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The escape response of food-deprived cod larvae (*Gadus morhua* L.)

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Abstract

The escape response of Atlantic cod larvae (*Gadus morhua*) 25 and 47 days post hatch (dph) – either fed or deprived of food for three days – was studied. Larval escape responses were provoked by water movement from the suction of a fixed-position pipette. Escape latency, distance, speed, burst speed, and vertical and lateral escape angles were quantified using motion tracking software designed for 3-D silhouette video recordings. Escape performance, expressed as escape distance and escape speed, improved with age. The escape angles were normally distributed and highly variable, ranging from -170° to 170° and -40° to 105° for lateral and vertical escape angles respectively. No food deprivation-induced effects in any of the behaviours were found, suggesting that there are no condition-related behavioural effects (size-independent effects) in escape response performance after 3 d of food deprivation. This may reflect a negligible difference in the cost/benefit equation for fed vs. food-deprived larvae in performing an escape response when under attack.

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1. Introduction

The two main mortality agents acting upon marine fish larvae are predation and starvation (Bailey and Houde, 1989). These factors are not independent, as food-deprived or food limited larvae likely show changes in behaviour and growth rate which may affect, sequentially, encounter rate, the probability of attack, and the probability of being captured after initiation of an attack (McNamara and Houston, 1987; Miller et al., 1988; Smith, 1997). The latter probability depends upon, amongst other things, the performance of the escape response, which is the ability of the

larva to move at the right time, in the right direction, and with a speed and distance sufficient to escape from the predator (Fuiman and Magurran, 1994; Scharf et al., 2003). At any given size, the escape success of fish larvae depends upon developmental improvements in both the sensory organs used to perceive predators and their neuro-locomotory system, both of which are affected by food deprivation.

The escape response of marine fish larvae has been described in detail for herring (*Clupea* sp.), and is characterized by a positive relationship between escape speed and larval size, and by decreased latency in response to predators (indicative of an improving ability to evaluate risk) (Blaxter and Batty, 1985; Yin and Blaxter, 1987a; Batty, 1989; Batty et al., 1993; Fuiman, 1993). The behaviour changes markedly when herring larvae reach approximately 26 mm, at which point a rapid development of the sensory organs occurs (Fuiman, 1993).

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Williams et al. (1996), and Sugisaki et al. (2001), reported a positive relationship between escape speed and larval length when describing developmental changes in behaviour of several species, including Atlantic cod (*Gadus morhua*). The relationship between nutritional state and swimming activity, which could affect encounter and attack, is generally dome-shaped; activity first increases before decreasing when the larvae deplete their energy stores (Yin and Blaxter, 1987b; Skiftesvik, 1992; Munk, 1995; Skajaa et al., 2003). The escape response rate – to tactile stimulation, pipette suction, or fish predators – is either a dome-shaped (Yin and Blaxter, 1987a) or a negative relationship with increasing duration of food deprivation (Neilson et al., 1986; Booman et al., 1991; Chick and Van den Avyle, 2000). Yin and Blaxter (1987a) reported that escape speed in herring (*Clupea harengus*) of different ages, and newly hatched cod and flounder (*Platichthys flesus*), initially increased with increasing time of food deprivation, peaked when the larvae were close to the point of no return (PNR, when 50% of the larvae will die even when offered food), and subsequently decreased.

Food deprivation can result in both size-related and size-independent (i.e. condition-related) effects. Since marine fish larvae can grow extremely rapidly (sometimes doubling their size in 4–5 d), size-related effects of food deprivation are the result of slower growth and development (Blaxter, 1986; Kiørboe and Munk, 1986; Fiksen and Folkvord, 1999). Such size-related effects are manifested only as a relative difference in behaviour between food-deprived (smaller) vs. fed (larger) individuals, and may indirectly affect predation mortality by, for example, (i) increasing the duration of any given ontogenetic stage, thereby prolonging the period during which larvae are vulnerable to predation, or (ii) altering the probability of a larva's being selected for attack by a predator that can choose from amongst different quality prey (Bailey, 1984; Folkvord and Hunter, 1986; Miller et al., 1988; Paradis et al., 1996). Behavioural changes associated with energy conservation, intensified prey search, and/or reduced overall condition are examples of condition-related effects of food deprivation (Munk, 1995; Ross et al., 1996; Chick and Van den Avyle, 2000; Puvanendran et al., 2002; Skajaa et al., 2003). Condition-related effects could be manifested as differences in behaviour between larvae that have been deprived of food for different periods of time and/or as deviations in behaviour from that exhibited by well-fed larvae of the same size and developmental state. Condition-related effects may influence predation rate directly by affecting the probabilities of encounter, attack and catchability by, for example, a change in swimming activity, vertical

migration, or evasive behaviour. During prolonged food deprivation, behavioural effects will always eventually become condition-related. Few attempts have been made to separate size-related vs. condition-related effects of food deprivation on the susceptibility to predation of marine fish larvae (Chick and Van den Avyle, 2000; Skajaa et al., 2004).

