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Ontogenetic- and condition-related effects of starvation on responsiveness in herring larvae (*Clupea harengus* L.) during repeated attacks by a model predator

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Abstract

The escape behaviour of fed and food-deprived herring larvae (20–30 mm SL) was studied during repeated attacks with a glass probe in order to investigate the causal mechanisms of starvation-induced changes in reaction to predators. Two experiments were set up: one where the fed and starved larvae were of the same age but of different sizes and one with groups of similar size but different ages. Biochemical and morphological condition measures described the condition of individual larvae. Starved larvae showed a lower responsiveness than fed larger larvae of the same age, and the responsiveness in this experiment decreased with decreasing nutritional status of the larvae. However, starved larvae that were of the same size but older than fed larvae showed a higher responsiveness, which could be explained by differences in development of sensory systems. A combination of condition and developmental factors thus explained the differences between starved and fed larvae. Both starved and fed larvae showed decreasing responsiveness over time with repeated attacks with no difference between starved and fed larvae in the relative change over time. The results emphasise the importance of taking into account individual age and/or development when the nutritional condition of wild-caught larvae is incorporated in survival models.

Keywords: Escape behaviour; *Clupea harengus*; Herring larvae; Condition; Predation mortality; Responsiveness

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1. Introduction

The mortality of most marine fish species is extremely high and variable during early life stages (Smith, 1985; Houde et al., 1994; Houde, 1997a), and predation and starvation are believed to be the two main agents of mortality (McNamara and Houston, 1987; Miller et al., 1988; Bailey and Houde, 1989). Several observations indicate that predation is the most important direct agent of mortality of fish larvae (see Hunter, 1984; Heath, 1992; Gleason and Bengtson, 1996). There are many predators in the sea, and the diversity of predators is highest during the early life stages of the prey (Hunter, 1984; Bailey and Houde, 1989; Houde, 1997b). The nutritional status of fish larvae in the wild indicates low starvation mortality (O'Connell, 1980; Blom et al., 1997) except at first feeding (Theilacker, 1986; Theilacker and Porter, 1995; Grønkjær et al., 1997). Furthermore, larval mortality during the stage of endogenous feeding cannot be due to starvation (Ware and Lambert, 1985; Purcell et al., 1987; Dorsey et al., 1995).

Starvation may, however, be an important indirect mortality agent (Bailey and Houde, 1989) as nutritional status could influence different aspects of larval survival. Besides causing changes in the ability to maintain a preferred depth in the water column (Blaxter and Ehrlich, 1974), starvation leads to decreased growth rate (Ehrlich et al., 1976; Yin and Blaxter, 1986; Bisbal and Bengtson, 1995), slower development (Kamler et al., 1990; Høie et al., 2000), and changes in behaviour for energy-saving or energy-searching purposes (Blaxter and Ehrlich, 1974; Skiftesvik, 1992; Munk, 1995; Ross et al., 1996; Sogard and Olla, 1996; Chick and Van den Avyle, 2000). Larvae with low nutritional status will thus be smaller than well-fed larvae of the same age and will tend to remain at an earlier ontogenetic stage and be less developed with regard to sensory and locomotory capacities. Furthermore, starved larvae with reduced energy reserves have less energy to allocate to predator avoidance. Predation mortality can be affected by changes acting both before and after an encounter with a predator. Reduced growth and changes in behaviour for energy-saving purposes might decrease predation by reducing the encounter rate between predator and prey (Chick and Van den Avyle, 2000) and the attack rate towards less conspicuous starved larvae (Fuiman, 1989; Litvak and Leggett, 1992; Skelly, 1994). Alternatively, more risk-prone behaviour may increase the encounter rate through a higher rate of activity. A change in motivational state may be reflected in the behavioural options during predatory threat. Slower development, energy-saving behaviour, and less focus on predator avoidance can reduce escape success for a starved larva once encountered by a predator (Bailey and Yen, 1983; Chick and Van den Avyle, 2000). Reduced growth rate caused by starvation will also increase the time a larva spends within a specific stage or size class, and as there are more potential predators for small larvae, starved larvae might suffer a higher cumulative rate of mortality, depending on the predator regime (Cushing, 1975; Shepherd and Cushing, 1980).

Most research on fish larvae predation has focused on the effects of larval size and development on predation mortality, and the mortality is usually found to decrease with increasing larval size or to be dome-shaped (e.g., Folkvord and Hunter, 1986; Cowan and Houde, 1992; Cowan Jr et al., 1996; Lundvall et al., 1999; but see Pepin, 1993; Pepin and Shears, 1995). Several studies have investigated the interaction of starvation and predation mortality directly. Starved fish larvae are generally more susceptible to predation than fed

