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Vertical distribution of larval cod (*Gadus morhua*) in experimental temperature gradients

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ABSTRACT

Behavioural responses to gradients of temperature and light in the pelagic can potentially regulate the distribution and survival of early life stages of fish. Vertical temperature gradients (strong, mild and no thermocline, range 4–8 °C) were established in transparent experimental plastic bags (15 cm diameter and 1 m depth) to investigate changes in vertical distribution of larval cod in response to temperature and light conditions. The vertical position of the larvae (10 cm intervals) was recorded at three different developmental stages: after yolk absorption (10 days post hatch, dph), at established feeding (26 dph) and metamorphosing larvae (47 dph). Observations were first made after 2 h in light, and then after 2 h in darkness the upper and lower part of the bag was sampled by pursing the plastic bag at the level of the thermocline. Additional experiments with reverse light settings were done on consecutive days. At 10 dph larval cod were all found in the upper few cm of the water column regardless of temperature or light settings. At 26 dph larvae were generally found deeper in the water column, but with a larger variation in response to light exposure. In darkness fewer larvae was found in the colder water, depending on the strength of the thermocline. At 47 dph the cod actively avoided the coldest water in the light. However, these patterns disappeared in the dark. Larvae distribution did not change with reversed light settings at any of the developmental stages. Larvae in the upper part of the column were significantly heavier throughout the experiment. Our results indicate an ontogenetic change in the response to a thermal gradient through the first two months after hatching, as well as a complex response to different light settings at later stages.

1. Introduction

Over large horizontal scales in the sea, larval fish are often perceived as relatively immobile organisms. However, vertically, physical and biological parameters will change with depth in distances within reach of larval fish by directional swimming (Leis, 2007), and vertical behavior may be seen as a behavioural strategy to mediate drift, growth and predation risk (Fiksen et al., 2007).

The growth potential of larval fish is mainly a function of temperature and size (Folkvord 2005). Positioning according to temperature in the water column may allow larvae to regulate behaviourally their metabolism and growth (Neverman and Wurstbaugh, 1994). Furthermore, vertical position can have great effect on the horizontal distribution of larvae since different depths will often result in different drift trajectories (Vikebø et al., 2005, 2007). Thus, identifying and responding to gradients and different water masses could be of great importance for survival of fish throughout early life stages. Laboratory observations have demonstrated that fish larvae aggregate according to temperature, light and prey (Olla and Davis, 1990; Batty, 1994), while field observations demonstrate how larval fish change their vertical position and aggregate according to physical gradients (e.g. Lough and Potter, 1993; Grønkjær and Wieland, 1997; Munk et al., 1999). However, the ontogeny of these responses remains to be explored in detail (but see Olla et al., 1996).

The drift patterns of cod larvae are studied extensively in modelling exercises (e.g. Hinrichsen et al., 2001; Vikebø et al., 2005; Huret et al., 2007; Opdal et al., 2008). These models typically keep the larvae at fixed depths. However, recent work suggests that changes in vertical distribution are not only of great importance to dispersal (Vikebø et al., 2007), but also to links between predator and prey interactions (Fiksen et al., 2007). Also, vertical profiles of temperature and light play a pivotal role in defining growth and mortality in these models (e.g. Kristiansen et al., 2007, 2009). Clearly, further field and laboratory studies are needed to develop and validate reliable biophysical models for commercially important fish stocks that seek to explore growth, dispersion and, ultimately, survival during the early life history stages (Gallego et al., 2007).

Here, we explore the vertical distribution of cod larvae in experimental vertical temperature gradients. A controlled lab experiment with vertical water columns was used to investigate changes in the vertical distribution of cod larvae at different developmental stages as a function of temperature and light. Larvae were exposed to three

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types of vertical temperature stratifications (strong, mild and no thermocline) just after yolk absorption (10 dph), at established feeding (26 dph) and at the time of metamorphosis (47 dph), both in light and darkness.