The predation mortality component of the individual-based models (IBMs) that are developed to estimate larval fish survivorship usually incorporate only the size-related effects of food deprivation (Pepin, 1989; Cowan et al., 1996; Cowan et al., 1997; Paradis et al., 1999; Paradis and Pepin, 2001). To the best of our knowledge, condition-related effects of starvation have not been included in IBM's, although such effects have been demonstrated in behaviours which may affect events early in the predation sequence (encounter rate and probability of attack, Munk, 1995; Ross et al., 1996; Chick and Van den Avyle, 2000; Puvanendran et al., 2002; Skajaa et al., 2003). The aim of the current study was to determine whether predation and starvation are related at the level of the larval escape response (late in the predation sequence), to characterize possible age-related effects, and to separate size-related vs. condition-related influences of starvation.

2. Materials and methods

The cod larvae used in the experiment were raised from two stocks of Norwegian coastal cod. The eggs were collected from tanks populated with approximately 20 adult cod broodstock (50:50% males and females) at the Institute of Marine Research — Austevoll Aquaculture Research Station (southern Norway) and 45 cod (approximately 50:50% males and females) from the cod farm Tromsø Marin Yngel (northern Norway). The eggs were transferred to the Department of Fisheries and Marine Biology at the University of Bergen where they were reared. They were kept at 5–6 °C until approximately two days before hatching when the temperature was gradually raised to 10 °C two days after hatching. The larvae from the two stocks were reared in green tanks (1 × 1 m, 500 l) at 10 °C. The light intensity and day length were controlled to mimic the seasonal and daily cycles in Bergen (60° N) using a computer controlled system, Lysstyr[®] (Hansen, 1990). The larvae were fed natural zooplankton collected at Espegrend field station outside Bergen. Every morning, the abundance of zooplankton in each tank was recorded and adjusted to about 2000 prey/l. The zooplankton consisted mainly of nauplii and copepods filtered and retained by 80–250 µm mesh size filters initially, and as

Table 1

Details of the experimental trials on the escape responses of fed and 3-D food-deprived Atlantic cod larvae (*Gadus morhua*)

Age group (dph)	25				47			
	Food-deprived		Fed		Food-deprived		Fed	
Replicate aquaria	1	2	1	2	1	2	1	2
Larva in aquarium (no.)	60	60	60	60	50	50	50	50
“Attacks” (no.)	45	93	69	60	70	70	70	70
Duration of trial (min)	60	59	56	38	44	57	66	36
Reactions to analysis (no.)	18	21	27	22	22	25	25	25
Captured larvae (no.)	0	2	2	3	2	0	0	0

the larvae reach 12 mm, by 80–1000 μm mesh size filters (see Otterlei et al., 1999, for details about the rearing procedure).

Three days before an experimental trial, approximately 300 larvae from each of the two stocks were mixed in two smaller tanks (60 \times 60 \times 50 cm, 180 l, otherwise similar to the rearing tanks) where one group continued to be fed while the other one was food-deprived. After one day they were carefully transferred to Austevoll Aquaculture Research Station, where experiments were conducted in April and May 2001. At Austevoll, the larvae were transferred to two black cylindrical tanks (\varnothing : 74 cm, 80 l), in which they were either fed or food-deprived, as previously. According to Skajaa et al. (2003), a significant condition-related change in behaviour could be expected after 3 d of food deprivation in cod larvae, and an ontogenetic change in ability to perform trade-offs between starvation and predation was evident when the larvae were close to metamorphosis. Therefore, experiments were conducted on fed larvae, and on larvae food-deprived for 3 d, at the ages of 25 and 47 dph (Table 1).

Behavioural observations were conducted in glass aquaria (20 \times 20 \times 20 cm) surrounded by black contact paper (except for 15 cm openings that allowed viewing from the sides) (see Fig. 1 in Browman et al. 2003). A white sheet surrounding the aquaria minimised disturbance from the observer. The behaviour of larvae was recorded using a 3-D silhouette imaging system described in detail by Browman et al. (2003).

To initiate escape behaviour, an attack from a suction predator was simulated using a glass pipette (6 mm inner diameter) connected to a peristaltic pump by a manual valve and a flexible silicon tube. The vertical angle of the pipette was 45°, and the suction plume (the area within where the suction of the pipette initiated responses, Fig. 1) was within the observation field of view. When the valve was closed, the peristaltic pump formed a vacuum within the silicon tube. Opening the valve created a suction of water into the pipette (an attack) that caused the larvae in the vicinity of the pipette's opening

to initiate an escape response. The volume of water displaced by the pipette during each simulated attack was a constant 16 ml. During some of these attacks, larvae were sucked into the pipette and caught. The rapid removal of water created an underwater wave which in some cases initiated escape responses; these responses were easily recognised (constant placement relative to the suction plume and longer escape latency) and were not included in the analysis. In most trials, it was necessary to replace approximately 1 l of water halfway through the trial. A ladle was used to carefully add water to the aquarium and the trial did not continue until water movement in the aquarium had ceased.

