Vol. 475: 191–201, 2013
doi: 10.3354/meps10129

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published February 14

Effect of food deprivation on distribution of larval and early juvenile cod in experimental vertical temperature and light gradients

K. W. Vollset[1,4,*], I. A. Catalán[2], Ø. Fiksen[1], A. Folkvord[1,3]

[1]Department of Biology, University of Bergen, 5020 Bergen, Norway
[2]Department of Ecology and Marine Resources, Mediterranean Institute for Advanced Studies (IMEDEA-CSIC/UIB), C/ Miquel Marques 21, CP 07190, Esporles, Balearic Islands, Spain
[3]Institute of Marine Research, Nordnes, 5817 Bergen, Norway
[4]*Present address:* Laboratory of Freshwater Ecology and Inland Fisheries (LFI), Uni Environment, 5006 Bergen, Norway

ABSTRACT: How and when food limits the growth and survival of larval fish are essential questions which link oceanography and fisheries science, and theory suggests that food availability may affect survival through behavioural responses. We tested the effect of food deprivation on the vertical distribution of larval cod *Gadus morhua* in light and temperature gradients during larval and early juvenile stages. Groups of fed (ad lib) and unfed (16 h) larval cod at 16, 30 and 37 d post hatch (dph) and early juvenile cod at 44 dph were repeatedly observed in experimental columns with temperature gradients (110 × 15 cm, 6 to 8°C) for a period of 3 h while the directionality of the light was manipulated. At earlier stages (16 and 30 dph), larvae were distributed mainly in the upper part of the column regardless of the water stratification. The presence of a thermocline reduced the likelihood of cod being observed in the lower part of the column at 37 and 44 dph, but this response was not affected by feeding history. Unfed larvae distributed deeper in the column than fed larvae, but this effect was small (on average, 4.3 cm difference in a 115 cm column). Video analysis of individual swimming behaviour at 44 dph indicated that, while unfed cod would not stay longer or swim deeper into the columns than fed cod, there was an increased number of fish swimming up and down in the column, suggesting an increased motivation to sample new environments. We conclude that short-term food deprivation (16 h) has a small but significant effect on larval vertical distribution, most likely as a consequence of increased prey-search behaviour, but that temperature and ontogeny were the key factors in determining distribution patterns.

KEY WORDS: Thermoregulatory behaviour · Adaptive behaviour · Feeding history · Larval fish · Vertical columns · *Gadus morhua*

INTRODUCTION

Hunger and food availability have major effects on an individual's behavioural decisions by altering the prospects for growth and the likelihood of starvation and predation mortality (Houston & McNamara 1999). Theoretically, there is an optimal activity level depending on food availability and risk of predation or starvation (McNamara & Houston 1987), often found to depend on energy reserves or feeding history (Houston et al. 1993). Foraging and activity are frequently associated with an increased risk of predation (Lima & Dill 1990, Kiørboe 2011), often modified through habitat selection or activity level. In plankton, this trade-off has been studied extensively through vertical migration behaviour, also considered to be highly dependent on feeding history and condition (e.g. Huntley & Brooks 1982, Pearre 2003).

*Email: knut.vollset@uni.no

For larval fish, vertical positioning has a strong bearing on drift patterns and dispersal, and it is important to understand the capabilities, cues and trade-offs larvae face during their drift phase (Kingford et al. 2002, Fiksen et al. 2007, Vikebø et al. 2007). Thus, larvae are modelled less as passive particles and more as rational actors driven by heuristic rules that maximise growth and minimise predation according to environmental (e.g. temperature and light) and biological cues such as feeding success, gut fullness and perceived predation risk (Kristiansen et al. 2007, 2009, Fiksen & Jørgensen 2011). As biophysical models become more mechanistic and death and growth rates are linked to environmental variables with strong vertical gradients, the models need kernels to determine vertical distribution and migrations.

Two of the most conspicuous vertical cues in the ocean are temperature and light. In larval cod from the Norwegian coast, maximum temperature-dependent growth potential (under unlimited prey conditions) occurs at about 14 to 16°C (Otterlei et al. 1999), conditions rarely experienced by cod larvae in the field. Field surveys suggest that feeding cod larvae are confined almost exclusively to within or above the thermocline in stratified waters (Lough & Potter 1993), and several experiments have demonstrated that well-fed gadoid larvae respond to a weak thermocline by generally choosing the warmer side of it (Olla & Davis 1990, Vollset et al. 2009a). Larval gadoid fish are also known to respond to light, and aggregate on one side of horizontal gradients of light (Colton & Hurst 2010). In vertical gradients, young cod larvae (4 to 25 d post hatch [dph]) distribute deeper in the water column when light levels are increased from darkness to 1250 lux (Skiftesvik 1994). Furthermore, field data suggest that larval gadoids often conduct diel vertical migration (Lough & Potter 1993).

