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The intestinal evacuation rates of larval herring (*Clupea harengus* L.) predating on wild plankton

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Abstract

The gut content, feeding rate and intestinal evacuation rate were monitored by direct visual observations of individual herring larvae feeding on wild plankton. The time to evacuate the gut was in the range of 40 minutes-3 hours which is in contrast to gut evacuation times of 7-9 hours reported by other authors for herring larvae feeding on *Artemia* nauplii. The evacuation process was divided into 4 distinct phases with copepod prey. Larval age did not significantly affect the evacuation rate. In non-feeding larvae the last 1-2 copepods were retained in the gut for several hours.

Introduction

The aim of this experimental series was to improve our understanding of the mechanisms responsible for the regulation of the gut evacuation in herring larvae. Specifically, the influence of the feeding rate and of the gut content on the intestinal evacuation rate was studied and quantified. Furthermore, direct visual observations of the passage of individual food particles along the gut showed that the evacuation process is divided into 4 distinct phases for copepod prey. A study on the gross anatomy of the larval gut was made in order to see whether or not the gut could be divided into different regions.

Unlike experiments reported by previous authors wild plankters were used as prey as they constitute the natural food of herring larvae. In the literature data are available on the gut evacuation time of herring larvae preying upon newly hatched *Artemia* nauplii (Werner & Blaxter 1981) but until now there has been no information on the gut evacuation rate and of the variables affecting it. On the basis of the results from the present study as well as from the results reported with *Artemia* prey a hypothesis is presented on the mechanisms regulating the gut evacuation in larval herring.

Materials

The aquariums were circular, grey, 50 liter PVC tanks. The salinity was 29.7‰ and the experimental temperature for feeding larvae was 9.5 °C, s.d. 0.4, n = 38. The tanks were illuminated at 1000 lux at the water surface from 5 a.m. until 8.30

p.m. For the study of the gut evacuation rates larvae of Minch herring were used. Eggs were fertilized on September 20, 1981 and incubated at 10 °C until hatching on the second and third of October. The larvae were fed *Brachionus* for 7 days and from that time onwards they were offered wild plankton only. The experimental animals were aged from 22-52 days. For the study of the phases of the gut evacuation Baltic herring larvae aged from 26-40 days were used. In the evacuation experiments the prey consisted of wild plankton collected in the strait between Denmark and Sweden. Only plankters retained on a 180 μm net were used. The food thus consisted of copepodites and adult copepods. The plankters were dominated by *Acartia tonsa* and *Temora longicornis*. The zooplankters were fed a mixture of *Rhodomonas, Tetraselmis* and *Isochrysis*. The range of the experimental prey densities was from 0.011-0.198 copepods per ml. In the study of the phases of the gut evacuation the prey consisted of either wild plankton or newly hatched *Artemia* nauplii.

Methods

The method used was a direct observation of the gut content, food intake and intestinal evacuation time of individual larvae. The experimental procedure was as follows:

A larva from the stock tank was selected and transferred to the test aquarium. The criteria for the selection of larvae were:

1. the number of copepods in the intestine could be counted by means of the unaided eye, and
2. the foremost particle in the gut (the T_0 copepod) had just been ingested and was easy to identify

In the test aquarium the number of successful attacks was monitored. When the copepod which had occupied the most anterior position in the larval gut at the onset of the experiment was defaecated, the larva was caught and its gut content checked against the number of successful attacks. Larval standard and hindgut lengths were measured, and the number and dimensions of the prey organisms in the intestine were noted. The principle variables recorded were thus:

1. the number of copepods in the larval gut at the onset of the experiment
2. the number of copepods ingested during the experimental period
3. the time elapsed from the transfer of the larva to the test aquarium until the foremost copepod of the start gut content was defaecated, i.e. the gut evacuation time.

From these variables it is possible to compute the feeding rate (number of copepods ingested per hour) and the evacuation rate (number of copepods evacuated per hour). A prerequisite for using this experimental design is that the particles cannot interchange their positions in the larval gut. Such an interchange of positions has never been observed.