Before an experimental trial, 50–60 larvae from either the food-deprived or fed groups were transferred to the test aquarium and acclimated for 30 min before observations began. In the fed group, no effort was made to filter out zooplankton from the water when larvae were transferred.

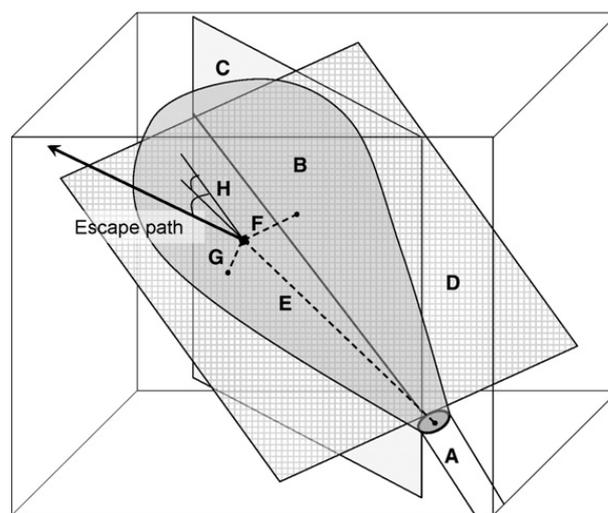


Fig. 1. Three-dimensional presentation of the experimental arena, showing the pipette (A), the suction plume (B, not to scale), the vertical plane (C), and the central plane (D). The arrow indicates an example of an escape path of an Atlantic cod (*Gadus morhua*) larva, from which the following variables were measured; distance to pipette (E), distance to vertical plane (F), distance to central plane (G), and lateral and vertical escape angles (H). The length of the arrow indicates the escape distance, which was used to calculate escape speed.

However, the density of zooplankton in the test aquarium was lower than that in the rearing tanks. Therefore, the possibility that we were observing an effect of ingesting a large meal (potentially confounding the effect of food deprivation) was unlikely. After acclimation, the larvae were observed visually through an opening in the white sheet, and whenever a larva seemed to move into the suction plume an attack was initiated. One experimental trial lasted until approximately 40 responses had been recorded. For both age groups, two replicate trials for each nutritional group were used. Experimental trials reported here are summarized in Table 1.

Every test day, 20 larvae were sampled from the two rearing tanks. Standard length (SL, snout to end of notochord) was measured in a stereomicroscope on live larvae anaesthetised with Metacain before being placed individually into Eppendorf vials, emersed in liquid nitrogen, and thereafter stored at -80°C . Individual larvae were later analysed for dry weight (DW) and total content of RNA and DNA according to methods described in Imsland et al. (2002). A Student's *t*-test was run to compare $\log\text{DW}:\log\text{SL}$ (log-transformed to obtain linearity and homogeneity of variance) and the RNA:DNA relationship between food-deprived and fed larvae in each age group.

Videotaped observations of escape responses were analysed frame-by-frame using motion tracking software developed for taking 3-D measurements from data obtained from the silhouette imaging system (TRAK-FISH and MANTRAK, JASOC Scientific, Victoria, B.C., Canada; see Browman et al. (2003) for details).

An escape response was easily recognised as the larvae showed a marked increase in swimming speed right after an attack was initiated. Swimming mode also changed from a stop-and-go swimming pattern to continuous swimming and the larvae usually swam in a straight line during the escape response. The following measurements were made for all escape responses: 1) *Distance to pipette*: the distance (mm) from the snout of the larvae (density of neuromast cells, which is likely responsible for sensing the "attack", is highest around the head) to the mouth of the pipette at the start position of the escape response. 2) *Escape latency*: the time (ms) from initiation of suction to the start of the escape response. 3) *Escape distance*: the distance (mm) from where the larva initiated the escape response to where it ended (defined as the point at which the larva's escape velocity declined markedly, although it was not quite completely stationary. 4) *Escape speed*: the swimming speed (mm s^{-1}) calculated from the escape distance. 5) *Initial escape speed*: the swimming speed (mm s^{-1}) calculated during the first 160 ms of the escape response (burst speed). The first 160 ms was used to calculate