larvae. Purcell et al. (1987) found that unfed herring larvae were more vulnerable to jellyfish predation than fed larvae of the same age. Gamble and Hay (1989) showed that starved late yolk sac-stage herring larvae were more susceptible to predation by *Aurelia aurita* than fed larvae, but only when the larvae have almost reached the point of no return (PNR, where 50% are too weak to feed). Yin and Blaxter (1987a) found that the response rate and escape speed of herring, cod, and flounder first increased with starvation and then irrevocably declined 1–2 days before PNR. For a variety of other fish species (hake, cod, anchovy, walleye, striped bass), it has been shown that starved larvae are more susceptible to predation than fed larvae (Bailey and Yen, 1983; Bailey, 1984; Neilson et al., 1986; Booman et al., 1991; Jonas and Wahl, 1998). The results depend, however, on the level at which the information is aggregated and analysed (Bertram, 1996). In some cases, it is advantageous to be small (Litvak and Leggett, 1992; Cowan Jr et al., 1996), and a poor nutritional status may thus occasionally lower vulnerability to predation. Because of the complex effects of starvation, it has often been difficult to identify the causal mechanisms of predation mortality in relation to nutritional status. Margulies (1990) suggested that the difference in predation of white perch larvae (*Morone americana*) with differing feeding histories was caused by starvation-induced growth depletion. Others have found starved larvae to be more susceptible to predation than similarly sized fed larvae, indicating that other effects of starvation than growth depletion were important (Rice et al., 1987; Chick and Van den Avyle, 2000). Except for this, little information of the causal mechanisms of predation mortality related to nutritional status exists (Chick and Van den Avyle, 2000). In most of the cited studies, relatively young larvae have been used, and often the low nutritional groups consist of larvae that have never been fed (but see Yin and Blaxter, 1987a; Margulies, 1990; Jonas and Wahl, 1998), and there is a lack of information on how starvation affects antipredation behaviour in older larvae.

The aim of the current study was to investigate the causal mechanisms behind behavioural differences affecting predation mortality in starved and fed herring larvae approaching metamorphosis. To separate size- and condition-related effects, two experiments were set up. In the first, the fed and starved larvae were of similar age but different size, and in the second, the starved larvae were older but the groups were similar in size. To obtain information about the condition and motivational state of the larvae, we repeatedly attacked individual larva with a model predator and recorded the responsiveness of the larvae. Biochemical and morphological condition measures were noted for each individual and used to evaluate the relative importance of size- and condition-related effects on the behaviour. By obtaining individual condition measures, the results of the current experiment might be used as input in individual based models (IBMs) where the condition of wild-caught herring larvae is incorporated.

2. Materials and methods

Eggs and sperm were obtained from wild-caught herring in March and April 1999 (two females, five males both times) and incubated in hatching trays in the laboratory. The first group had a mean incubation temperature of 5.7 ± 0.2 °C. For the second group, we used two incubation temperatures so that half the larvae hatched 5 days after the others

(6.2±0.1 and 8.0±0.5 °C). After hatching, each of the three hatching groups was transferred to two green rearing tanks using a total of six rearing tanks (1×1 m, 500 l) and kept at 8.1±0.4 °C. The light intensity fluctuated according to the seasonal and daily cycles in Bergen (60°N) using a computer-controlled light system, Lysstyr® (Hansen, 1990). The larvae were fed natural zooplankton collected at the Espegrend field station outside Bergen. Every morning, the density of zooplankton in each tank was recorded and adjusted to about 1500 prey l^{-1}. The zooplankton consisted mainly of nauplii and copepods filtered and retained by 80–250 μm mesh-size filters. As the larvae grew, the upper mesh size was increased to 1000 μm (for details on the rearing procedure, see Folkvord et al., 2000).

Five days before a trial, the larvae to be tested were transferred to two smaller tanks (60×60×50 cm, 180 l, otherwise similar to the rearing tanks), in which the larvae in one tank were fed as previously while no food was added in the other tank (5 days of starvation at the given temperature yielded biochemical condition measures similar to those found in the field). The larvae from the two rearing tanks with the respective hatching groups were mixed in the transfer. The age of the herring larvae when they were used in the experiment ranged from 54 to 67 dph (days post-hatching), and they were in the size range (20.9 to 32.7 mm) where the ontogeny related to sensory development is rapidly advancing (Blaxter and Batty, 1985; Batty, 1989; Fuiman, 1989).

The observations were conducted in a flow-through aquarium measuring 90×20×17 cm (Fig. 1). A laminar-type water flow was used to reduce the time the larvae swam into the aquarium wall (a position found quite often during rearing), as larvae tend to swim towards water currents. The water inlet and outlet were separated from the test chamber by plankton filters to stop the larvae from entering these areas. The sides were marked with vertical lines every 5 cm and the bottom had grid lines separated by 1 cm. The water inlet end of the aquarium was raised by 6 cm relative to the outlet end to obtain a declining

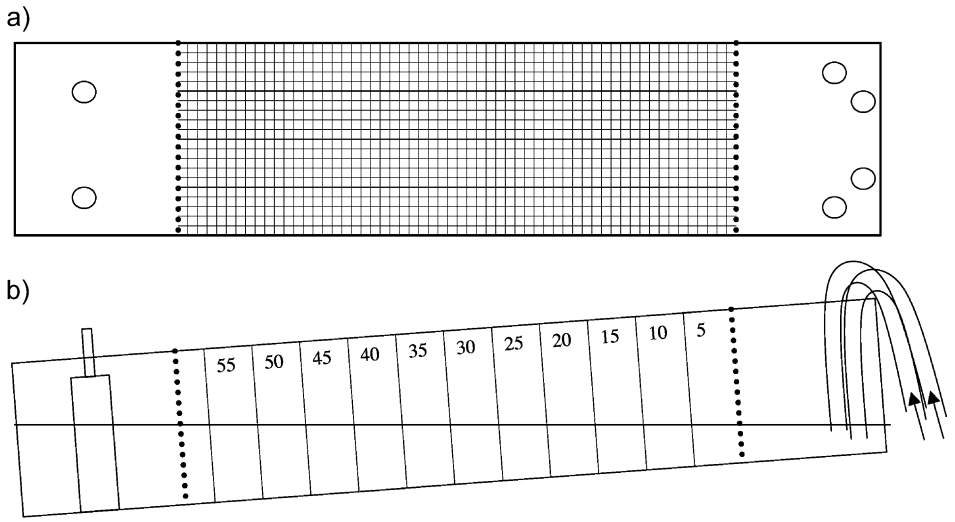

Fig. 1. Top view (a) and side view (b) of test aquarium with water inlet on the left side.

