Vol. 24: 161–165, 1985 | MARINE ECOLOGY – PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published July 11

Variations in the ingestion rate of algal cells with morphological development of larvae of *Paracentrotus lividus* (Echinodermata: Echinoidea)

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ABSTRACT: Ingestion rates of larvae of the sea urchin *Paracentrotus lividus* (Lamarck) increase during development. The pattern of the increase in ingestion rates with larval development is similar to that of a growth curve representable by the logistic equation $I = A/1+e^{B+Cx}$, where I = ingestion rate; A = maximal ingestion rate; B = integration constant; C = specific ingestion rate; x = age of the larva.

INTRODUCTION

During their exotrophic phase larvae of echinoids pass through several morphological stages characterized by the development of new appendages, or 'arms', situated near or outside the circumoral area. According to Strathmann (1971), beating of the cilia on the ciliated band lining the appendages produces a flow which collects particles in suspension and draws them towards the mouth opening. Formation of new larval arms near the mouth increases the area of the ciliated band which collects the food particles. One may suppose that increasing the length of the ciliated band would result in a greater rate of particle ingestion.

The present paper describes variations in the ingestion rate by larvae of the sea urchin *Paracentrotus lividus* (Lamarck) fed on a diet of 1 of 2 haptophyceaen algae: *Hymenomonas elongata* (Droop) Braarud or *Monocrysis lutheri* (Droop) Braarud. *H. elongata* supports complete larval development (Fenaux et al. unpubl.). *M. lutheri* is a good food for larvae of molluscs (Lucas 1978) but only mediocre as a food for larvae of echinoids (Hitnegardner 1969). The rates of ingestion of these 2 algae by the larvae were compared and related to differences observed in their morphological development according to the food-type ingested. Equations are proposed that describe *H. elongata* ingestion rates by plutei, in relation to larval development.

METHODS

Measurements of ingestion were made on *Paracentrotus lividus* plutei at times corresponding to 5 stages of larval development: (1) plutei with 4 arms at the third day after fertilization; (2) development of posterodorsal arms; (3) development of preoral arms; (4) development of epaulettes; (5) development of pedicellars and the adult rudiment.

Cells of *Hymenomonas elongata* were grown in filtered sea water enriched with 2 % Erd Schreiber (Provasoli 1968). Diameters and average volumes of cells of *H. elongata* were 8.0 to 13.5 µm and 780 μm^3; those of *Monocrysis lutheri*, 3.7 to 4.2 µm and 31.7 μm^3.

Initial density of larvae was 1 to 1.5 plutei ml^{-1}. Larvae and algae were kept in suspension by continuous agitation of the vessels. The volumes of available algal cells were 9 to 14 $\times 10^5$, 24 to 37 $\times 10^5$ and 43 to 61 $\times 10^5$ μm^3 ml^{-1} for *Hymenomonas elongata*, and 7 to 14 $\times 10^5$ and 18 to 24 $\times 10^5$ μm^3 ml^{-1} for *Monocrysis lutheri*, corresponding to about 1,000, 3,000, and 5,000 cells of *H. elongata*, and 22,000 and 57,000 cells of *M. lutheri* per pluteus, for one day. Eighty per cent of the sea water of the flasks was removed every day and replaced by fresh filtered sea water. To re-adjust to the initial algal volume after the grazing of larvae and the loss of algal cells in filtration, new fresh cells were added.

The number of larvae was counted once or twice during the culture period and again at its end. The

0171-8630/85/0024/0161/$ 02.50

equation $N_t = N_o \cdot e^{-kt}$ was used to calculate mortality rate and to make corrections in order to allow estimations of the number of living plutei and the actual volume of available algae pluteus^{-1} d^{-1} in each of the feeding experiments (N_o and N_t = number of larvae at time t_o and at the subsequent time t; k = rate of mortality). The estimate of daily consumption of cells by the plutei was based on the change in the number of algal cells in each of the 2 vessels containing both larvae and algal cells, compared with that in the control vessels that contained only algae. Rate of filtration was calculated from the equation:

$$\bar{F} = v\ (1/t \ln C_o/C_t - a) \text{ ml larva h}^{-1} \quad (1)$$

(Frost 1972, Conover 1978) where v = volume of water per larva; t = duration of the experiment (19 to 24 h); C_o and C_t = initial and final concentrations of algae in the vessels containing plutei and algae; a = correction factor to account for growth of the algae in the control bottles:

$$a = 1/T' \ln \frac{C'_o}{C'_t} \quad (2)$$

where T' = duration of incubation of algae; C'_o and C'_t = initial and final concentrations of the algae in the control bottles. The ratio $\bar{R}$ is equal to $\bar{F} \times C$ with

$$C = \frac{C_o - C_t}{t\left(\frac{\bar{F}}{v} + a\right)} \text{ (Lucas 1982).} \quad (3)$$

The ingested algal volume is equal to this ratio multiplied by the average volume of the algal cells.