Studies of juvenile fish have indicated that food deprivation provokes both increased prey-search behaviour and energy-saving behaviour (e.g. decreased thermal choice and activity level; Sogard & Olla 1996), with an initial outburst of prey search followed by a decrease in activity level and lower temperature affinity. There is a clear trade-off between these mechanisms, since increased prey search involves higher activity levels. Also in the vertical realm, temperature and prey encounter are often strongly correlated for visual feeders due to thermal stratification and light attenuation. In larval fish, this trade-off is likely to be strongly driven by the need to grow through critical stages (Folkvord 2005). Based on optimality arguments, Fiksen & Jørgensen (2011) concluded that increased prey availability improves survival rather than growth for larval fish, particularly at food levels above a minimum level. Their argumentation assumes that larval cod choose to increase prey search (and consequently increase predation risk) rather than to conserve energy in the face of food deprivation. This is in line with the work by Munk (1995), which demonstrated an increase in activity level with decrease in stomach fullness. This could also explain the lack of correlation between calculated feeding conditions and growth in larval fish from field data (Pepin et al. 2003).

In the present study, we attempted to couple state-dependent behaviour in cod larvae and early juveniles with the 2 most important vertical cues in the environment: temperature and light. In this context, larval state is defined as physiological condition determined by stomach fullness. A system of experimental vertical columns developed earlier (Vollset et al. 2009a) was used to test whether larval cod alter their response to temperature gradients according to food deprivation. In addition, to assess whether a potential state-dependent vertical behaviour was affected by light level and gradients, the light was periodically projected from above and below. Depth-stratified sampling of individuals at the end of each trial also enabled us to test whether the vertical distribution was independent of relative size at a given age or stage.

MATERIALS AND METHODS

Larval rearing

Eggs used in this experiment came from a wild-caught brood stock of cod *Gadus morhua* originating from Møre, Norway (62° 50′ N, 6° 00′ E), and was the same as used in Vollset et al. (2009a). The eggs were transported to the High Technology Centre in Bergen (HiB), Norway, where they were held in 80 l incubators at 6°C, and transferred to 3 rearing tanks (500 l) with an initial density of 6 ind. l^{-1} at 50% hatch, which was defined as Day 0 post hatch (dph). The rearing system has already been documented in a number of earlier publications (e.g. Otterlei et al. 1999), and we only briefly describe it here. Temperature was kept between 8 and 9°C throughout the experimental period. The larvae were fed live wild zooplankton collected from a Hydrotech® filter that concentrated zooplankton from seawater pumped up from 8 m depth in a fjord. Concentration and zooplankton size were altered throughout the experiment to match the larval ad lib requirements as explained in Vollset et al. (2009b). To assess the quality and performance of the offspring used in this experiment, a group of 30 larval fish was randomly sampled weekly for standard length (SL, mm, precision: 0.1 mm, ImageJ v. 1.41) and dry weight measurements (DW, µg, precision: 1 µg) after 24 h at 60°C in a Thermax® oven. Overall growth and survival in the rearing tanks were similar to earlier experiments from the same laboratory (Folkvord 2005). Larval groups did not show signs of pre-yolk absorption mortality, also indicating that viable larval groups were used.

Overall experimental design

The experimental treatments for the vertical distribution data (Table 1) consisted of (1) fish age (16, 30, 37 and 44 dph), (2) column stratification (thermocline [T] and isothermal column [IS]), and (3) recent feeding history (fed [F] and unfed [UF]). These trials, with corresponding treatments, were carried out at 2 light settings (light from above [A], and light from below [B]). As in a parallel study on herring larvae (Catalán et al. 2011), each feeding treatment was replicated 8 times in T columns and 4 times in IS columns at each of the 4 larval ages. Two columns from both 16 and 44 dph were not available for analysis, yielding a total of 92 columns used in the experiment.

The youngest age group (16 dph) corresponded to the end of the first feeding stage, where non-feeding larvae would die of starvation (Ellertsen et al. 1980). At 30 dph, individuals were in the late larval stage, while at 37 dph, average size was close to 12 mm, corresponding to the onset of metamorphosis (Otterlei et al. 1999). By 44 dph, most of the cod had lost the larval fin fold and had visible dorsal and ventral fin structures, indicative of the early juvenile stage (Otterlei et al. 1999).

Experimental columns and thermocline treatment

Response studies of cod larvae in vertical gradients of light and temperature were carried out in 2.2 m long transparent plastic bags (hereafter termed columns) hung from a metal frame. The columns were filled up to 110 cm and submerged into large aquaria (60 × 60 × 100 cm) that functioned as water baths to create a stable thermocline at around 55 cm water depth. The columns were held in a temperature-regulated room that kept the upper parts of the columns and the surrounding air constant at 8°C. In the T treatments, 6°C water was circulated around the lower half of the column in the water bath to create a thermocline, while in the IS treatments, 8°C water was circulated to maintain isothermal columns. The thermal gradients were constantly logged every other second in selected columns, with 1 sensor above and 1 sensor below the thermocline (Tempscan, Comark®), and temperature was stable throughout the experiment. The temperature range to which these fish larvae are normally exposed in the sea (6 to 8°C) was chosen to avoid the unwanted effect of putative sub-lethal thermal stress (Somero 2002). No aeration was provided due to the small larval density, short experimental time and the need to keep stable thermoclines. Salinity was stable throughout the experiment, with a mean of 32.2 ± 0.4.