burst speed as it included the acceleration component of the escape response and because it also allowed an escape velocity measurement for those larvae that were within the field of view at the beginning of the response but not at the end (33 out of 245 larvae). 6) *Lateral escape angle*: the lateral angle of the escape direction, measured relative to the linear axis of the pipette and independent of laterality (Fig. 1). 7) *Vertical escape angle*: the vertical angle of the escape direction, measured relative to the linear axis of the pipette. Escape angles were measured from -180 – 180° , where downwards escapes were assigned negative values. To test for variation in vertical escape angle, a vertical residual angle was calculated as the angle's deviation from the mean vertical escape angle. Vertical residual angles, and lateral escape angles, were log transformed due to deviation from normality. As the position of the pipette was fixed, and the attack angle relative to the larvae varied, the influence of the relative attack angle was evaluated by addressing 8) *Distance to vertical plane*: the shortest distance (absolute value) from the start position of the larvae to the vertical plane in the centre of the suction plume (*x, y* plane, splitting the plume into a left and right half, Fig. 1). 9) *Distance to central plane*: the shortest distance from the start position of the escape response to the plane in the centre of the suction plume splitting it into an upper and a lower half. Negative values indicate that the larva is below the central plane.

Effects of age and nutritional state on escape performance for both age groups were analysed using nested ANOVA (STATISTICA, StatSoft, 2001), where age group and nutritional group were nested within replicate aquaria (see Table 1). Effect of age on escape angle was

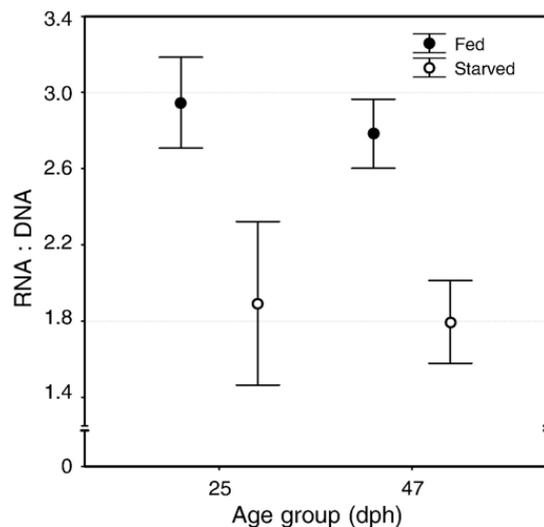


Fig. 2. Mean RNA:DNA ratio (error bars: 95% conf. int.) in fed and 3-D food-deprived Atlantic cod (*Gadus morhua*) larvae.

analysed using nested ANOVA with age nested within replicate. Because of interactions between age group and effects of food deprivation, the effect of distance to central plane, vertical plane, and pipette on the escape angle were analysed for the two groups separately by using ANCOVA. The categorical variable was food deprivation level nested within replicate aquaria while distance to pipette and distance to the central and vertical plane were the continuous variables. Further, to visualize size-related and condition-related effects of starvation, the estimated mean values of the different variables for a virtual larva – three days younger than the tested larvae (corresponding to the time of food deprivation) – are also presented in the figures. These estimates were made by extrapolation from the regression equation of the polynomial fit of the mean values for the two age groups of fed larvae in addition to some preliminary results using 10 dph old larvae (not reported here).

3. Results

The daily growth rate in weight for the larvae between 25 and 47 dph was 9.5%, which was similar to the growth rate observed in the batch from which the larvae was sampled (Skajaa et al., 2003). Nutritional status, indicated by RNA:DNA and logSL:logDW, was significantly lower for food-deprived than for fed larvae in both age groups (25 dph: $t_{38} = -3.81$, $p < 0.001$, $t_{37} = -7.24$, $p < 0.0001$, 47 dph: $t_{38} = -7.30$, $p < 0.0001$, $t_{38} = -8.26$, $p < 0.0001$, Fig. 2).

The distance to the pipette at which escape responses were initiated ranged from 6.6 to 83.6 mm. The amount of time that larvae had spent in the treatment tank had no effect on the trends observed for any of the behaviours studied.

3.1. Escape latency

The mean escape latency for the response to pipette suction was 10.8 and 9.5 ms for fed 25 and 47 day old