The ingested algal volume varied with the rate of larval development. The logistic function

$$I = \frac{A}{1+e^{B+Cx}} \quad (4)$$

was used to represent the change in the ingestion rate with respect to the development of the larvae fed cells of *Hymenomonas elongata* (I = ingested volume; A = maximum ingestion; B = integration constant which defines the curve relative to the origin; C = specific ingestion rate; x = larval age). The data used for the logistic function are listed in Table 1.

RESULTS

Larvae fed *Monocrysis lutheri*

Development of the larvae was asynchronous and in numerous cases occured with an increase only in larval length. Mean daily rate of ingestion for larvae supplied a volume of algal cells of 7 to 14 × 10^5 µm^3 ml^{-1} d^{-1} showed small variations (Table 2). Only 25% of the larvae supplied a volume of 18 to 24 × 10^5 µm^3 ml^{-1} developed posterodorsal arms after 11 d. Thus, mean daily rate of ingestion of this set of cultures was slightly higher after Day 11. (Table 2).

Larvae fed *Hymenomonas elongata*

Mean daily rate of ingestion by larvae aged 3 to 7 d was low. Large variations occured among larvae of the same age and from the same parents as shown by

Table 1. *Paracentrotus lividus*. Rates of ingestion of *Hymenomonas elongata* by larvae of different ages

Volume of available cells ml^{-1} d^{-1} of *Hymenomonas elongata*	9 to 14 × 10^5 µm^3	24 to 37 × 10^5 µm^3	43 to 61 × 10^5 µm^3
Age (d)	Ingestion rate	Ingestion rate (10^5 µm^3 pl^{-1} d^{-1})	Ingestion rate
3	0.67 ± 0.15	3.18 ± 2.04	0.87 ± 1.31
4	0.94 ± 0.21	2.21 ± 2.01	1.27 ± 1.78
6		2.32 ± 0.31	2.84 ± 1.81
7	1.42 ± 0.11	6.78 ± 2.88	3.37 ± 0.07
8	1.75 ± 0.15		3.73 ± 0.72
9	5.40 ± 0.06	11.00 ± 1.31	9.31 ± 4.55
10	5.50 ± 0.02	16.26 ± 3.54	17.74 ± 4.06
11		22.10 ± 0.51	29.93 ± 3.91
12			45.99 ± 0.18
13		25.21 ± 7.59	41.16 ± 5.23
14		27.15 ± 2.33	41.72
15		22.33 ± 2.25	46.39 ± 7.66
16	7.35 ± 0.35	27.65 ± 3.46	35.36 ± 4.39
17		26.33 ± 0.97	36.95 ± 0.91
18	6.54 ± 0.34		
24	7.31 ± 0.65		

Table 2. *Paracentrotus lividus*. Volume of cells of *Hymenomonas elongata* and *Monocrysis lutheri* ingested by larvae during their development. Volumes of available algal cells are: 9 to 14 × 10^5, 24 to 37 × 10^5 and 41 to 63 × 10^5 μm³ ml^{-1} d^{-1} for *H. elongata*; 7 to 14 × 10^5 and 18 to 24 × 10^5 μm³ ml^{-1} d^{-1} for *M. lutheri*. *N* = number of mated pairs of *P. lividus*. Values are means ± 1 SD