Table 1. *Gadus morhua*. Experimental design used for analysis of (a) fish distribution and (b) larval dry weight (DW). All experimental columns were subject to the same light settings: from above (setting A) at 0 to 60 min from start, from below (setting B) at 60 to 130 min, and setting A again at 130 to 200 min. Columns were terminated and pursed (upper and lower half separated by squeezing the column at mid-height level) after 200 min and individuals recovered for DW measurements. Measurement times of distribution were after 30, 70, 100, 140 and 170 min. Four replicates were used for the isothermal (IS) columns and 8 for the thermocline (T) columns, half of these from the morning and half from the afternoon. dph: days post hatch, F: fed, UF: unfed

Response variable	Treatment	No. of levels	Levels
(a) Distribution	Age groups	4	16, 30, 37, 44 dph
(% of fish in lower	Thermocline	2	IS, T
half, median depth)	Feeding	2	F, UF
(b) DW	Age groups	4	16, 30, 37, 44 dph
	Thermocline	2	IS, T
	Feeding	2	F, UF
	Column level	2	Upper half, lower half

Feeding treatment

Sixteen hours before each trial, cod were evenly sampled from the 3 replicated rearing tanks and placed into two 40 l aquaria. Here they were split into the 2 feeding treatments UF and F, with no prey and 2000 prey l^{-1}, respectively. The individual response to gut fullness was assumed to be strongest in the period after the stomach was emptied and before starvation caused deterioration in

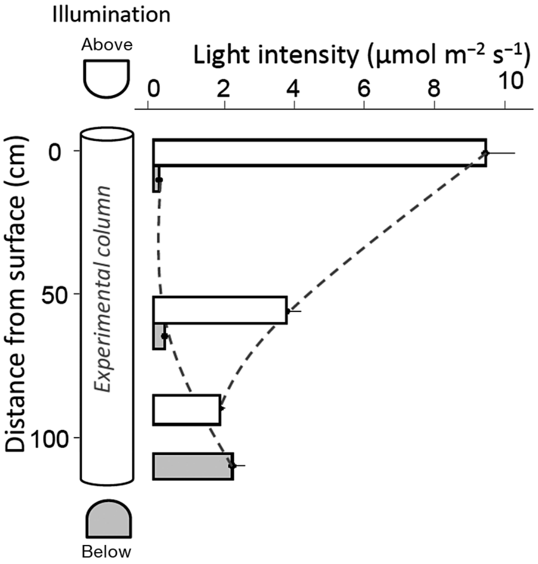

Fig. 1. Mean light intensity (±95% CI) at 3 different depths of the experimental column, under both light regimes: light from below and light from above. Adjacent bars are offset for visual clarity

the physiological state. Thus, 16 h was chosen on the basis that, in the early stages, cod are known to starve within 2 to 3 d (Jordaan & Brown 2003) and empty their stomachs within 6 to 10 h (Ellertsen et al. 1980). The aquaria, located in the same room as the experimental columns, were gently aerated with a small air stone and had approximately the same light conditions as the fish in the rearing tanks. One hour before the trial, a sub-sample of 10 individuals was analysed for stomach contents to validate that the 2 groups F and UF had full and empty stomachs, respectively. No identifiable prey items were observed in the stomachs of unfed specimens, while the number of identified prey items in the stomachs of fed individuals ranged from 4 to 28.

Light settings

Each column had 2 light sources, which made it possible to direct light from above (setting A) or below (setting B) the column with a light switch (Fig. 1). The rationale behind this setup was to reverse the light–temperature gradient, and assess whether the potential response to food deprivation was consistent in both light settings. An inert green dye (Baker Green, 0.22 ml l^{-1}) was added to the water column, yielding a steeper light gradient and diffuse light (average attenuation coefficient: 1.39 m^{-1}). The colour would remain suspended throughout the experiment, and no adverse behaviour in 10× concentration of the dye was observed in preliminary trials.

Procedures and data collection

For each replicate (column), 20 fish were inserted into each unit from the top. The light setting was first set to A. Fish distribution was then observed after 30 min by placing a centimetre scale alongside the column and reading the exact vertical placement of all visible individuals into a Dictaphone. After 60 min, light was then turned to setting B and the distribution read after 70 and 100 min. Light was then turned back to setting A after 130 min and new measurements were made after 140 and 170 min. After the last measurements (at 200 min), the bags were pursed (upper and lower half separated by squeezing the column at mid-height level) at the level of the mid-water level and thermocline, as explained in Vollset et al. (2009a) and Catalán et al. (2011), and the individuals were filtered and counted out from the upper and lower half of the column, respectively. On average, 98.7% of the added fish per column were accounted for (19.7 out of 20). The cod were frozen for subsequent DW measurements as described above ('Larval rearing'). In total, 1586 larval DWs were obtained after excluding individuals that were damaged or broken during pursing or weighing. The weights of the larvae from the upper and lower halves of the individual columns were used to document any depth-related differences in larval weights in the various treatments.

Behavioural analysis

In order to gain knowledge on individual larval behaviour and to explain the vertical distributions observed, 2 video cameras (Panasonic WV BP550) recorded a section of 2 replicated pairs of F and UF columns from each thermocline treatment. Contrasts through the plastic bags were only sharp enough for analysis at 44 dph with light setting A. Each camera recorded a section of approximately 16 × 33 cm of the 2 columns. This was the largest field of vision that could be covered while still being able to observe individual fish. The section was chosen to include the mid-water level of the columns (defined as where the columns were submerged in the water bath) to be able to observe how individuals responded to the presence or absence of a thermocline. Video recordings were digitised and stored at 1 frame s^{-1} intervals. A total of 1047 individual trajectories in 2 dimensions were visually tracked at 1 s resolution using the MTrack© plugin from ImageJ. Three behavioural measures relevant to vertical distribution were chosen: (1) mean time in the field of vision spent by larvae entering below the mid-water level (t_b), (2) mean maximum vertical displacement below the mid-water level by individual larvae (D_b), and (3) mean number of larval swimming trajectories below the mid-water level (N_b). The *a priori* expectations were that larvae would spend less time in the mid-water level (lower t_b) in the T columns than in the IS columns. Further, unfed larvae were expected to be more willing to cross the mid-water level and thermocline (higher D_b and N_b in UF groups than F groups).