2. Material and methods

2.1. Larval rearing

Eggs used in this experiment came from a wild brood stock originating from Møre (62° N). Cod spawned in 32 m^2 spawning tanks with between 30 and 50 adult cod (E. Otterlei pers com. Sagafjord AS, Stord) and the eggs were transported to the High Technology Centre in Bergen (HIB), Norway. The eggs were incubated in 80 L conical tanks at 6 °C, and were transferred to rearing tanks (500 L) with an initial density of 20 larvae/L at 50% hatch on the 13th of April 2007, which was defined as 0 days post hatch (dph). From 2 dph larvae were fed natural zooplankton, which were collected with a Hydrotech® Filter at Espegrend Marine Biological station and transported to HIB every other day where they were held in storage tanks for maximum 2 days. Zooplankton were counted and fed out every day around noon (2000–3000 plankton/L), and consisted of large amounts of nauplii and copepods which are ideal food for larval cod (van der Meeren and Næss, 1993). The volume water in the tanks were increased from 300 to 500 L during the course of the experiment. The tanks had stagnant water with aeration, and were siphoned regularly. Oxygen and temperature were measured daily using an oximeter (WTW LF 330). Day length followed the normal photoperiod for 60° N, and temperature was stable around 8 °C throughout the experiment. Ten larvae were sampled weekly from each tank, photographed and frozen individually. The pictures were analyzed using Image J, measuring standard length, and yolk sac volume, while each larva was weighed after being dried for 24 h at 60 °C in a Thermax oven. Due to lack of fish for the last column trial (see below), equal numbers in each replicate (10) of additional fish from a slightly different temperature treatment was used. These fish were initially held at 6 °C until 20 dph, and then held at 8 °C. We justify this by the fact that they at this time had experienced the same temperature for a period of 17 days, during which they approximately had a 25-fold increase in weight, and they were not significantly different in size.

Three replicates of 100 larvae each were monitored for 10 days as non-feeding trials to assess larval quality. These trials showed no significant post yolk absorption mortality, and after 10 days the larvae had completely absorbed their yolk sac. Growth in the rearing tanks was somewhat suboptimal in the last part of the experiment in comparison with work in the same system (Folkvord, 2005) (Based on end sample: 10% day^{-1}).

2.2. Vertical columns

The vertical position of larvae at 10, 26, and 47 dph was observed in vertical columns developed from transparent plastic bags (Fig. 1). The bags were 2 m long with a diameter of 15 cm and were mounted in a metal frame. The height of the water in the bags was 1 m to ensure that the shape of the actual water column was uniform. The lower half of the water column (50 cm) was submerged in a large glass tank ($60 \times 60 \times 100$ cm) which served as a water bath to generate the thermocline and temperature differential between the upper and lower half of the water column. Temperature in the room was held at 8 °C while temperature in the aquariums were held at 4, 6 or 8 °C, to create a strong and a mild thermocline in addition to a control (no thermocline). The columns were illuminated from above with natural spectrum light tubes mounted 120 cm above the water surface. Light levels were relatively uniform in the water column (top: 6.4 $\mu Em^{-2}s^{-1}$ and bottom: 2.6 $\mu Em^{-2}s^{-1}$). These light levels correspond to a depth of