In order to facilitate the observation of individual copepods in the larval intestine, a light from a microscope lamp was focused on the larval hindgut at intervals of 5-10 minutes. Only few larvae showed signs of stress when the light found them (i.e. increased swimming speed or abolished already started attacks), and the results from these fish were excluded.

The T_0 copepod, which in most cases was the foremost particle in the larval gut at the onset of the experiment, had to be monitored from ingestion until it was defaecated. This was made possible by the fact that *Temora longicornis* showed up in a brilliant copper red colour when reflecting the white light. No other genus in the plankton shared this property, so single *Temora* were used as markers. In experiments on the gut evacuation of non-feeding larvae individual larvae with a known gut content were transferred to aquariums void of prey and the gut content was monitored at intervals of about 30 minutes.

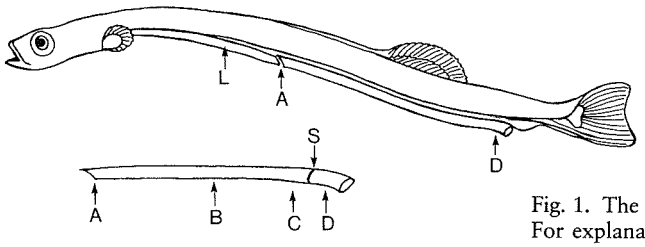

Fig. 1. The gross anatomy of a herring larva. For explanation of symbols, see text below.

Results

The gross anatomy of a larva is illustrated in Fig. 1 (above). 'L' designates the liver, at the caudal tip of which position 'A' shows the transition from the narrow foregut to the hindgut. Fig. 1 (below) is a more detailed sketch of the larval hindgut. 'A' is the most anterior end of the hindgut, which gradually widens in diameter through positions 'B', 'C' and 'D'. At 'S', which is the cephalic demarcation of region 'D', a strongly developed muscular sphincter is located.

The gut evacuation experiments with feeding larvae were aimed at the rejection of the following null hypotheses:

H_0 the gut evacuation rate is independent of the number of prey in the intestine at the start of the experiment

H_0 the gut evacuation rate is independent of the feeding rate during the experiment

The results are given in Table 1.

As the evacuation rate was found to be a linear function of the gut content, and as the evacuation rate was dependent of more than one variable, multiple regressions were performed *ad modum* Fisher (Mather 1965). As probability point for the rejection of the hypothesis that the regression coefficients did not depart significantly from zero, I have chosen 0.05.

Table 1.

