capture would be expected to be greatest during periods of high prey density since the number of prey ingested is directly related to prey density (Lasker, 1976; Purcell, 1981b, 1982; Clayton & Lasker, 1982; Bailey & Batty, 1983). Dense concentrations of marine zooplankton routinely occur near the substrate (Emery, 1968; Alldredge & King, 1977), which raises the possibility that increased ingestion rate during periods of high prey capture may enhance feeding capability (i.e. number of prey ingested) when prey are present in large numbers.

Zooplankton feeding may be viewed as the integration of several distinct behaviors, some of which may be affected by the feeding process itself. In these experiments, various treatments affected the number of zooplankton captured (Table 1) but not ingestion time (Table 2), suggesting that the capture phase of zooplankton feeding is distinct from the ingestion phase of the feeding process. Ingestion time was positively correlated with the number of prey captured and ingested (Fig. 1) but calculations indicate that multiple captures resulted in a decrease in the ingestion time for each prey

item. Thus ingestion time was unaffected by treatments that reduced zooplankton capture but was modified by the individual in response to multiple captures. Only further studies of the various phases of zooplankton feeding, and their relationship to each other, will yield a thorough understanding of the controlling mechanisms of zooplankton feeding.

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