Statistical analyses

Two separate analyses were applied to analyse the distribution of larval fish in the experimental columns. First, the proportion of larvae observed in the lower half of each column was analysed on a column basis using a quasibinomial generalised linear model (qbGLM) in R (Zuur et al. 2009, Catalán et al. 2011) for all ages towards the end of each light regime (at 100 min for B and 170 for A; Table 1). This model was chosen since it was appropriate to use for the underlying binomial data, individual larvae being in the lower half of the columns or not. Although column average values were used, i.e. the proportion of larvae in the lower half of the column, the relatively low number of individuals per column (20) precluded a reasonable normal approximation to an ordinary GLM analysis, especially in the youngest age group, where the observed proportions were close to zero (Zar 1999). Video observations indicated that the proximity of larvae did not affect an individual's behaviour. This is a basic assumption for the use of a quasibinomial distribution. The multifactorial qbGLM had Age (16, 30, 37 and 44 dph), Thermocline (IS, T) and Feeding (F, UF) as main factors. Model selection was conducted by applying a saturated model and hierarchically removing non-significant higher-order terms, starting with the highest-order terms (Zuur et al. 2009). When significant age interactions were present, age-specific models were run to determine Thermocline and Feeding effects at each age level.

Further, the distribution in the column according to the observed depth of each individual was analysed by calculating the median depth in each column and applying a factorial GLM model on column-specific median depths with Age, Thermocline and Feeding as main factors. The rationale behind applying 2 analyses of fish distribution was 2-fold. First, scarcely pigmented larvae are hard to observe in the upper part of the column because of the water-to-air refraction in circular columns (Vollset et al. 2009a), and precluded analysis of a more precise average depth analysis for the youngest stage (16 dph). Second, the proportion of individuals in either half of the columns does not indicate whether the response is temperature-dependent, since a significant response could take place above the mid-water level and thermocline. The median vertical depth of all larvae in each column was used as input in the analysis and considered adequate for a normal approximation in the GLM. When treatment factors with more than 2 levels were significant (e.g. Age effect and interaction effects), a Tukey HSD post-hoc test was employed to determine which levels were significantly different (Zar 1999). The analyses were carried out separately for the time periods 100 and 170 min, corresponding to light settings B and A, respectively.

To test for differences in cod weights in the upper and lower parts of the columns, multifactor GLM was used, where log-transformed individual larval DW was used as response variable and Column level (upper half vs. lower half of the column) was added as a main effect in addition to Age, Thermocline and Feeding, in addition to the interaction terms of Column level and the other main effects. The behavioural measures t_b, D_b and N_b from the video analyses were also tested by factorial GLM (Thermocline × Feeding) on column means. All behavioural measures were log-transformed to conform to homoscedasticity. In line with previous practice, all non-significant higher-order interactions were removed and GLM assumptions were evaluated. Tukey HSD post hoc tests were applied when appropriate, and as for all other tests, 0.05 was used as the level of significance.

RESULTS

Effects of age and thermocline

Larvae were distributed differently in IS and T columns with age (qbGLM tests, Age × Thermocline interactions, $p < 0.002$). Overall, larval cod were distributed deeper with age in the IS columns, but were mainly restricted to the upper part of the T columns (Figs. 2 & 3). Significantly more cod larvae were distributed in the upper part of the IS columns compared to the T columns at 37 and 44 dph at 100 min (light setting B, Thermocline effect, qbGLM, $F_{1,21} > 25.7$, $p < 0.001$; Fig. 2) and at 44 dph at 170 min (light setting A, Thermocline effect, qbGLM, $F_{1,19} = 18.4$, $p < 0.001$; Fig. 3). These differences were also apparent as higher average median larval depths in the IS columns compared to the T columns at 37 and 44 dph at 100 min (light setting B, Tukey HSD, GLM, $F_{2,63} = 20.69$, $p < 0.05$) and at 44 dph at 170 min (light setting A, Tukey HSD, $p = 0.005$; Fig. 4).

Effects of feeding history

F and UF cod did not distribute equally in the columns throughout the experiment (qbGLM, Age × Feeding interactions, $F_{3,83} > 2.91$, $p < 0.05$). F fish were more abundant in the lower part of the columns at 16 dph and in the upper part at 30 dph at 100 min (light setting B, qbGLM, $F_{1,20} = 6.15$, $p = 0.023$, and $F_{1,22} = 5.69$, $p = 0.027$; Fig. 2), while there was no significant difference at 37 and 44 dph. At 170 min, F fish were less abundant in the upper part of the column at 30 dph (light setting A, qbGLM, $F_{3,82} = 5.41$, $p = 0.030$; Fig. 3), while no significant differences were observed in the other age classes. Feeding treatment had a significant additive effect on the median depth of larval cod in the columns from 30 to 44 dph (Table 2). The overall average difference in median displacement between F and UF groups was relatively minor, with UF fish on average being 4.3 cm deeper than F fish. This was, however, significant across age, thermocline and light settings (GLM, Feeding effects, $F_{63,1} > 4.60$, $p < 0.036$; Fig. 4).