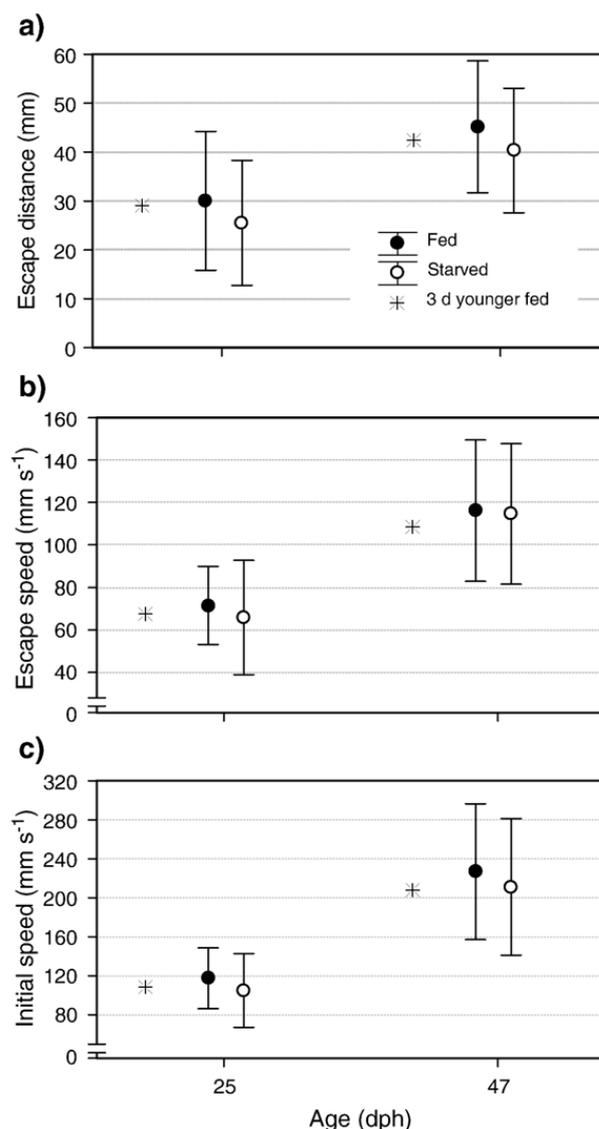


Fig. 3. Mean escape distance (a), escape speed (b), and initial escape speed (c) (error bars: SD) for fed and 3 d food-deprived Atlantic cod (*Gadus morhua*) larvae. Asterisks indicate the estimated escape distance and initial escape speed of a simulated 3 d younger fed larva.

larvae respectively (Table 2). There was no significant effect of either age or food deprivation on mean escape latency. There was a positive relationship between

Table 2

Mean (+/-SD) values for size ($n \sim 20$) and escape reaction variables ($n \sim 39-50$) observed and quantified in fed and 3-D food-deprived Atlantic cod (*Gadus morhua*) larvae

Age group (dph)	25		47	
	Food-deprived	Fed	Food-deprived	Fed
SL (SD) (mm)	7.3 (0.8)	7.7 (2.1)	13.2 (1.1)	13.7 (1.8)
Latency (ms)	10.0 (4.3)	10.8 (3.4)	9.2 (3.8)	9.5 (2.5)
Escape distance (mm)	25.5 (12.8)	30.0 (14.2)	40.3 (12.7)	45.2 (13.5)
Escape speed (mm s ⁻¹)	65.8 (26.9)	71.5 (18.3)	114.6 (33.2)	116.1 (33.8)
Initiating speed (mm s ⁻¹)	105.2 (37.8)	117.5 (31.2)	211.3 (70.0)	226.2 (69.5)

escape latency and distance to pipette ($r^2=0.318$, $p<0.0001$).

3.2. Escape distance

The mean escape distance was significantly higher for the 47 dph age group compared to the 25 dph larvae ($F_{2, 145}=23.75$, $p<0.0001$, Fig. 3). There was no significant difference between nutritional groups. The distance to pipette when the attack was initiated did not affect the escape distance of the larvae. The escape distances for the 3 d food-deprived larvae were comparable to the estimated value for 3 d younger larvae (Fig. 3).

3.3. Escape speed

Escape speed and initial escape speed were strongly correlated ($r^2=0.94$, $p<0.0001$) and there was a positive linear relationship between both of these variables and age ($F_{2, 145}=53.41$, $p<0.0001$ and $F_{2, 179}=85.32$, $p<0.0001$ respectively) (Fig. 3).

The mean initial escape speeds for fed larvae were 117.5 and 226.2 mm s⁻¹ for the 25 and 47 dph age group, respectively (15.3 and 16.5 body lengths s⁻¹). Variability in escape speed increased with age, to an extent greater than what was expected from the increase in the variability in size (Table 1). There was no significant difference between nutritional groups, and the distance to pipette did not affect either escape speed or initial escape speed. There was a significant effect of distance to the vertical plane on both speeds ($r^2=0.076$, $p<0.01$ and $r^2=0.087$, $p<0.001$, respectively) — the escape speed was higher when the larvae were positioned closer to the central line within the suction plume. The initial escape speeds in the 3 d food-deprived larvae were comparable to the escape speed estimated for 3 d younger larvae (Fig. 3).