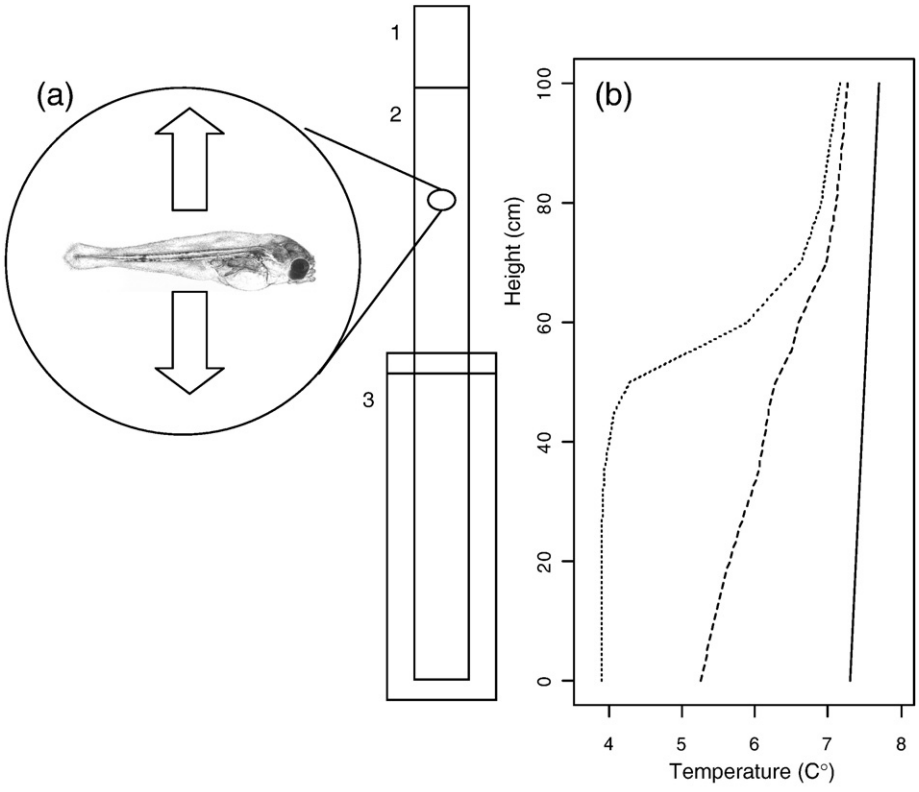

Fig. 1. (a) Simplified illustration of experimental design showing the plastic bag (1), the water level (2) and the level of the thermocline (3), (b) the plot of the characteristics of the thermoclines scaled to the illustration. Illustration of cod larvae by Laura Vollset.

approximately 18–23 m with a surface irradiance of 250 μE $\mu Em^{-2}s^{-1}$ and a light attenuation coefficient of 0.2, typical spring time-values in Norwegian coastal waters. The wall behind the observer was covered with a dark plastic, and the observer wore dark clothes while observing, to minimize light scattering and disturbance. For each trial, fish larvae were sampled from the main rearing tank by focal sampling with a ladle. Larval fish were acclimatized in the observation room for at least 2 h before observations. Observations in columns started at approximately 10 am for the first 4 replicates and 5 pm for the remaining replicates.

2.3. Experimental setup

For each trial 24 fish were inserted into each water column from above. At 10, 20 and 120 min after transfer the vertical distribution of the larvae in the column was measured by placing a centimetre scale along side the column and reading the position of each larva. Then the light was turned off and after 2 h the bag was pursed at the level of the thermocline. The light was then turned on and the upper and lower sections of the water column were filtered separately to retrieve the larvae. The fish larvae from each section were counted and preserved in 96% ethanol for later examination. At day 10 and 26 dph the control and strong thermocline treatments were replicated six times and the weak thermocline treatments were replicated four times. Due to technical issues however, two replicates of the control and strong thermocline treatments were lost, resulting in a total of 14 replicated units available for analysis. The last trial was conducted over a period of 3 days (46–48 dph) to give a total of 16 replicates of each treatment in a period when cod are likely to experience stratified water in the sea.

To separate the effect of light from the effect of temperature and geotaxis, additional trials were conducted on day 11, 28 and 48 dph, with the lower part of the water column illuminated from the side while the upper part was covered with black plastic. The larvae were held in the water columns for two hours before sampling the water columns as described above. Light levels in this experiment were lower to create a gradient of light from below (top: 0.04 $\mu Em^{-2}s^{-1}$ and bottom: 0.60 $\mu Em^{-2}s^{-1}$). This light regime was replicated four times at control and strong thermocline conditions.

At 10 dph all larvae not observed in the water column were considered to be floating at the surface because light refraction at the surface made it difficult to observe all individuals from below. This assumption was confirmed by counting surface oriented larvae from above in four replicates. All observations were done by the same person throughout the experiment.

At the beginning of each trial, the water temperature was measured at the surface, at the thermocline and at the bottom in each column by submerging a temperature probe (WTW Conductivity Meter LF 330) slowly into the column. This was done at least 2 h before the trials so that the thermocline would stabilize. Preliminary studies showed that it would take less than 1 h to completely stabilize the thermocline if the temperature of the water added to the bag was initially 8 °C. In addition, once during the experiment a temperature probe was lowered through the water column, pausing every 5 cm for 2 min to map the shape of thermoclines. Salinity was stable throughout the experiment with a mean of 32.2 ± 0.4 ppt. All persevered larvae from the columns were rinsed in fresh water and subsequently dried in a Thermax oven at 60 °C. A total of 2496 larvae were used in the experiment.

A sharp thermocline was created in the strong treatments while a weaker thermocline was created in the mild treatment (Fig. 2). The mild thermocline was somewhat variable in temperature, but the slope of temperature was stable throughout the experiment (approx. 1.6 °C over 100 cm). Furthermore, a minor gradient occurred in the no thermocline treatments (less than 0.6 °C over 100 cm). The mild and no thermocline treatments were somewhat colder at 26 dph than at the other sampling days and comparison should thus be interpreted with care.