Volume of available cells ml^{-1} d^{-1} of *H. elongata*		9 to 14 × 10^5 μm³			24 to 37 × 10^5 μm³				43 to 61 × 10^5 μm³			
Age (d)	Stage	Ingestion rate (10^5 μm³ pl^{-1} d^{-1})	Filtration rate (ml^{-1} pl^{-1} d^{-1})	N	Stage	Ingestion rate (10^5 μm³ pl^{-1} d^{-1})	Filtration rate (ml^{-1} pl^{-1} d^{-1})	N	Stage	Ingestion rate (10^5 μm³ pl^{-1} d^{-1})	Filtration rate (ml^{-1} pl^{-1} d^{-1})	N
3	4 arms	0.67 ± 0.94	0.15 ± 0.21	1	4 arms	3.18 ± 2.04	0.14 ± 0.10	3	4 arms	0.87 ± 1.31	0.03 ± 0.01	3
7	4 arms	1.42 ± 1.11	0.11 ± 0.08	1	6 arms	6.78 ± 2.88	0.26 ± 0.12	4	6 arms	3.37 ± 0.07	0.08 ± 0.05	3
9	6 arms	5.40 ± 0.06	1.70 ± 0.01	1	8 arms	11.00 ± 1.35	1.50 ± 0.35	2	8 arms	9.31 ± 4.55	0.38 ± 0.22	2
10	6 arms	5.50 ± 0.06	1.69 ± 0.13	1	8 arms	16.26 ± 3.54	1.20 ± 0.43	4	8 arms	17.74 ± 4.06	0.54 ± 0.16	3
11					epaulettes	22.10 ± 0.51	2.84 ± 0.72	2	epaulettes	29.93 ± 3.91	1.13 ± 0.40	3
13					ad. rudiment	25.21 ± 7.59	1.53 ± 0.76	3	ad. rudiment	41.16 ± 5.23	1.54 ± 0.28	2
16	8 arms	7.35 ± 0.35	1.96 ± 0.06	1	ad. rudiment	27.65 ± 3.46	2.66 ± 0.47	3	ad. rudiment	35.35 ± 4.39	1.39 ± 0.39	2
24	epaulettes	7.31 ± 0.65	2.40 ± 0.18	1								
Volume of available cells ml^{-1} d^{-1} of *M. lutheri*		7 to 14 × 10^5 μm³			18 to 24 × 10^5 μm³							
3	4 arms	1.88 ± 0.32	0.34 ± 0.07	1	4 arms	2.54 ± 0.94	0.11 ± 0.04	1				
7	4 arms	2.22 ± 0.08	0.35 ± 0.00	1	4 arms	3.30 ± 0.42	0.15 ± 0.02	1				
9	4 arms	2.09 ± 0.10	0.32 ± 0.02	1								
10	4 arms	1.84 ± 0.23	0.38 ± 0.07									
11	4 arms	1.83 ± 0.08	0.30 ± 0.01	1	6 arms: for	5.00 ± 1.70	0.27 ± 0.11	1				
14	4 arms	2.51 ± 028	0.33 ± 0.05	1	25% larvae	7.23 ± 0.04	0.60 ± 0.03	1				
31	4 arms	2.40 ± 0.00	0.20 ± 0.01									

standard deviations (Table 2). With a daily supply of 24 to 37 × 10^5 or 43 to 61 × 10^5 μm³ ml^{-1}, the mean rate varied from 2.2 to 6.8 × 10^5 and from 0.9 to 3.4 × 10^5 μm³ pl^{*-1} d^{-1}. Between Day 7 and Day 9, the mean ingestion rate increased until it reached a plateau which stabilized at a level that depended on the available quantity of algae (Fig. 1). For larvae supplied with 9 to 14 × 10^5 μm³ cells ml^{-1}, the rate of ingestion increased slightly between Day 9 and Day 16, ranging from 5.4 to 7.4 × 10^5 μm³ algal cells pl^{-1} d^{-1}. From Day 16 to Day 24, there was little variation in the volumes ingested, which averaged 7.0 × 10^5 μm³ algal cells pl^{-1} d^{-1}. Mean volume of available algal cells at the end of the feeding experiments was only 1.39 × 10^5 μm³ ml^{-1}.

For larvae supplied 24 to 37 × 10^5 μm³ cells ml^{-1}, the rate of ingestion increased greatly between Day 7