water flow through the aquarium, with the highest water current close to the inlet. The water depths in the test chamber were 3.5 and 7.5 cm at the inlet and outlet, respectively. The larvae were expected to be swept out of the area with the strongest current close to the inlet and to stay closer to the centre of the aquarium, where the outcome of an attack was easier to record.

Mean current velocity and Typical Turbulent Velocity (TTV) were measured at 27 locations in the aquarium (one location was excluded due to inconsistent data) with a transit-time difference current meter (Minilab from Sensordata). The measurements were adjusted to the mean current velocity as measured by means of a dye marker. Mean current velocity and TTV in the aquarium were 1.8 cm s^{-1} (S.D. 0.14) and 0.12 (S.D. 0.058), respectively. Both variables generally decreased with distance from the inlet and with depth, but the differences were quite small except for a high TTV close to the inlet. The difference in current velocity between the two locations with the highest and lowest mean current velocities was 0.55 cm s^{-1}.

On the day of the trial, individual herring larvae were carefully transferred in a beaker to the flow-through aquarium (one larva for each observation). The order of the larvae tested was randomised, with every pair of larvae consisting of one fed and one starved larva (starved for 5 days). One minute after transfer, the larva was exposed to repeated attacks with a handheld black 4-mm-thick glass probe. The attacks were directed behind the head of the larva, at approximately 90° to its body axis. The probe approached the larva perpendicularly and parallel to its side (it was not the tip but the side of the pipette that touched the larva). The attack started about 5–6 cm from the larva. The speed corresponded to 4–5 larval body lengths s^{-1} and was kept as constant as possible within and between attacks. The attack was characterised as unsuccessful if it failed to meet the criteria of speed, touch, or direction of probe during the attack. The attack ended either when the probe was in contact with the larva or when the larva showed a reaction. The interval between two attacks varied considerably as attacks were only conducted when the larva was in free water (not in contact with the sides or bottom of the aquarium). The time of the attack (seconds after start of trial), the position of the larva, whether the larva reacted or not, and the escape distance (cm) were recorded for each attack by the use of a dictaphone. A reaction to an attack was categorised as: (1) reaction before contact: marked increase in swimming speed before contact with the probe, usually swimming in a straight line; (2) contact reaction: marked increase in swimming speed after contact with the probe; or (3) wriggling reaction: the larvae reacted to the touch by wriggling its body vigorously. The escape distance was the estimated distance (cm) relative to the bottom grid that the larvae swam with increased swimming speed (distances >9 cm grouped to 10+ cm). The position of the larva was recorded as the distance from the filter at the inlet end when attacked (grouped into 5-cm intervals). It was also noted whether the larva was in the vicinity of the wall, bottom, or surface (within one cm but not in contact). The trial was terminated if the larva swam out of the experimental chamber (to the filter at the outlet end of the aquarium) or after 4 min. The larva was then removed from the aquarium using a pipette. The standard length (SL, snout to end of notocord) was measured in a stereomicroscope on live larvae anaesthetised with Metacaine before they were stored individually in Eppendorf vials in liquid nitrogen and thereafter at −80 °C. Individual

larvae were later analysed for dry weight (DW) and total amount of RNA and DNA according to methods described by Suneetha et al. (1999).

Two experiments were carried out. In Exp. I, the two nutritional groups were of the same age (larvae from first hatching group), while in Exp. II, they were approximately the same size but of different ages (larvae from second hatching group, Table 1). General Linear Models (GLM) were run with trial day as the continuous predictor to test if the larval size of the two nutritional groups was suitable for the intended study in each of the two experiments (using the data analysis software system STATISTICA, StatSoft, 2001). Trials were run on 2 consecutive days with 1 or 2 days between trials. The two experiments lasted for 5 (4 test days) and 9 days (6 test days), respectively.

The responsiveness (proportion reacting) and median escape distance were calculated for each larva. The data for Exp. I and Exp. II were analysed separately by ANCOVA with starvation group as categorical predictors and trial day as continuous predictor. Larvae attacked less than four times (36 out of 372 larvae), and unsuccessful attacks (270 of 4246 attacks) were excluded from the analysis. Natural log transformations were used for DNA and DW to obtain linearity and homogeneous variance. The residual of SL and log DW (morphometric condition measurement) and the residual of the log RNA and log DNA relationship (biochemical condition measurement) were used to describe the nutritional status of the individual larvae. Regression analyses were carried out in order to determine if any of the condition measures (explanatory variables) could explain some of the variation in responsiveness and escape distance (dependent variables) between starved and fed larvae. The size-related effects on responsiveness were analysed by using log DNA (measure of cell number in the larvae) as the explanatory variable in the regression analysis. DNA is a measure of cell number and probably represents larval development better than SL. In order to further clarify the effects of size and condition, regression analyses were performed on starved and fed larvae separately in both Exp. I and Exp. II and on larvae from a limited size range in Exp. I (dependent and explanatory variables as above).