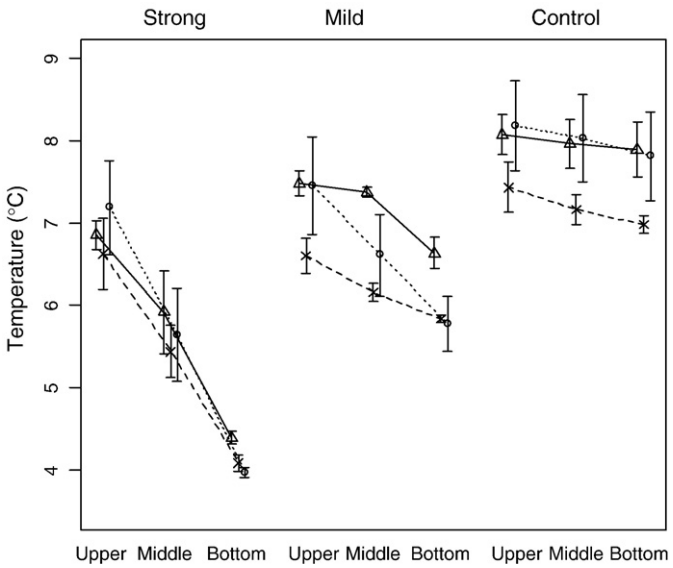

Fig. 2. Temperature in vertical columns in the upper and lower sections, and at the thermocline (middle), at 10 dph (solid lines), 26 dph (dashed lines) and 47 dph (dotted lines). The data are represented for each of the temperature treatments strong, mild and control respectively.

3. Data analysis

All data analysis and graphical presentations were done in R 2.2.1©. The effect of thermocline in light and darkness was tested separately using a Generalized Linear Model (GLM) with the number of larvae in the lower 50 cm of the column as the response. A quasibinomial family function was applied due to over-dispersion. To correct for multiple tests on the same replicated trials a Bonferroni correction was applied. There were minor differences in the vertical distribution 10, 20 and 120 min after introduction of the larvae, and the data presented for light will hereafter be distribution after 120 min. All observational replicates where more than eight fish were unaccounted for, or had more than 26 fish (due to counting error) were removed from the analysis to avoid biased data (10 replicates were removed from a total of 100). All error bars in the graphs represent 95% confidence intervals based on bootstrapping.

A size- and temperature dependent growth model (Folkvord, 2005) was applied to analyse differences in growth in different temperature treatments. Since all larvae from the columns were preserved in alcohol, a conversion between actual dry weight and dry weight from larvae preserved in alcohol was set to a simple 1:0.8 (MacKenzie et al., 1990), to get a more realistic growth estimate from the model. Although larger larvae, with a higher fat content, will be more affected by alcohol preservation, the weight correction and growth remain suitable means of estimating growth in the different age groups.

4. Results

4.1. Vertical distribution

4.1.1. After yolk absorption (10 dph)

Almost all the larvae were found in the upper water film at 10 dph in all light treatments, and we found no significant differences between thermocline treatments in light or dark treatments (GLM, $p > 0.05$) (Figs. 3 and 4). We found no indications that larvae moved deeper when light was placed under the thermocline (Fig. 5).

4.1.2. Established feeding (26 dph)

At 26 dph larvae moved deeper, but did not actively avoid the thermocline in light (Figs. 3 and 4). No clear pattern emerged from the

behavior of the larvae, as they were sometimes in patches, and sometimes evenly distributed throughout the column. In darkness, however, a significant difference between temperature gradients was found (GLM, ANOVA, $p<0.025$), with less larvae in the lower part in the mild and strong thermocline columns (Fig. 5). This was also the case when light was placed under the columns (GLM, ANOVA, $p<0.025$) (Fig. 5).

4.1.3. Metamorphosing larvae (47 dph)

At 47 dph larvae avoided the thermocline in light (GLM, ANOVA, $p<0.025$) (Figs. 4 and 5). There was a clear relationship with the temperature and number of larvae in the lower part of the column, with fewer larvae in colder water. There were also indications of avoidance of the lower part of the columns in the controls. Whether this was an artefact of the light settings or the small temperature