Larval behaviour

The video analyses corroborated the visual observations and added further explanatory power to the observed distributions for 44 dph. Presence of a thermocline significantly reduced t_b (GLM, thermocline effect, $p < 0.02$; Fig. 5). On average, larvae spent 29 and 72 s in the field of visison in the T and IS columns, respectively. At the same time, average maximal D_b of larvae was lower in the T columns (GLM, Thermocline effect, $p < 0.02$). No significant

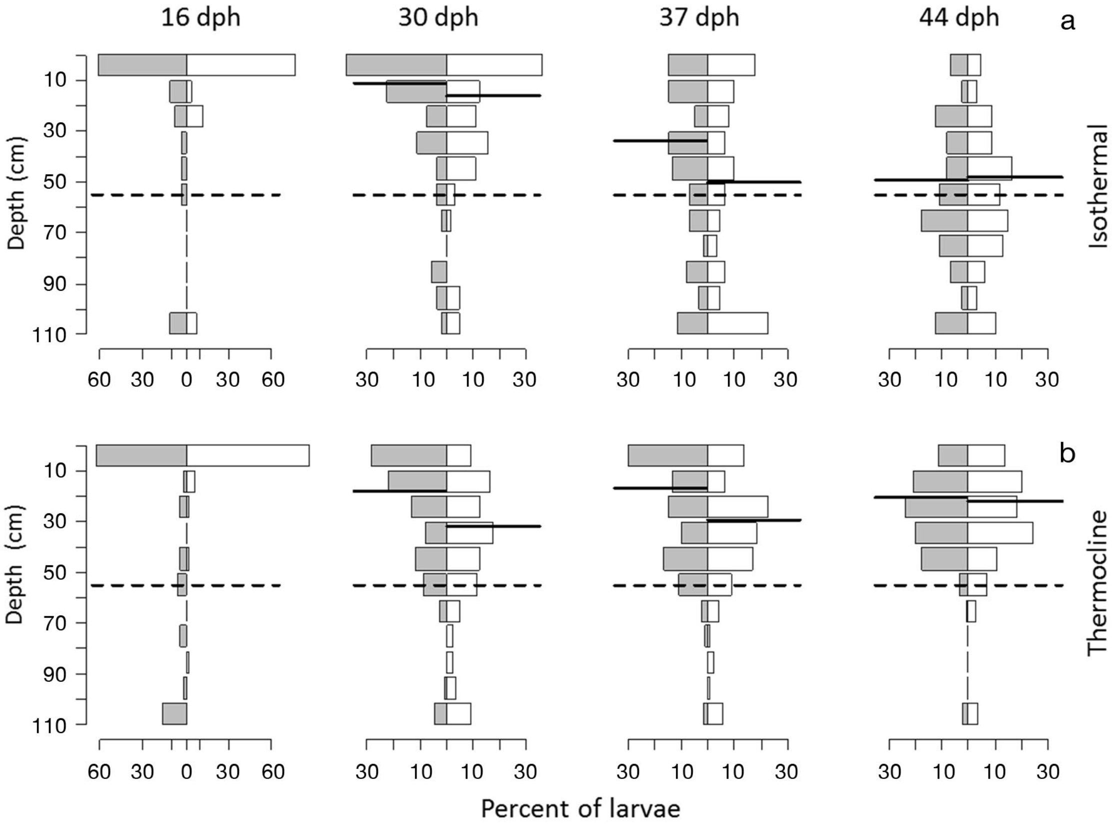

Fig. 2. *Gadus morhua*. Vertical distribution of larvae in experimental columns at 100 min for fed (grey bars) and unfed (white bars) larvae. Solid line indicates median depth of larvae, while dashed line indicates position of the thermocline. Light is from below (setting B). Histograms are in 10 cm bins with a total height of 115 cm for age 16, 30, 37 and 44 d post hatch (dph) in (a) isothermal and (b) thermocline columns