3.4. Escape angles

There were no differences in either lateral or vertical escape angles, nor vertical residual escape angle, between the age groups. The lateral escape angle was

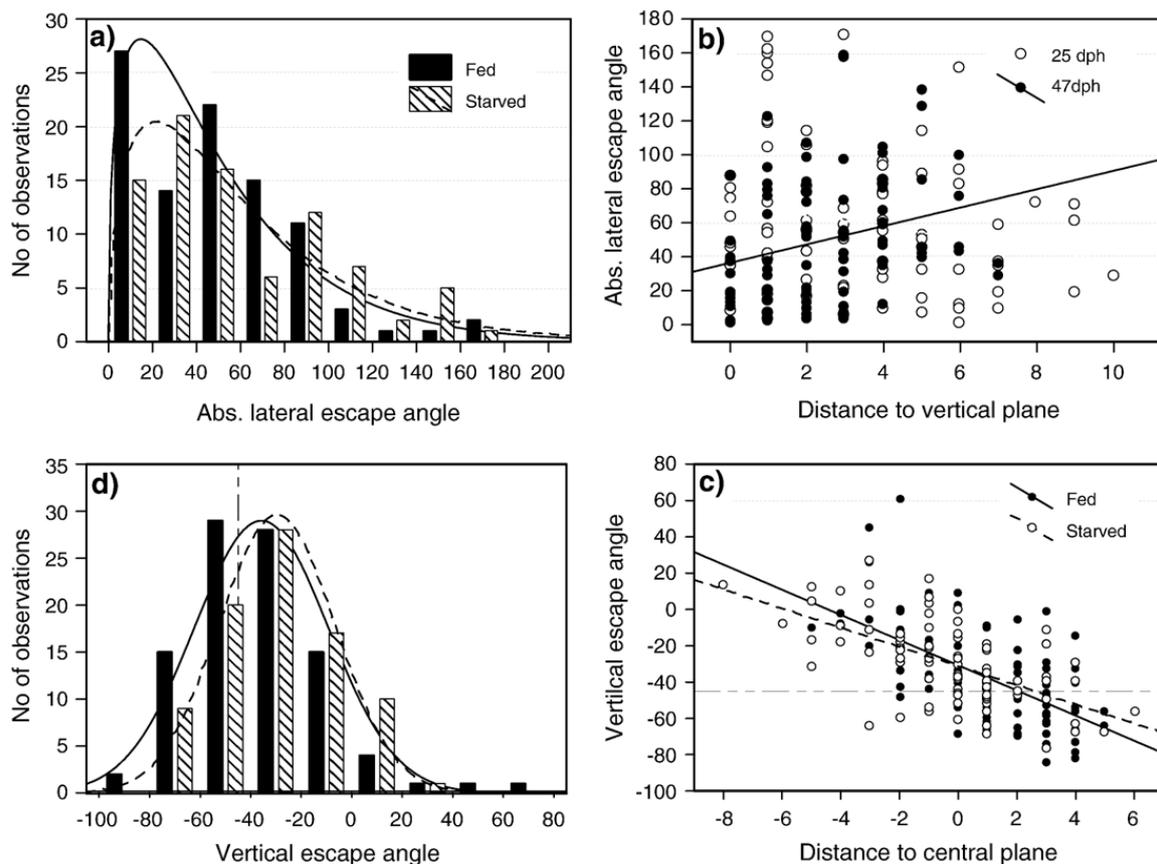


Fig. 4. Frequency distribution and correlation of escape angles of fed and 3 d food-deprived Atlantic cod (*Gadus morhua*) larvae where the attack direction is set to 0°. Shows lateral absolute escape angle (a), and correlation between that and distance to vertical plane for 47 dph larvae (b), vertical escape angle (c), and correlation between that and distance to central plane (d). Double dotted gridline (in c and d) indicates where the escape direction of larvae is horizontal. Gamma distribution line (a), normal distribution line (c), and linear regression lines (d) are shown for fed and food-deprived larvae respectively.

highly variable, ranging from 0 to 170°, and was symmetrically distributed on both sides of the pipette (Fig. 4a). Most larvae escaped at angles different from the attack angle, and the mean lateral escape angle relative to the angle of the pipette was $54.9^\circ \pm 39.9^\circ$ (SD). In the oldest age group, the lateral angle depended upon distance to the vertical plane ($r^2=0.13$, $p<0.05$, Fig. 4b); a larger lateral escape angle was observed when the larva was positioned further away from the vertical plane. In neither of the age groups were the absolute lateral escape angles affected by the distance to the pipette.