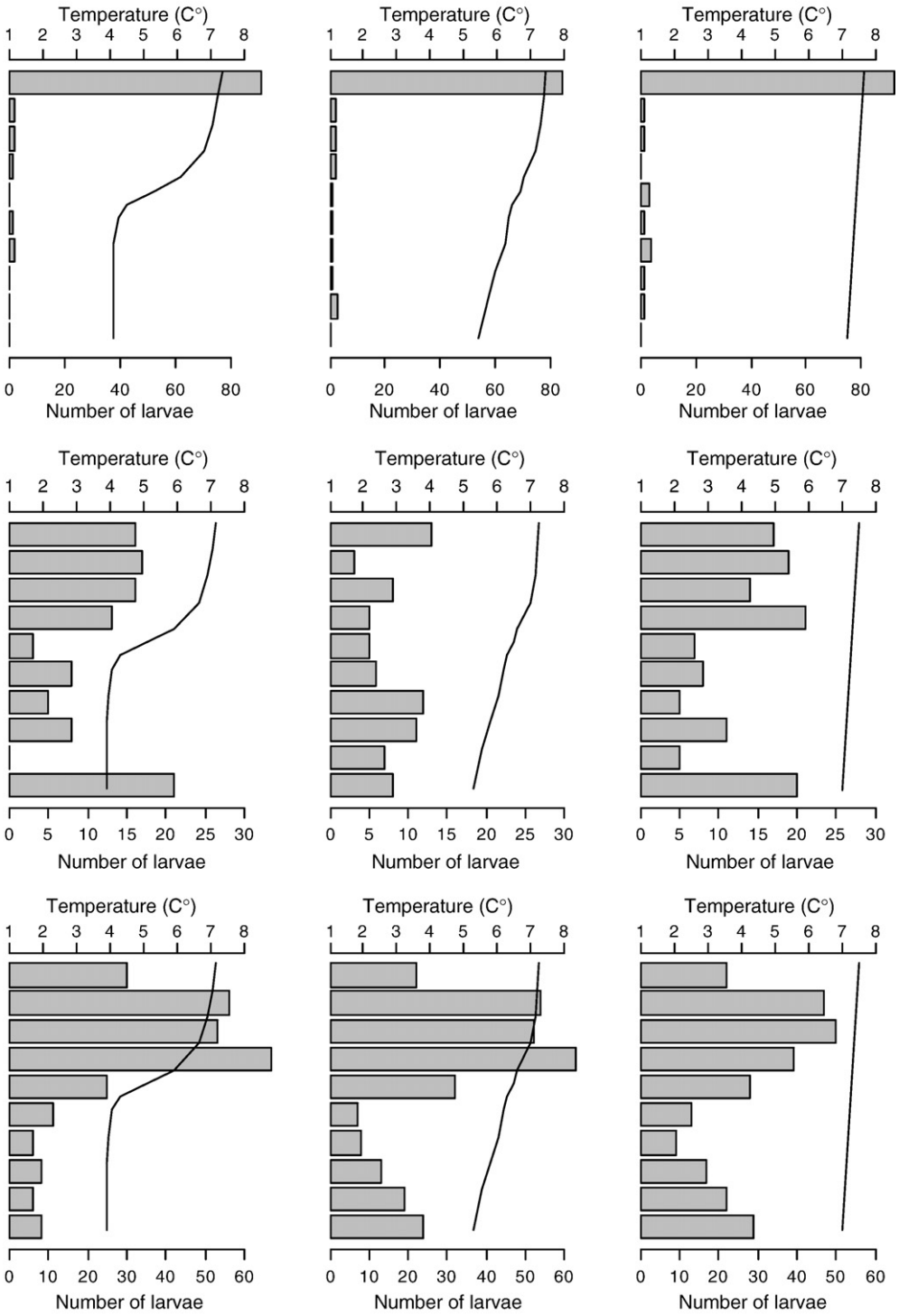

Fig. 3. Histogram with 10 cm vertical distance/depth bins of overall distribution of larvae in the three different temperature treatments at 10 DPH (three upper panels), 26 DPH (three middle panels), 47 DPH (three lower panels) in light. Data are pooled observations from all replicates for the respective day and treatment. Solid lines represent the temperature in the different treatments.

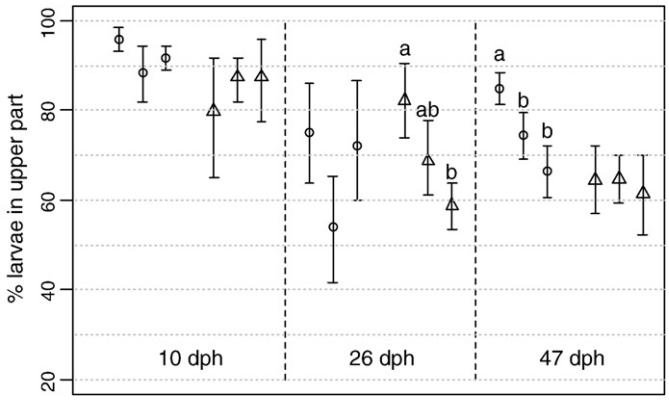

Fig. 4. Plot of percent larvae above the thermocline in light (circles) and the following dark (triangles) treatment for 10, 26 and 47 dph. For each light treatment within each day the data are represented for the strong, mild and no-thermocline treatment from left to right. Bars represent 95% confidence intervals. Different letters indicate significantly different treatments within day and light settings.