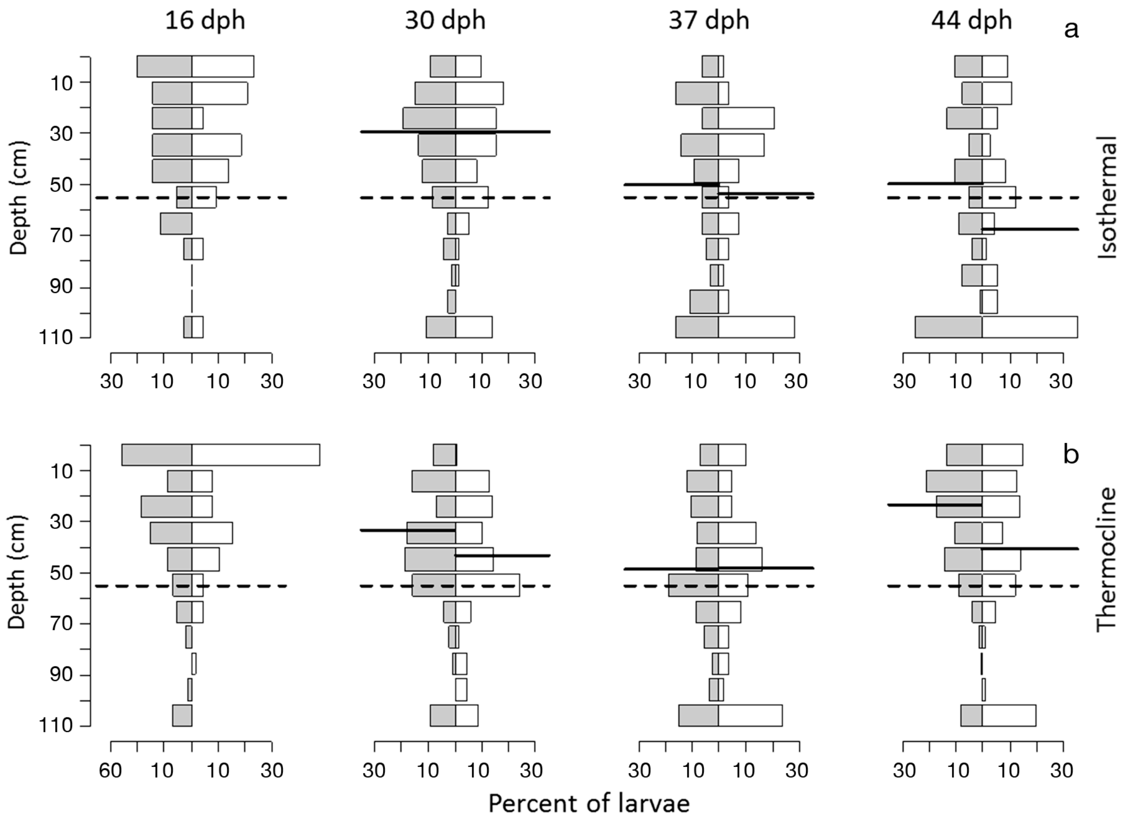

Fig. 3. *Gadus morhua*. Vertical distribution of larvae in experimental columns at 170 min for fed (grey bars) and unfed (white bars) larvae. Solid line indicates median depth of larvae, while dashed line indicates position of the thermocline. Light is from above (setting A). Histograms are in 10 cm bins with a height of total 115 cm for age 16, 30, 37 and 44 d post hatch (dph) in (a) isothermal and (b) thermocline columns

differences in t_b or D_b were found between F or UF larvae. However, UF larvae appeared more frequently in the field of vision than F larvae (N_b), swimming into the lower part of the column and then returning to the upper part (GLM, Feeding effect, $p < 0.03$; Fig. 5).

Larval weight

Relatively heavier larvae tended to be recovered in the upper half of the column among younger larvae, while the opposite was the case for older larvae (GLM, Age × Column level interaction, $F_{3,1576} = 3.16$, $p = 0.024$), although this difference was not significant at any specific age (Tukey HSD, $p > 0.2$). The lack of other significant GLM interaction terms suggests that the relative weight of larvae in the upper versus lower half of the column was unrelated to feeding or thermocline treatments. The main feeding treatment resulted in larvae in the F group being 6.3% heavier than larvae in the UF group after the latter group had been subjected to an added 16 h of food deprivation (GLM, Feeding effect, $F_{1,1576} = 8.49$, $p < 0.004$). This weight difference amounted to about half the average daily larval weight increase (around 12%) observed during the experimental period (16 to 44 dph).

DISCUSSION

Behavioural responses to temperature have been studied in various species, from insects to mammals (e.g. Swiergiel & Ingram 1986, Smolinský & Gvozdik 2009). Earlier work has shown that late-stage larval cod respond to the presence of a thermocline (Vollset et al. 2009a), while hunger increases both larval swimming behaviour (Munk 1995) and their willingness to enter into areas inhabited by predators (Skajaa et al. 2003). Furthermore, work on juvenile fish suggests that observed patterns of vertical migration can in some cases be explained by individuals moving into colder stratified layers at night to conserve energy (Neverman & Wurtsbaugh 1994, Sogard & Olla 1996). In the present study, we demonstrate that