The possible effect of a starvation-induced difference in responsiveness for similar aged larvae was modelled. The differences in responsiveness observed for starved and fed

Table 1
Characteristics of larvae used in the two experiments

Date	Trial	Description	Fed group			Starved group (5 days)		
			Age (days)	*n*	Mean SL mm (S.D.)	Age (days)	*n*	Mean SL mm (S.D.)
07. June	1	Exp. I: Similar age,	61	13	24.7 (0.9)	61	14	23.1 (1.2)
08. June	2	different size	62	17	24.7 (1.8)	62	17	23.6 (1.5)
10. June	3		64	15	25.3 (1.5)	64	17	24.1 (1.2)
11. June	4		65	16	25.7 (1.7)	65	15	24.1 (1.5)
21. June	5	Exp. II: Different age,	54	19	26.6 (1.5)	59	19	26.2 (2.0)
22. June	6	similar size	55	16	27.0 (0.9)	60	19	26.7 (1.4)
24. June	7		57	16	27.7 (1.2)	62	19	27.7 (1.6)
25. June	8		58	19	28.3 (0.9)	63	15	28.3 (1.2)
28. June	9		61	18	29.7 (1.3)	66	19	28.6 (1.2)
29. June	10		62	15	29.8 (1.2)	67	18	29.2 (1.2)

Total number of larvae used was 336.

larvae in the current experiment were used to estimate the percentage difference in the numbers of starved and fed larvae that are alive after a series of attacks. The number of fed or starved larvae alive after n attacks (L_n) was calculated as:

$$L_n = L_{(n-1)} - \left(rL_{(n-1)}(1-s) + (1-r)L_{(n-1)}(1-p)\right)$$

where r=responsiveness for fed or starved larvae respectively, s=response success, and p=predator error.

Differences in responsiveness between different feeding groups and time intervals (time since start of trial and time since last attack) were tested for both experiments using repeated-measurements ANOVA (StatSoft, 2001).

3. Results

The age and mean size of the individual groups in the two experiments were suitable for the intended study (Table 1). In Exp. I, starved larvae were significantly smaller than fed larvae (GLM, $p<0.05$), while in Exp. II, there was no statistically significant size difference. The difference in Exp. I corresponded to 5 days of growth (daily growth found by the regression of SL versus age using fed larvae in the corresponding experiment). The larvae in Exp. II were larger than in Exp. I although their ages were the same (for starved larvae) or less (for fed larvae). The growth rate prior to the experiment had thus been higher for the larvae in Exp. II (see Discussion), as was the case also during the experiment (0.41 vs. 0.28 mm/day). In Exp. II, we recorded more larvae with inflated swim bladders in the starved group than in the fed group (Chi-square=25.8, df=1, $p<0.001$). The morphometric (residual of SL and log DW) and biochemical condition measurements (residual of the log RNA and log DNA relationship) were positively correlated (r=0.53, $p<0.001$), and both reflected the starvation treatment of the groups (Fig. 2).

Both starved and fed larvae reacted to attacks by the glass probe. The mean number of attacks on individual larvae was 11.8 (S.D.=5.6) with the interval between attacks ranging from 1 to 200 s (mean=16, S.D.=21). There were no differences between fed and starved larvae in the number of attacks, indicating that they spent about the same time in free water masses. The contact reaction comprised 80% and 86% of the reactions, reaction before contact 11% and 10%, and wriggling reactions 9% and 5% of the reactions for fed and starved larvae in the two experiments, respectively. There were no differences in the frequencies of the different reaction types between fed and starved larvae in Exp. I. In Exp. II, there was a statistical difference (log-linear analysis, Pearson Chi-square=16.06, $p<0.001$), but the difference never exceeded 6% in any of the reaction categories, and the data were grouped together in the ANCOVA.

3.1. Experiment I

The responsiveness was lower for starved larvae compared to the fed larvae in Exp. I (Fig. 3), in which the groups were of the same age but differed in size (ANCOVA, $F_{1,121}$=17.66, $p<0.0001$), and the responsiveness was positively correlated with both

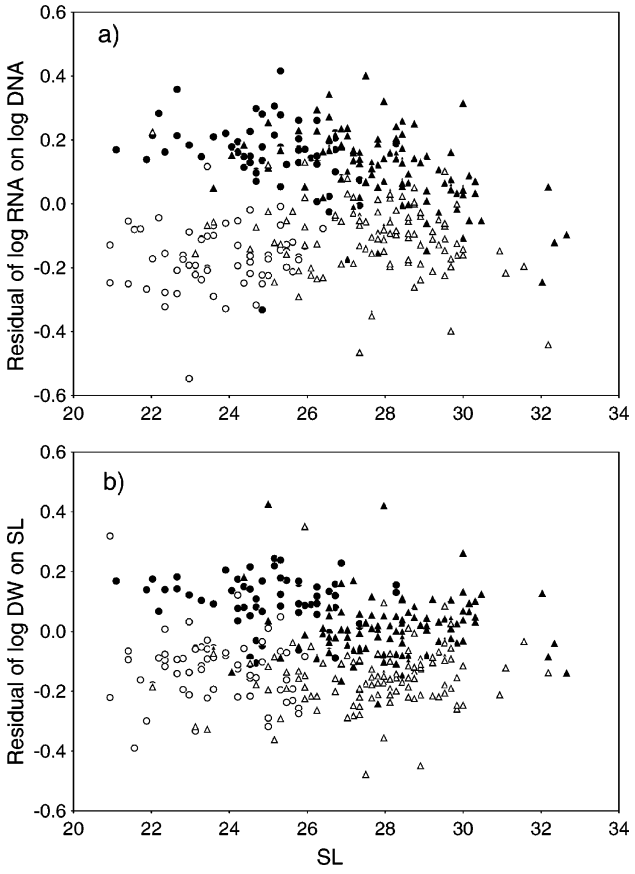

Fig. 2. Biochemical (a) and morphometric (b) condition of individual starved (empty symbols) and fed larvae (filled symbols) in Exp. I (circles) and Exp. II (triangles).