gradient in the controls is hard to determine, but the clear pattern of avoidance of the lower part of the column in thermoclines indicates that temperature had a clear affect on the distributional patterns at this stage. In darkness this pattern disappeared (GLM, ANOVA, $p > 0.05$), however. With light from below larvae distributed higher in the strong thermocline but this tendency was not significant (GLM, $p > 0.05$), and larvae was generally higher in the water column when compared to the main trials.

4.2. Size and growth aspects

Larvae in the upper part of the columns were 4.1, 8.8 and 13.2% heavier than larvae in the lower part of the column at 10, 26 and 47 dph, respectively (ANOVA, $p < 0.05$). There was no threshold body weight for which the larvae would position themselves in the upper part of the column, but rather a general trend within each column that heavier larvae would stay in the upper part.

Specific growth predicted from the STDG model (Folkvord, 2005) using the temperature exposure of larvae suggest that the expected growth-difference above and below the thermocline would be highest for metamorphosing larvae in our experiment (Table 1). These larvae were closer to the maximum growth potential (Folkvord, 2005), and experienced the largest temperature difference in the column. However, inherent difference in growth potential was actually larger for larvae at established feeding, when standardizing weight (mean) and temperature.

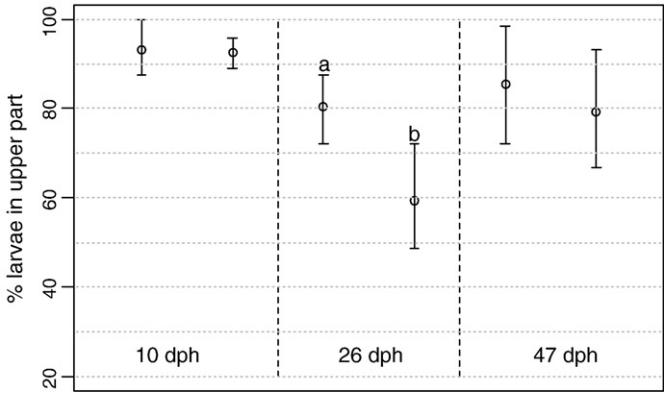

Fig. 5. Plot of percent larvae above the thermocline with light from below, for 10, 26 and 47 dph. For each light treatment within each day the data are represented for the strong, and no-thermocline treatment from left to right. Bars represent 95% confidence intervals. Different letters display significant different treatments within day and light settings.

Table 1
Estimated difference in SGR for larvae located in the upper and lower sections of the water column (upper–lower = difference) for the strong, weak and non-stratified treatments for each sampling day based on Folkvord (2005) STDG model.

	Yolk absorption	Established feeding	Metamorphosing
Strong	9.8–6.4 = 3.4	12.2–8.4 = 3.8	12.5–7.6 = 4.9
Mild	10.6–9.1 = 1.4	12.6–11.4 = 1.2	13.0–10.6 = 2.4
Non-stratified	11.5–9.8 = 1.7	14.2–13.2 = 1.0	14.0–13.8 = 0.2
Strong	8.7–5.5 = 3.2	13.0–7.9 = 5.1	12.7–7.8 = 4.9
Mild	8.8–7.7 = 1.1	13.0–11.4 = 1.7	12.8–11.2 = 1.7
Non-stratified	9.9–9.9 = 0	14.8–14.8 = 0	14.3–14.3 = 0

Upper part of table is based on actual weight at depth and measured temperature difference, lower part is based on mean weight and equal temperature difference.

5. Discussion

Our results indicate an ontogenetic change in response to a thermal gradient during the first two months after hatching, and also indicate that this response is not purely an additive with different light settings.