I	II	III	IV	V	VI	VII	VIII	IX	X
	Age			Start gut content					
No.	in days	I	ET min.	total	in A-B	in C-D	FR	ER	ERT_0
1	22	7	77.5	4	1	3	5.4	3.1	0.779
2	22	7	44	1	1	0	9.5	1.4	1.364
3	23	11	40	1	1	0	16.5	1.5	1.500
4	23	17	44	2	1	1	23.2	2.7	1.364
5	27	10.5	89	1	1	0	7.1	0.7	0.674
6	27	7	71	5*	2	3	5.9	3.4	0.845
7	30	6	60	2	1	1	6.0	2.0	1.000
8	30	12	66	12	full gut		10.9	10.9	0.909
9	30	9	77	3	1	2	7.0	2.3	0.779
10	31	10	100	3	1	2	6.0	1.8	0.600
11	32	3	91.5	2	2	0	2.0	1.3	0.659
12	32	9	112	3*	3	0	4.8	1.1	0.536
13	33	6	70	2*	2	0	5.1	0.9	0.857
14	33	3	83	4*	2	2	2.2	2.2	0.723
15	33	4	168	3	2	1	1.4	1.1	0.357
16	34	8	77	4	1	3	6.2	3.1	0.779
17	38	4	76	5	scattered		3.2	3.95	0.789
18	38	8	75	3	1	2	6.4	2.4	0.800
19	39	6	64	2	1	1	5.6	1.9	0.938
20	39	6	99	5	1	4	3.6	3.0	0.606
21	40	4	72	2	1	1	3.3	1.7	0.833
22	40	8	71	3	1	2	6.8	2.5	0.845
23	40	7	81	2	1	1	5.2	1.5	0.741
24	41	9	68.0	5**	3	2	7.9	2.6	0.882
25	44	4	82	2	1	1	2.9	1.5	0.732
26	44	16	66	2	1	1	14.5	1.8	0.909
27	44	15	51	5	3	2	17.6	5.9	1.176
28	45	9	57	2	1	1	9.5	2.1	1.053
29	45	10	75	11	scattered		8.0	8.8	0.800
30	45	7	77	1	1	0	5.5	0.8	0.779
31	46	3	106	2	1	1	1.7	1.1	0.566
32	46	4	93	4	1	3	2.6	2.6	0.645
33	47	3	81	3	scattered		2.2	2.2	0.741
34	47	2	134	3	1	2	0.9	1.3	0.448
35	47	1	122	1	1	0	0.5	0.5	0.492
36	47	1	179	2	1	1	0.3	0.7	0.335
37	51	3	77	6*	2	4	2.3	3.9	0.779
38	52	1	145	6	3	3	0.4	2.5	0.414

Column III : I = Ingestion in number of copepods

– IV : ET = evacuation time of the timed particle (the T_0 copepod)

– V : start gut content. For larvae numbers 8 and 29, the total start gut content is estimated.

* designates, that the timed copepod was number 2 counted from the cephalic end of the row of particles.

** designates, that the timed copepod was number 3 counted from the cephalic end of the row of particles.

– VIII : FR = feeding rate = 60 × (III/IV) copepods per hour.

– IX : ER = evacuation rate of the start gut content (particles anterior to the T_0 copepod excluded) = 60 × (V/IV) copepods per hour.

– X : ERT_0 = evacuation rate of the T_0 particle = 60 × (1/IV) copepods per hour.

A multiple regression was done with the gut content (x_1) and the feeding rate (x_2) representing the independent variables; the dependent variable being the gut evacuation rate (y) defined as the number of copepods evacuated per hour, the evacuated copepods including the timed particle (the T_0 copepod) and all particles caudal to it.

The estimates of the coefficients of regression (b_1 and b_2) and their standard deviations (sb_1 and sb_2) are:

$$b_1 = 0.7898101 \qquad sb_1 = 0.0389807$$
$$b_2 = 0.1147912 \qquad sb_2 = 0.0185983$$

As n = 38, there are 35 degrees of freedom. The significance of the departure of b_1 and b_2 from the value of zero is:

$$t_{35} = \frac{b_1 - 0}{sb_1} = 20.262 \quad \text{thus } p < 0.001$$

$$t_{35} = \frac{b_2 - 0}{sb_2} = 6.172 \quad \text{thus } p < 0.001$$

b_1 and b_2 depart significantly from zero, and both null hypotheses are thus rejected. The following H_1 hypotheses are accepted:

H_1 the gut evacuation rate is dependent of the number of copepods in the gut at the start of the experiment

H_1 the gut evacuation rate is dependent of the feeding rate during the experiment.

Further regressions showed that the larval age did not significantly influence the evacuation rate ($0.60 > p > 0.50$).

The feeding rate could not be correlated with the average prey density in the tank. This may, in part, have been due to the occurrence of patchiness of the prey organisms in the aquariums.

With non-feeding larvae 17 experiments were run in order to estimate the time to empty the gut. The gut evacuation time was highly variable but it was more prolonged for non-feeding larvae than for feeding ones. The mean evacuation time for feeding larvae was 1 hour 25 minutes, s.d. 31 minutes, while the evacuation time for 14 of 17 non-feeders exceeded 2.5 hours. 6 of these larvae took from 4 to more than 7.3 hours to evacuate their gut content. The results indicated, that the evacuation was a linear function of time with the exception of the last 1-2 copepods which were retained in the gut for several hours. Fig. 2 shows an example of this.