the biochemical and morphometric condition measures ($F_{1,121}$=22.36, r^2=0.15, p<0.00001 and $F_{1,121}$=19.05, r^2=0.13, p<0.00005). Responsiveness also correlated with larval size expressed as DNA ($F_{1,121}$=8.64, r^2=0.059, p<0.005, Table 2) but to a lesser extent than the condition measures. When the regression analysis was run on the starved larvae alone, both DNA and the morphometric condition measure correlated with responsiveness ($F_{1,60}$=7.94, r^2=0.1, p<0.01 and $F_{1,60}$=4.85, r^2=0.06, p<0.05), while there was no correlation between the biochemical condition measure and responsiveness. For the fed larvae, neither one of the condition measures nor DNA correlated with responsiveness. When multiple regression analysis was run on larvae in the size range 22–26 mm SL (where fed and starved larvae overlapped), both the condition measures correlated more strongly with responsiveness compared to the size-related measurement DNA and morphometric measurement: $F_{1,92}$=2.82, p>0.05 and $F_{1,92}$=20.57, p<0.00005, respectively; DNA and the biochemical condition measure: $F_{1,92}$=5.91, p<0.05 and $F_{1,92}$=16.84, p<0.0001, respectively). The mean

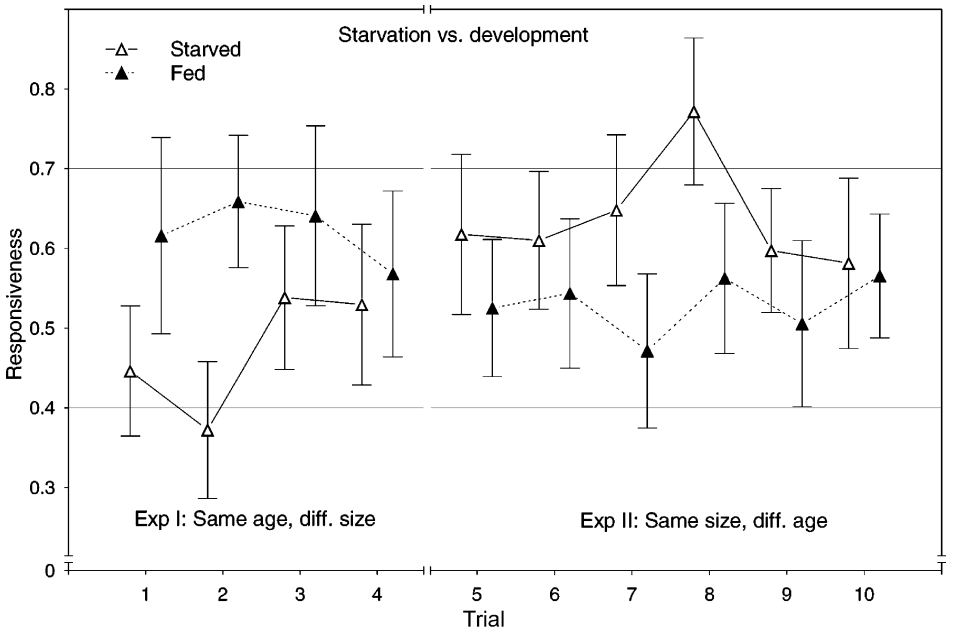

Fig. 3. Mean responsiveness (±95% confidence interval) on each trial day in Exp. I and II. Position of points shifted on the *x*-axis for clarity.

responsiveness of fed and starved larva was 0.62 and 0.47, respectively. No differences between trial days were found.

3.2. Experiment II

In Exp. II, in which the two feeding groups were similar in size, the opposite result was found and the starved larvae had higher responsiveness than the fed larvae (ANCOVA, $p<0.0005$; Fig. 3). In this experiment, the responsiveness showed a weak negative correlation with the biochemical condition measurement ($F_{1,208}=2.82$, $r^2=0.03$, $p<0.01$) and was not correlated with the morphometric condition measurement or to DNA. When the regression analysis was run separately on the starvation group, there were no correlations between responsiveness and any of the condition measures or DNA. The

Table 2
Summary of ANCOVA performed for the two experiments and the different condition measures

Responsiveness	Experiment I			Experiment II		
	Morph.	Biochem.	Size measure	Morph.	Biochem.	Size measure
All larvae	<0.00001	<0.00005	<0.005	n.s.	<0.01	n.s.
Starved larvae	<0.05	n.s.	<0.01	n.s.	n.s.	n.s.
Fed larvae	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Size 22–26 mm	<0.000005	<0.00005	<0.005			

mean responsiveness of fed and starved larva was 0.53 and 0.63, respectively. No differences between trial days were found.