* pl = pluteus

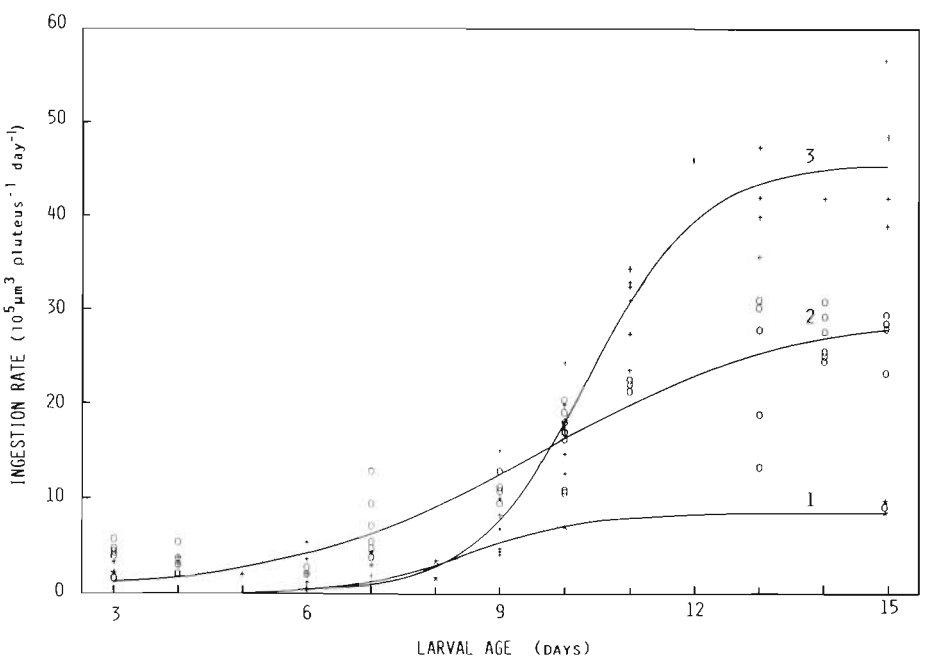

Fig. 1. *Paracentrotus lividus* plutei. Ingestion rate per larva per day as a function of age and of concentration of *Hymenomonas elongata*. I = A/1 + e^{B+Cx}, where *I* = ingestion rate; *A* = maximal ingestion rate; *B* = integration constant; *C* = specific ingestion rate; *x* = age of larva. Concentrations: (1) 9 to 14 × 10^5; (2) 24 to 37 × 10^5; (3) 41 to 63 × 10^5 μm³ ml^{-1} d^{-1}

and Day 14 ranging from 6.8 to 27.2 $\times 10^5$ μm^3 cells pl^{-1} d^{-1}. From Day 14 to Day 17, the rate of ingestion was about 27 $\times 10^5$ μm^3 algal cells pl^{-1} d^{-1}. Mean volume of available algae at the end of the feeding experiments, for larvae aged 10 to 17 d, was about 1.7 $\times 10^5$ μm^3 ml^{-1}.

For larvae supplied 43 to 61 $\times 10^5$ μm^3 ml^{-1}, the rate of ingestion increased greatly from Day 9 to Day 15 ranging from 9.31 to 46.40 $\times 10^5$ μm^3 cells pl^{-1} d^{-1}. As in the case of the larvae supplied 24 to 37 $\times 10^5$ μm^3 algal cells ml^{-1}, the first signs of competence towards metamorphosis (larvae exploring the substratum, podia being extended) appeared by Day 15. The number of available cells at the end of the feeding experiments was higher than 500 ml^{-1}. Nevertheless, between Day 13 and Day 16, the ingestion curve is stable (Fig. 1).

The curves of ingestion as a function of age and morphological development of the plutei, for the 3 concentrations of *Hymenomonas elongata,* show a first phase of small increase, a second phase of great increase, and a third phase where values remain stable. Ingestion rate varies as though it were a parameter measuring growth. Among the mathematical models tested (von Bertalanffy-Brody, Gompertz, logistic function) the logistic function is the one used. The parameters of the 3 equations corresponding to the 3 concentrations of available algae are indicated in Table 3.

DISCUSSION

Up to Day 7, the rate of algal cells ingested by the plutei fed *Hymenomonas elongata* and *Monocrysis lutheri* is minimal and does not depend on the number of cells available per pluteus. The development of posterodorsal arms marks the beginning of a definite increase in the ingestion rate. This was the case both in larvae offered the 3 concentrations of *H. elongata* cells as well as in some plutei (about 25 % of the population) in a culture fed 18 to 24 $\times 10^5$ μm^3 cells ml^{-1} of *M. lutheri.* The increase in ingestion rate was thus related to the increase in length of the circumoral band. In a study on the functional differences in nutritional activities, Lee (1983) emphasized the differences between ciliated band and epaulettes during filtration.

According to Strathmann (1971), beating cilia of the circumoral ciliated band create a current that draws suspended particles towards the mouth. Our study on the rate of ingestion of cells of *Monocrysis lutheri* and *Hymenomonas elongata* by plutei of *Paracentrotus lividus,* with respect to the development of the circumoral ciliated band, shows clearly that increase in ingestion rate depends on the formation of new larval arms which augment the length of the ciliated circumoral band.