In the study of the phases in the gut evacuation 19 experiments with copepod or *Artemia* prey were performed by direct observation. For herring of the age from 26-40 days the time elapsed from ingestion until an adult copepod reaches position 'A' is 7-11 seconds. The adult copepod will then stop at 'A' for 2-6 minutes before moving through the 'B' region in 3-5 minutes at a steady pace. Finally, it is lodged in the 'C'-'D' regions for 1-2 hours. Copepodites will exhibit similar phases during their passage of the herring gut but the first phases will be of shorter duration, i.e.

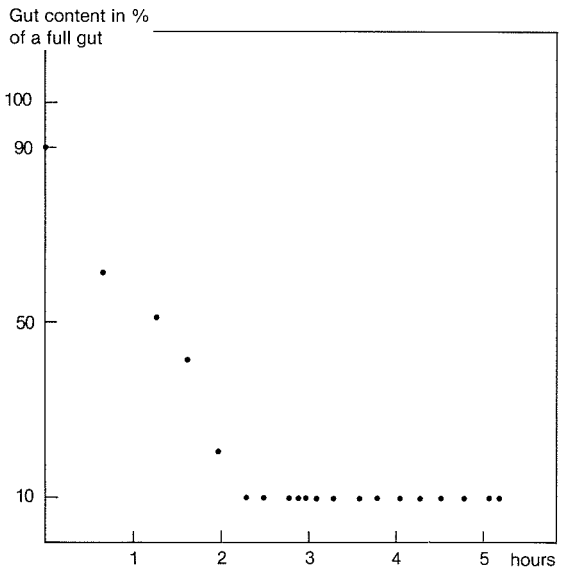

Fig. 2. Gut evacuation of a non-feeding larva.

it takes about 4 minutes from ingestion until a copepodite has traversed the intestine and has reached the 'C'-'D' regions. Half of this time is spent at 'A' position. A few experiments were run with newly hatched *Artemia* nauplii. The nauplii will pass from the herring mouth to the posterior part of the hindgut in 2.5-3.5 minutes, but contrary to copepods, *Artemia* nauplii have not been observed to stop at 'A' position.

Discussion

Gut evacuation time

Only a few authors have studied the gut evacuation time for feeding herring larvae. Werner & Blaxter (1979) conducted experiments on the gut evacuation time of feeding herring larvae of the age from 3-9 weeks. The prey organisms were newly hatched *Artemia* nauplii at 4 different densities, i.e. 0.03; 0.3; 3.0 and 30 *Artemia*/ml. At the 2 higher prey densities evacuation times of 3-6 hours were found, and at the lower prey densities the nauplii had not been evacuated after 7 hours.

For herring larvae of a similar age as those used by Werner & Blaxter (1979) and at prey densities of 0.011-0.198 copepods per ml, a mean gut evacuation time of 1 hour 25 minutes was found in this study. The most prolonged evacuation time found at all for feeding fish was 2 hours 59 minutes. Thus there does not exist any overlap between the evacuation times found by Werner & Blaxter (1979) and the evacuation times reported here. The abiotic factors in the experiments were identical except for tank size and a slight temperature difference of 0.3 °C. In previous experiments (paper in preparation), using 20 liter tanks and a method similar to that of Werner & Blaxter (1979), evacuation times of 1-2 hours were found with

Table 2. Gut evacuation times of larval herring.