The positions of the reacting larvae were symmetrically distributed above and below the central plane at the moment that the simulated attack was initiated. The vertical escape angle ranged from -84.2° to 60.4° (Fig. 4d). The mean vertical escape angle was $-33.6^\circ \pm 24.8^\circ$ (SD); since the angle of the pipette was 45° , the escape angle relative to the horizon was generally directed at a slight upward angle of 13.6° . In both age groups, the vertical escape angle depended upon the distance to the central plane (25 dph: $r^2=0.45$, $p<0.0001$, 47 dph: $r^2=0.33$, $p<0.0001$, Fig. 4c): a larva above the central plane more likely escaped downwards compared to a larva below the central plane. When correcting for the larva's distance to the central plane at the moment that the attack was initiated (thereby removing the effect of a varying attack angle), the vertical escape angle varied by approximately 80° (Fig. 4c). In the 25 dph old larvae the vertical escape angle was significantly lower in the food-deprived larvae compared to the fed larvae ($F_{2, 81}=3.59$, $p<0.05$).

3.5. Captured larvae

Sixteen larvae were caught by the pipette (seven, seven and two from the 10, 25 and 47 dph age groups respectively). Seven out of these larvae were food-deprived while nine were fed. The longest distance away from the pipette over which a larva was caught was 18 mm, with no difference in this distance for food-deprived vs. fed larvae.

4. Discussion

The RNA:DNA analysis confirmed differences in nutritional status between groups, and that the fed groups were initially in good condition (Grønkjær et al., 1997; Buckley et al., 1999). The RNA:DNA values recorded in the present study were within the range of values reported for cod larvae in mesocosm experiments in which no predators were present but the food level varied (Clemmesen et al., 2003). According to Jordean

and Brown (2003), 50% mortality due to food deprivation in cod larvae occurred after 5 d at 8°C . We consider 3 d of food deprivation to be biologically relevant, and not too short, even though a condition-related effect on behaviour might have been evident if the larvae had been food-deprived for a longer period. The water suction produced by the pipette is a proxy for an attack from a fish predator, but other predator-related stimuli would likely produce similar results (Yin and Blaxter, 1987a).

Despite this overall reduction in condition, escape responses were in general not affected by 3 d of food deprivation at 10°C . Except for a slightly lower vertical escape angle in the 25 dph group, there were no effects of food deprivation on any of the escape performance behaviours. The results suggest that the effects of food deprivation on behaviour acting late in the predation process, that is during evasive behaviour, are minimal or absent and that the escape response is conserved and prioritized even though other metabolic changes related to food deprivation are occurring (as indicated by the changes in RNA:DNA ratios). During the predation sequence, the risk of predation mortality increases rapidly; once an attack is initiated, the risk of getting caught is considerably higher than at the moment of encounter (Godin, 1997). The change in the risk of starving will, however, be minimal on this time scale. After initiation of an attack by a predator, the relative difference in the cost/benefit equation related to the performance of the escape response for food-deprived vs. fed larvae will, therefore, be negligible. Franklin et al. (1996) found that the energy used by herring larvae responding to attacks came from renewable (aerobic) sources, and indicated a low energy cost for single responses since the larvae were not easily fatigued. The results can be interpreted as indicating that food-deprived cod larvae do not apply energy-saving behaviour (condition-related effect) during the escape response, at least not before their energy reserves are depleted. If the fed larvae have grown and developed differentially (relative to those that were food-deprived), a size-related effect would, however, be expected. In the present study, such a size-related effect (indicated by the repeatedly lower mean values observed in food-deprived larvae) would have been small and, given the high variation in the data, its biological significance is uncertain.

Condition-related starvation-induced changes in behaviour occurring late in the predation process have been reported as changes in response rate (Neilson et al., 1986; Yin and Blaxter, 1987a; Booman et al., 1991; Chick and Van den Avyle, 2000; Skajaa et al., 2004). Regarding performance of the escape response, and in

contrast to the results reported here, Yin and Blaxter (1987a) found condition-related behavioural changes: the escape speed of starved larvae increased, reached a maximum of one to two days before the PNR, and was inversely related to time of food-deprivation (yolk-sac larvae of herring, cod and flounder, and 36 d and 60 d old herring larvae). It was argued that the increased escape speed (as well as increased responsiveness) was caused by a general increase in activity level observed during initial food deprivation (Yin and Blaxter, 1987b; Munk, 1995). As suggested by Billerbeck et al. (2001), this initial increase in activity with food deprivation could be due to the diminishing escape performance observed after consumption of large meals. In the present experiment, larvae were only food-deprived for 3 d, so changes in behaviour associated with initial and increasing time of food deprivation were not investigated. At the rearing temperature used in our experiments, 3 d of food deprivation would be approximately one to two days before 50% mortality (Jordaan and Brown, 2003), which is the time at which a peak in swimming activity would likely have been expected. As there were no starvation-induced differences in any age groups in the present study, we consider it unlikely that there was an initial increase in response rate.