5.1. Vertical distribution of after yolk absorption (10 dph)

The youngest larvae tested in our experiment (10 dph) congregated at the surface of the water column, and this result was independent of the light settings. This upward migration coincides with field observations for deep-spawning pelagic fish, whose larvae move upward to depths with light levels more suitable for first feeding (Kendall et al., 1994). Olla et al. (1996) observed that walleye pollock were positively phototactic at first feeding. Karlsen and Mangor-Jensen (2001) also correlated phototaxis of halibut larvae to first feeding. Our observations at 10 dph occurred after initiation of first feeding, but additional observations suggest that the upward movements of larvae were present within hours of hatching. Since neither darkness nor reversed light settings affected the surface oriented distribution observed at early stages, it is likely that cod regulate their vertical distribution partially by geotaxis, as shown for walleye pollock (Davis and Olla, 1994).

5.2. Vertical distribution of feeding larval cod (26 dph)

As the larvae developed and increased in visibility and size (26 dph), their behaviour changed and they were found distributed throughout the water column. The trial replicates at this stage were the most variable, obscuring any general pattern in distribution. It appeared that the larvae did not respond to the presence of a thermocline with colder water at this stage of development. However, when the light was turned off larvae were found in lower numbers in the colder water, and a similar pattern emerged when light settings were reversed. Batty (1994) observed that larval herring would actively swim up from the thermocline in darkness. This is a plausible explanation for cod as well; however, observations in the dark of larval cod exposed to a thermocline need to be done to resolve this question. The pattern observed in darkness (and low light intensities in reversed light settings) compared to in the light suggest a behavioural trade off between light induced behaviour (to increase food or decrease predator encounter) and thermoregulatory behaviour.

5.3. Vertical distribution of metamorphosing larvae

The difference in response observed in the light between larvae at 26 and 47 dph was clear: metamorphosing fish avoided the deeper and colder water of the columns. In the sea cod at this age would experience temperatures gradients of early summer. The larvae at this age range in size between 10 and 18 mm depending on temperature and food availability (Otterlei et al., 1999; Puvanendran et al., 2002),

they are pigmented, and have developed fins and muscle structure (Ellertsen et al., 1980). Their temperature optima shifts to slightly higher temperatures as they grow (Otterlei et al., 1999), and they are likely to be more sensitive and avoid colder water.

Helle and Pennington (1999) observed that the distribution of early juveniles (2–3 months) in the Barents Sea was correlated with zooplankton abundance, and that areas with cod had a higher temperature (average 7.64 °C) than areas without cod (average 6.91 °C). They suggested that increased probability of survival in areas with food and currents bringing them there by chance as possible explanations. Our results indicate that the cod before this stage can make behavioural choices according to temperature.

More larvae were found deeper and colder in the strong and mild thermocline treatments in the dark. This was a surprising observation, but may reflect the benefit of reduced metabolism in darkness to conserve energy. Neverman and Wurstbaugh (1994) observed that juvenile sculpin (*Cottus extensus*) (>30 mm) migrate into warmer waters to increase their digestion rate. The costs and benefits of vertical positioning to manipulated temperature exposure depend on night length, digestion rate and gut fullness. Olla and Sogard and Olla (1996) demonstrated that behaviour of larger juvenile walleye pollock (*Theragra chalcogramma*) can be predicted by a bioenergetic hypothesis (e.g. Brett, 1971). Whether larval cod also regulate their position with respect to temperature gradients, and thus regulate metabolism, based upon their gut fullness and condition, cannot be answered by our observations. However, there were indications of size differences between larvae in the upper and lower part of the water column, which might reflect a difference in condition and/or stomach fullness.

5.4. Growth advantage due to temperature choice

The maximum difference in growth between individuals in the lower and upper part of the vertical column was calculated for metamorphosing larvae from the STDG model (Folkvord, 2005). Predicted rates showed an increase in growth of up to 4.9% in the strong thermocline. Metamorphosing larvae were more affected by the temperature difference than the younger larvae, and a more pronounced behavioural response to temperature is expected (Kristiansen et al., 2009). However, inherent growth differences were greater for larvae at 26 dph when the data were standardized for size and temperature. Optimal temperature is a function of feeding conditions for juvenile and older stages of various species (Brett, 1971). Feeding conditions throughout our experiment were maximized to ensure good growth and condition. Thus, given a choice of ambient temperatures, we would expect larvae to position themselves in higher temperature to optimize growth.