Reference	Prey type	Evacuation time, hours	Temp., °C	Larval age or length
Blaxter 1962	*Balanus* nauplii	>9	7	10-12 mm
		>5	11	10-12 mm
		>4.5	15	10-12 mm
Blaxter 1965	*Balanus* nauplii	>8	8	12 days, 9 mm
		>4	15	12 days, 9 mm
Blaxter 1965	Squid meat	24-30	12	older larvae
Rosenthal & Hempel 1970	*Artemia* nauplii	4-10	10	11-14 mm
Fossum *et al.* 1979	Wild plankton	12-24	9	8 & 22 days
Werner & Blaxter 1979	*Artemia* nauplii 0.03/ml	>7	9.2	3-9 weeks
	0.3 /ml	>7	9.2	3-9 weeks
	3.0 /ml	3-5	9.2	3-9 weeks
	30.0 /ml	4-6	9.2	3-9 weeks
Werner & Blaxter 1981	*Artemia* nauplii 0.3 /ml	9.1	9.2	3-9 weeks
Bhattacharyya 1957	Copepods	1	field	?
	Lamellibranch veliger	4-6	field	?
Present study	Copepods at 0.011-0.198/ml	0.7-3	9.5	22-52 days, 12.5-18.1 mm

copepod prey for other strains of herring larvae, so the discrepancy in evacuation time with *Artemia* and copepod prey seems most likely due to the prey organism utilized.

A summary of the results hitherto reported in the literature on the gut evacuation time of herring larvae is given in Table 2.

It is noted that an evacuation time of 1 hour for copepod prey has been found only by Bhattacharyya (1957) in a field study.

Feeding rates

Werner & Blaxter (1979) have conducted a series of ration experiments with herring larvae of 4-5 weeks using *Artemia* nauplii at densities from 0.03-5.0 per ml. Prior to the experiments, the larvae were starved for 24-36 hours. On the basis of the results from these experiments Blaxter (1982) states that the feeding rates were within the range of 1.2-6.9 *Artemia* nauplii/hour. In the present study feeding rates from 1.4-23.2 copepods/hour were realized by larvae aged from 22-34 days at prey concentrations of 0.043-0.198 copepods/ml. The mean feeding rate of these fish was 7.45 copepods/hour. This value is well above the maximum feeding rate reported with *Artemia* prey even in spite of the fact that the copepod density was lower than the optimum density for *Artemia* prey (0.3-3.0 *Artemia*/ml). It is conceivable, that the larval feeding rate is higher with natural prey than is the case with *Artemia* nauplii.

Digestion

Except for their storage lipid copepods seem to be digested very well by larval herring. Microscopy revealed that the internal structures of copepods lodged in the 'D' region were completely dissolved. Rosenthal (1970) observed that *Artemia* were not digested as well as copepod nauplii and Werner & Blaxter (1981) noted that intact *Artemia* nauplii were defaecated at the higher prey densities. The molecules directly responsible for the degradation of the tissues of the prey may come from 2 sources; the digestive organs of the larvae (endogenous source) and proteases released from the prey (exogenous source). A number of authors (Szlaminska 1980, Kawai & Ikeda 1973, Kawai & Ikeda 1973) have reported protease (pepsin and trypsin) and carbohydrase (amylase and maltase) activity in fish embryos and larvae. It is thus established for a number of fish species that the endogenous source is available. The exogenous source, however, seems to vary according to the prey organism utilized. Dabrowski *et al.* (1976) have examined the proteolytic activity of several species of fish larvae and several genera of prey (herring were not included in the study). The ratio between the total proteolytic activity in the daily ration and the total proteolytic activity of the fish larvae was about 1 with copepod prey. With *Rotifera* and *Cladocera* prey the ratio was well below 1 (*Cladocera* and *Artemia* both belong to the Branchiopods). Furthermore, Rosenthal (1968) reports that the ratio of ectodermal to entodermal tissue masses is 3 to 1 in the first nauplius stage of *Artemia*, while the inverse ratio is found in the last metanauplius stage. Thus, the proteolytic activity in newly hatched *Artemia* nauplii is probably low.