Our study further shows that different algae are not treated in the same way by the larvae. Plutei supplied with 3 concentrations of *Hymenomonas elongata* cells showed synchronous development and acquired the ability to undergo metamorphosis irrespective of algal concentrations (Fenaux et al. unpubl.). A volume of 18 to 24 $\times 10^5$ μm^3 cells ml^{-1} of *Monocrysis lutheri* (i.e. a volume yielding optimal plutei growth in the case of *H. elongata*) gives poor results as regards the development of the larvae. Hinegardner (1969) noted the poor growth also in larvae of *Lytechinus pictus* and *Psammechinus milliaris* offered cells of *M. lutheri.* In contrast, Wilson (1981) found that both *H. elongata* and *H. carterae* (another haptophyceaen) supported good growth of echinoid larvae.

A complete but slow development (1 mo) occured with 9 to 14 $\times 10^5$ μm^3 cells ml^{-1} of *Hymenomonas elongata,* whereas the development takes only 16 d with 24 to 37 $\times 10^5$ and 43 to 61 $\times 10^5$ μm^3 algal cells (Fenaux et al. unpubl.). The 3 plateaus appearing at the last phases of ingestion rates have different significations. With 9 to 14 $\times 10^5$ and 24 to 37 $\times 10^5$ μm^3 algal cells ml^{-1}, the ingestion rate was limited by the volume of cells still available under the experimental conditions. With 43 to 61 $\times 10^5$ μm^3 cells ml^{-1}, the plateau appeared when the formation of the adult rudiment took place. With these data, it appears that an optimal diet of *Hymenomonas elongata* cells, in relation with the development of the larvae, is 1,000 cells ml^{-1} during Day 3 to Day 7, 3,000 cells during

Table 3. *Paracentrotus lividus.* Ingestion of cells of *Hymenomonas elongata* by plutei as function of larval age and prey concentration per individual per day

Volume of available cells ml^{-1} d^{-1} of *Hymenomonas elongata*	A + δ A	B + δ B	C + δ C	F	D.D.L.
43 to 61 $\times 10^5$ μm^3	45.596 ± 1.558	11.826 ± 1.362	−1.141 ± 0.136	994.2*	1 and 50
24 to 37 $\times 10^5$ μm^3	29.825 ± 1.945	4.905 ± 0.600	−0.512 ± 0.075	463.07*	1 and 53
9 to 14 $\times 10^5$ μm^3	8.646 ± 0.776	9.823 ± 2.702	−1.151 ± 0.327	80.03*	1 and 9

$I = A/1 + e^{B+Cx}$; * $p = 0.001$

Day 8 to Day 10 and 5,000 cells until metamorphosis. According to Lucas (1982), larvae of *Acanthaster planci* are unable to reduce their rate of ingestion: this leads to gut obstruction at high algal concentrations. In our experiments, a reduced rate of ingestion at the time when the adult rudiment is formed demonstrates a different behaviour, and sugests a maximum volume at the end of larval growth.

For the two 6-armed plutei of *Strongylocentrotus droebachiensis* and *Dendraster excentricus*, maximum filtration rates ($\mu l\ min^{-1}$) were respectively 2.3 and 0.5 (Strathmann 1971); they were 1.3 to 6.3 for the bipinnaria of the sea star *Acanthaster planci* (Lucas 1982). In our study the mean filtration rate of the 6-armed plutei of *Paracentrotus lividus* varied according to algal concentrations between 1.8 and 0.05. The recorded differences between the filtration rate of *P. lividus* larvae and the maximum filtration rates of *S. droebachiensis* and *D. excentricus* plutei may be due to the method used for the estimating the number of cells ingested: Particle Counter and direct observations under the stereomicroscope. The method used by Lucas for estimating ingested cells is the same as ours. The difference observed between the filtration rate of *A. planci* bipinnaria and *P. lividus* plutei can be explained in terms of differences in temperature. Rassoulzadegan & Fenaux (1979) showed for *Arbacia lixula* larvae that grazing rates increase with increasing temperature.

Acknowledgements. We thank P. Nival, F. Rassoulzadegan, J. L. Lawrence, B. Caram and 3 anonymous referees for critically reading our manuscript. This research was supported by Centre national de la Recherche Scientifique (ERA 228 and RCP 080658).

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This paper was presented by Professor J. Lawrence; it was accepted for printing on March 31, 1985