3.3. Escape distance

The escape distance ranged from 1 to more than 10 cm. No significant difference between starved and fed larvae in median escape distance was found in either Exp. I or Exp. II, and there was no difference between test days. Except for a positive correlation with the biochemical condition measure in Exp. I ($F_{1,114}$=4.41, r^2=0.03, p<0.05), there was no correlation between escape distance and the two condition measures.

3.4. Effect of repeated attacks

Both starved and fed larvae showed a waning in responsiveness in the course of the attack sequence. In both Exp. I and Exp. II, responsiveness differed between starved and fed larvae ($F_{1,>111}$>5.85, p<0.05; Fig. 4). The larvae showed a higher responsiveness during the initial attack (naive) compared to repeated attacks ($F_{1,>111}$>5.85, p<0.05; Fig. 4a and b), but there was no change in responsiveness with increasing time since previous

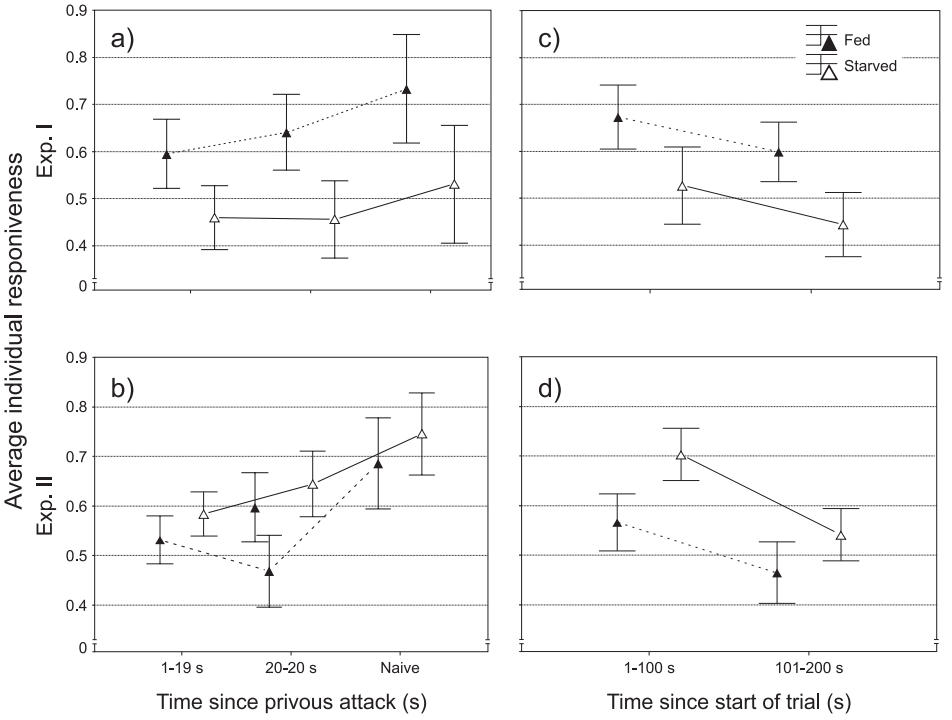

Fig. 4. Responsiveness relative to time since previous attack (a and b) and time since start of trial (c and d) for starved (empty symbols) and fed larvae (filled symbols) in Exp. I (a and c) and Exp. II (b and d). Position of points shifted on the x-axis for clarity.

attacks (grouped in time since previous attack 1–19 and 20–200 s). A waning in responsiveness was apparent when testing responsiveness versus time since start of the trial and feeding level in all groups ($F_{1,>111}>5.21$, $p<0.05$; Fig. 4c and d).

4. Discussion

Herring larvae in the size range 20–33 mm SL starved for 5 days were highly capable of reacting to an attack. The probability of reacting was lower in starved than in fed larva of the same age. If, however, the starved larvae are slightly older and more developed than the fed larvae, the difference in responsiveness is reversed, as the 5-day-older starved larvae showed a higher responsiveness than the similar-sized fed ones. The relatively small difference in responsiveness between starved and fed larvae indicates that the risk of not reacting to an attack is high relative to the risk of starvation (Ydenberg and Dill, 1986). Franklin et al. (1996) found that the energy used by herring larvae in reacting to attacks came from renewable (aerobic) sources and indicated a low energy cost for single reactions because the larvae did not fatigue easily. A relatively low cost of fleeing is supported by the relatively high responsiveness in the current experiment after starvation and after repeated attacks.

The starvation level of the larvae in the experiment may be regarded as moderate, as only 1 of 336 larva had a RNA/DNA value below 1.8, the critical level for 25-mm herring larvae suggested by Clemmesen (1994). The condition range of the larvae (RNA/DNA ratio from 1.7 to 5.1) was representative of the condition range found by Blom et al. (1997) in similar-sized wild herring larvae (approx. 2.1 to 5.9). Rather prolonged starvation has earlier been proven necessary for inducing behavioural changes that might affect predation mortality. Several studies of herring yolk sac larvae have shown that starvation increases vulnerability to predation, but only when the herring larvae were close to PNR (Bailey, 1984; Purcell et al., 1987; Gamble and Hay, 1989). Yin and Blaxter (1987a) found that responsiveness and escape speed of starved larvae (yolk sac larvae of herring, cod and flounder, and 36- and 60-day-old herring larvae) reached a maximum 1 to 2 days before PNR and declined with further starvation (PNR for 60-day-old herring larvae at 10 °C was 7 days). A similar trend has also been found for other species (Jonas and Wahl, 1998, but see Booman et al., 1991), and it is argued that the increase in responsiveness and escape speed was caused by a general increase in activity level observed during initial starvation (Wyatt, 1972; Yin and Blaxter, 1987b; Munk, 1995). As the temperature in the current experiment was 8 °C (compared to 10.5 °C in Yin and Blaxter, 1987b), we might expect PNR after 8 to 9 days of starvation. We have thus demonstrated starvation-induced changes in responsiveness for larval herring in a condition corresponding to 3 to 4 days before PNR.