Hypotheses on the regulation of the intestinal evacuation in larval herring

The causative factors producing the discrepancy in gut evacuation time between *Artemia* and copepod prey may be:

a. a difference in prey dimensions
b. a difference in prey type
c. a difference in larval feeding rate
d. a difference in larval gut content

a. *Prey dimension.* Dilatation of the gut is known to promote peristalsis in mammals. The effect is mediated through the autonomic nervous system. As fish possess an autonomic nervous system identical to that of mammals it is possible that a larger prey (i.e. adult copepods) promotes peristalsis more effectively than a smaller prey (i.e. *Artemia* nauplii). The question is as yet unresolved, but *Brachionus* of approximately the same width as newly hatched *Artemia* nauplii has a gut evacuation time of around 2 hours in herring larvae (paper in preparation). Factors other than the width of the prey may thus be important.

b. *Prey type.* Apart from the regulation of intestinal motility by the autonomic nervous system a number of gastro-intestinal hormones are known to play a major part in regulating peristalsis as well as the secretion of bile and pancreatic enzymes in mammals. These hormones have been isolated from a number of fish species, for

example *Esox* and *Gadus* (Dockray 1975), *Salmo* (Bayliss & Starling 1903), *Esox* and *Anguilla* (Barrington & Dockray 1972). The adequate stimuli for the secretion of these hormones are changes in pH or the release of degradation products from the food organisms (amino acids, polypeptides and lipid).

Concerning the release of these hormones, a shift in prey type could have three direct consequences: First, chemical differences in the composition of the exoskeleton between copepods and *Artemia* could have the effect that copepods are capable of invoking a release of larval gastro-intestinal hormones and thence of larval digestive enzymes, while *Artemia* do not carry the adequate signal for this release. In this context it is interesting, that *Artemia* have not been observed to stop at 'A' position, which in mammals and fish is the primary site for secretion of bile and pancreatic digestive enzymes into the gut. Second, differences in the permeability of the exoskeleton between the two prey types could result in the larval proteases gaining access to and hydrolysing the internal structures of copepods, but not of *Artemia*. Third, the proteolytic activity could differ between the prey types, adult copepods possessing a high activity, newly hatched *Artemia* a low one (cf. above).

It should be emphasized that the release of larval gastro-intestinal hormones, whether caused by a chemical stimulus from the exoskeleton or by degradation products from the organ systems of the prey, will affect the release of digestive juices as well as the motility of the alimentary tract; the point being that a prey organism which is not well digested may not be evacuated quickly.

c. *Feeding rate.* The multiple regression indicated, that the feeding rate did influence the evacuation rate. Furthermore, the feeding rates with copepod prey exceeded the hitherto reported feeding rates with *Artemia* nauplii constituting the prey.

d. *Gut content.* A discrepancy in the gut content at the start of the experiments with the 2 prey types will have an impact on the gut evacuation *rate*, but will only influence the gut evacuation *time* to a very limited extent, as a full gut is evacuated in approximately the same time as an almost empty gut.

The numerical value of the regression coefficient representing the influence of the gut content on the evacuation rate was 0.79. This means, that the addition of one extra copepod to a given gut content will increase the evacuation rate by almost 1 copepod/hour. My interpretation of this is, that the individual particles in the gut are simultaneously undergoing enzymatic degradation, i.e. they are digested in around 1-2 hours independent of the number of particles in the gut. If this is true, the enzyme–substrate ratio in the gut must be constant and thus independent of the number of particles in the intestine. In physiological terms this can be accomplished by secreting a fixed amount of enzymes per copepod at 'A' position.

The mechanism responsible for the retention of the last 1-2 copepods in the gut of non-feeding larvae may be: First, the parasympathetic nervous system is not stimulated (no feeding and therefore no distension of the gut), and second, there is a lack of food degradation products in the gut (gastro-intestinal hormones will not be released).

The regulation of the intestinal evacuation is a complex series of processes in vertebrates. Suffice it to say that probably the feeding rate, the prey type and dimension and the proteolytic activity inside the prey organism are most likely all important factors in regulating the motility of the alimentary canal in herring larvae.

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