5.5. Mechanisms underlying vertical distributions

Theoretically a fish can always move towards optimal depth for either growth or survival (Kristiansen et al., 2007). However, this also means that it must have knowledge not only about the conditions in the depth were it resides, but also of other depths. This is the question of information for theoretical optimality frameworks like the ideal free distribution (Fortier and Harris, 1989). Simple mechanisms such as phototaxis (Karlsen and Mangor-Jensen, 2001) or chemo-kinesis (Døving et al., 1994), may be important behavioural responses for larval fish that are limited by swimming ability and Reynolds number. In our experiment, such simple rules would lead to aggregations at one end of the column if our parameters were outside the optimal range of conditions for the larvae, and could perhaps explain the surface bound aggregation of the early stages.

Physical and biological conditions in the pelagic zone often form predictable vertical gradients that facilitate information about conditions at other depths. Our observations are “snapshots” of vertical distribution in time and do not reveal detailed information about individual behaviour or the mechanisms behind it. Further study is required in order to determine whether or not a fish larvae can sense what the temperature is, and move up or down accordingly, in order to locate a preferred temperature, perhaps further regulated by potential growth. Distributional patterns in temperature gradients at later stages — when buoyancy has less impact on vertical distribution relative to swimming ability — indicate that cod at this stage may regulate their temperature exposure behaviourally.

Buoyancy may influence vertical distribution at the very early larval stages. The main factors affecting the buoyancy of fish larvae are first the yolk sac and later in development the inflation of the swim bladder (e.g. Govoni and Hoss, 2001). In addition, the nutritional condition of the larvae can affect larval buoyancy (Ellertsen et al., 1980; Sclafani et al., 1993). In our experiment buoyancy was potentially very important since the height of the column accentuated the effects of small vertical displacements due to buoyancy differences. Larvae at 10 dph are neutrally buoyant at 32–33 ppt (Ellertsen et al., 1980), which is around the salinity measured in our experiment (32.24 ± 0.38). Sclafani et al. (1993) predicted that buoyancy could explain daily vertical migration at early life stages, with the assumption that larvae do not move at night and therefore sink. Based on their model the larval cod at 10 dph in our experiment should have a vertical velocity (w) of -3.3×10^{-3} m s^{-1} under our conditions, which means that the first feeding larvae would be negatively buoyant and should sink in our columns. Buoyancy can also be affected by incubation salinity (Nissling and Vallin, 1996), but this was unlikely to be a factor in our experiment, since salinity was the same during incubation, rearing, and the trials.

5.6. Experimental setup

Our system of submerged plastic bags was easy to handle, and has its main advantage in comparison with other experimental columns (e.g. Oulette and Allard, 2006; Ruzicka and Gallager, 2006) that it is easy to replicate. The system worked well for strongly pigmented larval stages, but had some disadvantages and potential bias at early stages, where not all larvae could be accounted for. The air to water refraction on the circular column made the larvae difficult to observe. However, this was not a problem in the lower part since this was submerged in an aquarium, and was one of the arguments for using the counts of the lower half of the column as the response variable in our statistics.

Our experimental setup was not designed to test the thermal physiological optima and limits (Somero, 2002), since we used temperatures well below the temperature growth optima for all stages (Otterlei et al., 1999). The rational behind this was that we wanted the larvae to have a choice between ambient temperatures that is within the range that they experience in the sea. From an aquaculture point of view it could be interesting to repeat the experiment with higher temperatures to see if the larvae would choose temperatures according to their maximum growth. This is important knowledge both for further optimizing rearing protocols and a more ethical welfare point of view. Our results are in line with other studies on temperature choice which indicates that larval fish in general choose higher temperature water layers when given a choice (Batty, 1994; Olla et al., 1996).

5.7. Relevance to the field

Our results are purely qualitative measurements of response to thermal gradients and light settings at different developmental stages, and as pointed out by Leis (2007), a direct comparison of laboratory behaviour to the field is not realistic. However, qualitatively identifying ontogenetic differences to gradients in the lab can be useful to discard or build up support for ecological hypotheses and models that try to identify important biological and physical factors that affect dispersion. It can, for example, be used to setup and test ecological hypothesis regarding if and when inner drivers (e.g. physiological)

(Gallego et al., 2007) can potentially affect a fish larva's response to important physical (vertical) gradients.

5.8. Concluding remarks

This work has shown that the vertical behaviour of larval cod with respect to temperature and light changes with ontogeny. An early surface oriented behaviour changes to a deeper distribution with age, and there are indications of differential responses to temperature with different light settings and ages. Our main results were the finding of response to thermoclines in darkness for feeding larvae and light for metamorphosing larvae. Further work should focus on the internal (e.g. physiological status) and external (e.g. predation risk and behavioural interactions) drivers of larval fish behavioural responses to physical gradients.

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