The most likely explanation for the difference in behaviour between starved and fed larvae of different ages is a combination of condition and development of the larvae. In Exp. I, the decrease in responsiveness of starved larvae was primarily due to the poorer condition of the larvae, as indicated by the morphometric and biochemical condition measures. Size, expressed as DNA, also explained some of the variation in responsiveness. This may be connected to differences in development due to a relatively strong correlation

between size and ontogeny, particularly within hatching groups (Zweifel and Lasker, 1976; Gerking and Rausch, 1979; Chambers and Leggett, 1987; Fuiman, 1994). Chick and Van den Avyle (2000) also concluded that a decrease in response to predators in larval bass (*Morone saxatilis*) on low feeding rations was an effect of larval condition rather than of size. In Exp. II, differences in development could explain the increased responsiveness of the starved larvae because neither of the condition variables had such an effect. We recorded more larvae with inflated swim bladders in the starved group, supporting that these larvae were more developed although they were no bigger than the fed larvae. The relative importance of development- and condition-based effects on responsiveness is likely to vary in the course of ontogeny because the effects of starvation vary over time and development is discontinuous (Balon, 1984).

The regression analyses on the individual starvation groups indicated that the condition of the larvae had to fall to a certain level before responsiveness was affected. The larvae in Exp. I, possibly because of their smaller size, seemed to be more affected by an equivalent starvation treatment than the larvae in Exp. II, as indicated by a larger difference in the residual condition measures between starved and fed larvae and by lower RNA/DNA values. Only the starved larvae in Exp. I showed a responsiveness that positively correlated with any of the condition measures. In the fed larvae in Exp. I and the starved and fed larvae in Exp. II, there was no such correlation, and the condition of these larvae had probably not yet fallen to a critical level. The condition, expressed by RNA/DNA, in which responsiveness began to decrease with larval condition, was approximately 2.5–3.0. This is higher than what is normally considered critical in terms of starvation in herring (Clemmesen, 1994).

Both the biochemical and morphometric condition measures explained the difference between starved and fed larvae in terms of responsiveness in Exp. I. We are aware of no other experiment in which individual larval condition could be associated with responsiveness in this way. Booman et al. (1991) used histological starvation criteria on the group level to link the nutritional condition of larvae to responsiveness to attacks. Neilson et al. (1986) found a difference in antipredator behaviour in Atlantic cod (*Gadus morhua*) related to prey density, but the condition measures (Fulton's K and body height standardised for length) did not explain the difference. In our study, however, condition did not explain why the starved larvae in Exp. II showed higher responsiveness than fed larvae although their condition was poorer. Yin and Blaxter (1987a) found an initial increase in response rate with starvation that might explain the higher responsiveness found for starved larvae in Exp. II, but plots of the various condition measures and responsiveness were not dome-shaped and thus do not support the idea of an initial increase in responsiveness during starvation. The higher responsiveness of the starved larvae in Exp. II, which were older but similar in size to the fed larvae, was therefore caused by differences in development. The ontogeny of larval herring in the rather limited size range used is characterised by a number of changes in sensory systems and escape capacity (Batty, 1989; Fuiman, 1989, 1993; Blaxter and Fuiman, 1990). The larvae in Exp. II came from the same fertilisation group but were incubated at different temperatures, hatched on different dates, and kept in separate tanks. Because differences in development between groups may be related to incubation temperature (Johnston, 1993; Johnston et al., 1998) and to the specific rearing tank conditions, the starved but older larvae might very

well have been more developed than the younger fed larvae. Development may also continue during starvation as slower-growing herring larvae have been shown to reach a given larval stage at a smaller size than faster-growing larvae (Høie et al., 2000).

Both nutritional groups in Exp. I and Exp. II showed response waning, represented by decreasing responsiveness with time tested and with time since previous attack. Although there were differences in the absolute responsiveness between starved and fed larvae within each experiment, there was no difference in the relative change in responsiveness. Overt behaviour is the outcome of internal (here nutritional status) and external cues (here model predator), where the internal ones refer to the animals' motivation (Colgan, 1993). Motivational changes in animals may occur over relatively short time intervals, e.g., in the course of a meal as it becomes satisfied or if it experiences energy depletion, with the relative change being affected by initial nutritional status. In this experiment, the initial nutritional status of the larvae differed between the two groups. The observation that the relative change did not differ between starved and fed larvae indicates that (1) the starved larvae did not become totally exhausted during the attacks and (2) the relative importance of the antipredatory behaviour after a series of attacks developed in a similar way in starved and fed larvae.