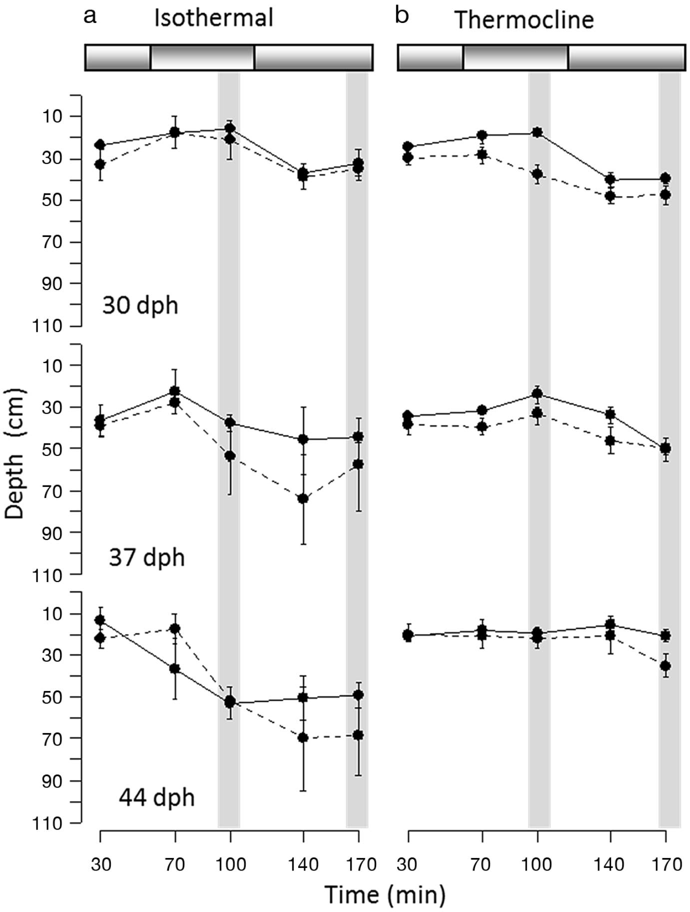

Fig. 4. *Gadus morhua.* Median depth distribution (cm from bottom, ±95% CI) of fed (solid line) and unfed (dashed line) larvae in (a) isothermal and (b) thermocline columns, at 30, 37 and 44 d post hatch (dph). Vertical shaded bars represent time periods used for statistical analysis. Horizontal bar on top indicates light settings at different time periods (increasing shading from top to bottom representing light from above, otherwise light from below)

early stages of cod do respond to a thermocline of only 2°C, and that, independent of stratification, older larval cod distribute deeper when unfed. The feeding-dependent response was, however, small, compared to other effects (e.g. time, age, thermocline), and there was no apparent interaction between feeding history and presence of thermocline. Video analysis suggested that early juveniles at 44 dph alter their behaviour when unfed by diving more frequently into the deeper part of the column. We suggest that unfed larval and early juvenile stages of cod are driven by an increased motivation to find prey while attempting to stay within higher temperatures.

According to the bioenergetics hypothesis (Brett 1971), temperature preference is dependent on energetic state to optimise expenditure of energy reserves when state (defined here as stomach fullness) is high, while conserving energy when state is low. If this is valid for larval cod, we should expect more unfed individuals to move into colder water relative to fed larvae. This would imply that an individual's temperature affinity decreases with decreasing state, as seen for older juvenile fish (Sogard & Olla 1996). Our results, however, do not suggest a different temperature affinity, but rather an additive distributional difference between feeding treatments. Larval fish must keep growing to grow out of the vulnerable size range, and saving energy or reducing starvation risk has few benefits when mortality rates are high (McNamara & Houston 1987, Folkvord 2005). To a larval fish, remaining in the warmer water near the surface, where nauplii prey are most likely to be found, is probably a common viable strategy (Fiksen & Jørgensen 2011).

The results from the present study imply that larval fish prefer warmer water independent of stomach

Table 2. *Gadus morhua.* Generalised linear model (GLM) table for median depth variable, for light from below (setting B) at 100 min and above (setting A) at 170 min, with corresponding Tukey HSD tests. Analyses include larval ages from 30 to 44 d post hatch (dph). Only levels significantly different from each other are listed, using level names as in Table 1. No Tukey test results are presented for main effects that were part of significant higher-order interactions

Median depth	SS	df	MS	*F*	p	Tukey HSD test
Light setting B at 100 min						
Thermocline	2152.5	1	2152.5	20.53	<0.0001	
Feeding	1268.6	1	1268.6	12.10	0.0009	UF > F
Age	3064.6	2	1532.3	14.61	<0.0001	
Thermocline × Age	4339.7	2	2169.9	20.69	<0.0001	IS44, IS37 > T30, T37, T44, IS44
Error	6605.7	63	104.9			
Light setting A at 170 min						
Thermocline	734.8	1	734.8	3.03	0.0865	
Feeding	1116.0	1	1116.0	4.60	0.0357	UF > F
Age	3004.8	2	1502.4	6.20	0.0035	
Thermocline × Age	3332.3	2	1666.2	6.88	0.0020	IS44 > T44, IS30
Error	15267.9	63	242.3			

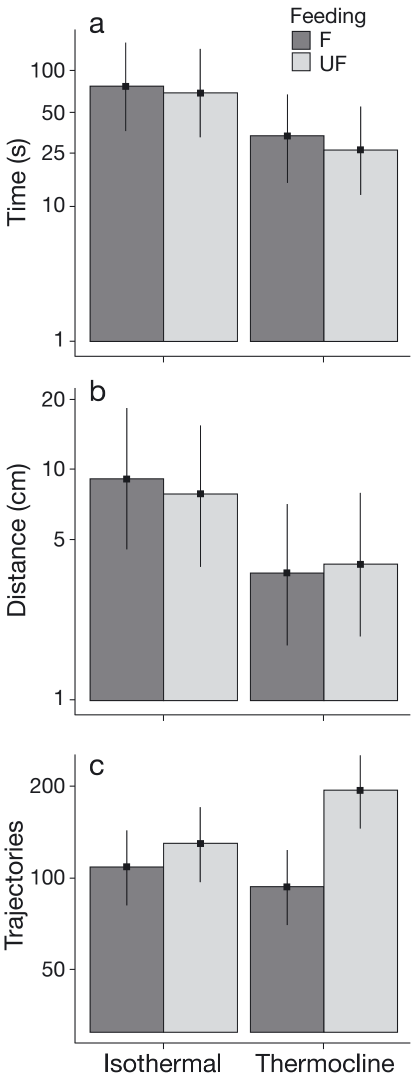

Fig. 5. *Gadus morhua*. Model results (main effects) from behavioural analyses. Mean ± 95% CI of (a) time spent below mid-water level and thermocline (t_b), (b) vertical descent below mid-water level (D_b), and (c) number of trajectories below mid-water level (N_b). Shaded bars, feeding treatment (F, fed; UF, unfed). Note that *y*-axes are log-scale