The increase in escape speed and distance with age observed here is consistent with earlier reports of age-related increases in the effectiveness of escape responses by fish larvae (Miller et al., 1988; Fuiman, 1993). The present study also indicates that at 47 dph, larvae had an increased ability to evaluate the position of the threat and to react more directionally to it compared to the 25 dph larvae as the lateral escape angle depended on distance to the linear axis of the pipette for the older larvae only. Fuiman (1993) reported a decreased variation in response angle of herring larvae with increasing age. A bimodal distribution of lateral escape angles (20–40° and 160–180°) was present in small herring larvae, while in older larvae shallower angles predominated. A decrease in escape latency with age was also observed, and it was suggested that the larger larvae showed improved responses to predator attacks as a result of sensory development (Fuiman 1993). No decrease in escape latency was observed in the present experiment, although this could be due to low sample size and high variability ($p=0.087$).

The initial escape speeds and distances observed in the current experiment are similar to those reported in cod and other fish larvae at comparable temperatures (Yin and Blaxter, 1987a; Miller et al., 1988; Williams et al., 1996). Previous reports of escape direction in fish larvae are all based upon two-dimensional observations,

and in most cases the data have been categorized as escapes towards or away from the predatory threat. Most commonly during fast-start escape responses in fish, the prey's flight path tends to be away from the source of the predatory stimulus, along the horizontal plane (Godin, 1997). In the present study, there was no difference in lateral escape angle between fed and food-deprived larvae, indicating that the food-deprived larvae were as consistent (or inconsistent) in the directionality of their escape responses as were fed larvae. Yin and Blaxter (1987a) reported no difference in lateral escape angle with food deprivation for newly hatched larvae of herring, cod and flounder. In the present experiment, the vertical escape angles of the food-deprived 25 dph larvae were lower than those of fed larvae, but the biological significance of this difference is uncertain.

The lateral escape angles found in the current experiment were highly variable and normally distributed. This supports the hypothesis that the best escape tactic is to randomly vary the escape direction (Godin, 1997). In juvenile and adult fish, escape angles vary greatly, but the preferred angle is between 50° and 0° (Domenici and Blake, 1993; Domenici and Batty, 1994; Meager et al., 2006). In larval herring, Fuiman (1993) reported a peak in lateral escape angles at 20–40° and 160–180°, with the larger angles only observed in smaller herring larvae (<15 mm). In the current experiment, most of the escape paths were directed away from the threat, and the mean lateral escape angle was 55°.

The vertical escape angles were also normally distributed and highly variable. The variability in the vertical escape angle – –40° to 105° relative to the horizon – is consistent with the argument that the best tactic to escape a predator is to randomly vary in the escape direction. The mean vertical escape angle relative to the horizon was 12°, but as this likely depends on the angle of the pipette, this mean may not be indicative of a natural response. The vertical escape angle depended on the position of the larvae within the plume at the initiation of the attack, which reflected inconsistency regarding the directionality of escapes. When the larva was positioned above the central plane at the initiation of the attack, its mean escape path was directed relatively more downwards compared to when the larvae were below the central plane. Downwards escape paths in larvae may be favoured when attacked from beneath because the axial musculature and a rigid body in most fish predators suggest that rapid changes in vertical angle may be limited compared to lateral turning.

The vulnerability of fish larvae to predation is related to size and growth rate (Folkvord and Hunter, 1986; Bailey and Houde, 1989; Houde, 1997). This relationship

is either dome-shaped or decreasing with increasing larval size, depending upon the predator involved (Cowan et al., 1996). One component influencing a larva's vulnerability to predation mortality is the escape success rate and, in this context, the performance of the escape response is significant (Fuiman and Magurran, 1994; Scharf et al., 2003). However, the relative importance of the escape response compared to other factors (encounter and attack rate) depends largely upon the predator involved. If the predator is relatively small, the outcome of a predatory attack depends comparatively more on the performance of the escape response than if the predator is bigger and more efficient (most larvae are caught regardless of the escape performance). The decreased growth rate associated with starvation will extend the duration of vulnerable ontogenetic stages thereby affecting where the larva is along the age–size axis of the predation vulnerability curve. The increased effectiveness in escape performance with increasing age suggested in the present experiment confirms that a decrease in growth rate due to food deprivation will extend the duration of the period during which larvae show less efficient escape manoeuvres. However, no behavioural effects caused by the larvae's deteriorating condition were evident in any of the escape performance behaviours investigated. This may reflect a negligible difference in the cost/benefit relationship between fed and food-deprived larvae in performing an escape response. In the context of parameterizing IBM's, condition-related effects of food deprivation on behaviours acting late in the predation sequence are, therefore, less likely than at earlier stages in the predation sequence. More research is needed to characterize the threshold condition levels at which behavioural effects on escape performance become evident, and to determine whether the stimuli associated with different types of predator attacks (vision, touch, sound, smell) differentially affect the escape response in food-deprived larvae.

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