At a similar age, a starved herring larva in nature should, according to our findings, have a 15% lower probability than a well-fed larva of reacting to an attack by a predator at the speeds employed in this study. The importance of this starvation-induced difference in responsiveness is visualised in Fig. 5, where the percentage difference in the numbers of starved and fed larvae that are alive after a series of attacks is found by using the observed differences in responsiveness for fed and starved larvae. Two variables that greatly influence the differences in survival rates are the proportion of successful escapes of a reacting larva (*escape success*) and the proportion of predator attacks that fail to catch a nonreacting larva (*predator error*). *Survival probability* is defined in terms of the combined effect of escape success and predator error. In laboratory experiments, predator errors of about 10–20% have been reported (Fuiman, 1989; Fuiman and Batty, 1994), but in a field situation, a relatively lower predator error should be expected due to a wider available range of prey, a nonmanipulated hunger level (of the predator) as well as a lack of confinement that restricts predator mobility. Fig. 5 suggests that under the most extreme conditions (predator error 0 and/or survival probability high), three times as many fed as starved larvae will survive after five predatory attacks. The more the effect of nutritional status on survival is pronounced, the more attacks the larvae experience and the higher the survival probability. The difference decreases as predator error increases, and even with a predator error of only 1%, the difference in survival between starved and fed larvae decreases markedly. In addition, larval size, predator species or type, predator satiation, and the relative difference in size between predator and larva are crucial to both predator error and escape success rates (Folkvord and Hunter, 1986; Bailey and Houde, 1989; Fuiman, 1994; Fuiman and Batty, 1994). The model emphasises the importance of being aware of the predator regime when modelling survival. Apart from reaction rate, we assumed no differences in reaction performances (escape speed, -distance, -direction, and -latency) between starved and fed larvae that would alter the probability of survival. No significant difference in escape distance was observed, but more accurate methods may give other results. Escape speed has been found to change with starvation (Yin and

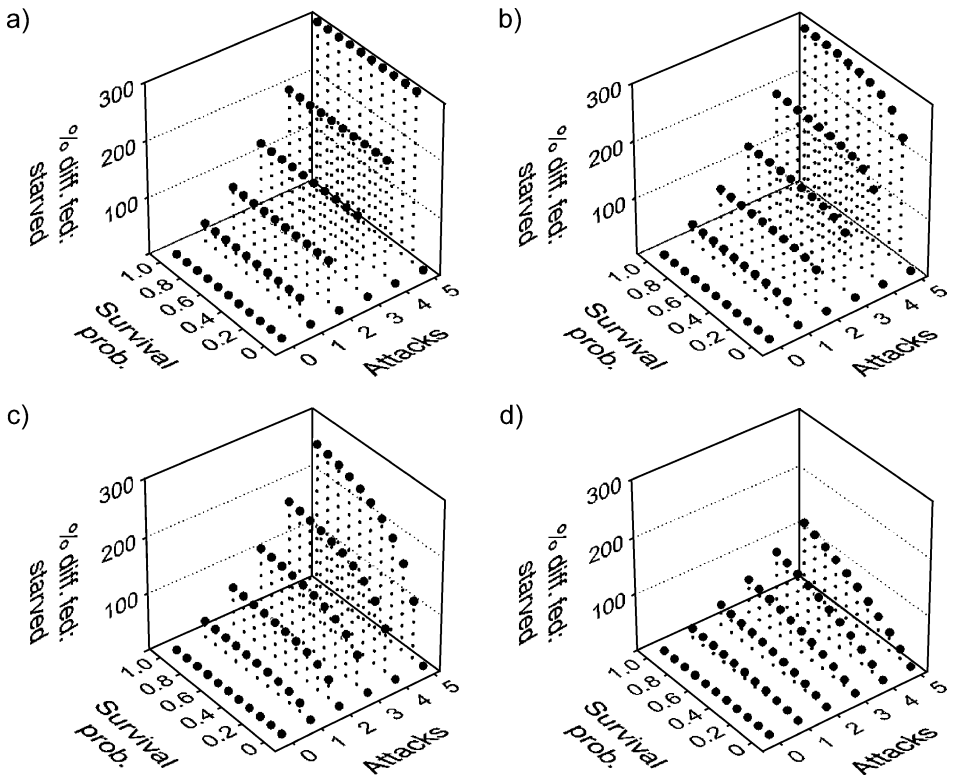

Fig. 5. Percentage difference between number of starved and fed larvae surviving after repeated attacks at various levels of survival probability (see text). Reaction rates of the two groups were those found in Exp. I (0.47 and 0.62, respectively). Predator failure rates (%) were set to 0% (a), 1% (b), 5% (c) and 30% (d).

Blaxter, 1987a), but it was difficult to convert this difference into survival probability in the model. The assumption of no difference in reaction performances between starved and fed larvae is probably conservative, and such differences should cause an even greater difference in survival.

The main results of this study were that starved herring larvae in the size range 20–33 SL are well able to react to an attack from a model predator, but that there is a difference between starved and fed larvae in reaction rate, caused by condition and/or development. The actual difference in survival will be highly dependent on the predator regime, which under certain conditions may lead to a considerably higher rate of survival of larvae with good nutritional status. The difference between starved and fed larvae found is based on differences in behaviour after an attack has commenced. Because of the higher costs involved in fleeing early in a predation process due to a higher fleeing frequency (Ydenberg and Dill, 1986), larger behavioural differences caused by starvation might be expected at this stage. In future experiments, the behavioural effects of starvation prior to attacks should therefore be investigated. There is also a need to study starvation-induced differences in responsiveness and success, as well as rate of predator error, invoked by different species or types of predators.

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