fullness. At the same time, increased prey-searching activity in unfed cod is expected (Munk 1995). This would imply that individual larvae would have to balance 2 contrasting demands: the need to seek out new areas to find prey, while simultaneously trading off the cost of crossing into colder water. The results of the present study, showing a deeper distribution of unfed fish in both the T and IS columns, support such a prey-searching trade-off (e.g. Sogard & Olla 1996). Data from the present study's video analysis at the early juvenile stage (44 dph) also support this hypothesis. Both time spent in the field of vision and vertical distance were strongly affected by the presence of the thermocline, indicating that larval fish avoided the colder temperature. However, more larvae would more frequently appear in the lower part of the column in the UF treatment (if only for a brief moment), indicating that unfed larvae more frequently sample the colder water. Huwer et al. (2011) suggested that larger cod larvae choose greater depths to find larger, more suitable prey and save energy in deeper, colder water. However, stratification in the Baltic can be between 16 and 6°C and should present a different trade-off from that experienced by the cod in the present experiment. At the same time, the fact that colder temperatures (6°C) are avoided in our study regardless of stomach fullness suggests that the choice of deeper waters is most likely a function of prey search rather than thermal preference.

Other hypotheses might also explain the ultimate cause of thermal preference. For example, temperature could be related to the high encounter rate of prey and predators, due, for example, to the temperature-dependent behaviour of fish and their prey and/or autocorrelation of stratified water with light. This means that temperature can hypothetically be used as a proxy to define optimal habitat (e.g. thin layers; Clay et al. 2004). However, such behaviour mechanisms are hard to disentangle from other hypotheses with regard to temperature-dependent behaviour. Furthermore, the fact that temperature has been shown to have such a large effect on physiology in growth experiments (e.g. Otterlei et al. 1999) suggests that fitness related to temperature affinity should at some level be affected by the growth and developmental aspects of increasing temperature.

To our knowledge, Ehrlich & Muszynski (1982) is the only study that has documented an affinity for lower temperature in unfed marine larval fish (Californian grunion *Leuresthes tenuis*). However, the unusual spawning strategy and lack of point-of-no-return in this species (May 1971) make the comparison with cod difficult. While behavioural responses according to temperature are well documented for marine larval fish (e.g. Batty 1994), research into state-dependent behaviour according to temperature is harder to find. Behavioural response to environmental variables in the vertical realm will affect both short-term (e.g. starvation, predation) as well as long-term (drift, settling area) survival benefits (e.g. Mullin 1993, Fiksen et al. 2007). Large-scale circulation features can be utilised by larval fish through simple behavioural rules and morphological characteristics (Vikebø et al. 2007). Thus, single-factor behavioural responses can be hard to identify and need to be studied over different scales and potential interactions with internal and external stimuli. In the present study we have included several important physical and biological factors to elucidate the potential state-dependence of larval temperature-dependent behaviour, but clearly more remains to be done on different levels of food deprivation and environmental conditions.

In contrast to findings from herring larvae (Catalán et al. 2011) in the same experimental setup, there was no apparent strong response to light directionality in the present study. This is in line with results from Pacific cod that showed no response to varying light levels at younger larval stages (Hurst et al. 2009). Cod larvae are known to avoid high intensities of light (Skiftesvik 1994). Earlier work in a similar setup demonstrated that late larval stages respond differently to thermoclines in light and darkness, but not to different directionalities of light (Vollset et al. 2009a). In the present study as well, larval cod did not appear to respond differently to either feeding or thermocline in the 2 different light settings, suggesting that they respond more to the intensity of light rather than to its directionality. A potential explanation is that larval cod regulate depth by geotaxis (Davis & Olla 1994) modified by light.

We designed the present experiment to represent a realistic temperature difference experienced by Northeast Arctic cod larvae drifting in the coastal current, but many cod larvae encounter much steeper temperature gradients. To really tease out the effects of temperature on larval cod vertical behaviour, a gradient bordering the upper and lower tolerance range would be informative in revealing the preferred temperature of starved or well-fed larvae through ontogeny. Another topic that needs future experimental attention is the risk-sensitive behaviour of larval fish — do they respond to the presence of predators (e.g. jellies or fish) by altering their habitat use (light exposure) or activity level in a similar manner as seen in some zooplankton species (e.g. Loose & Dawidowicz 1994)? Experimental studies in environmental gradients can elucidate how larvae behave when there are trade-off conflicts and which environmental cues are most important. As ocean models increase in spatial resolution, a reliable representation of behavioural responses of organisms will be crucial to improve the models' credibility.

Acknowledgements. We thank Vibeke Lokøy and Frank Midtøy for help with larval rearing and technical support. This work was funded by the University of Bergen and the Institute of Marine Research (KILO project). I.A.C. was partially supported by a 'José Castillejo' research mobility grant from the Spanish Ministry for Education and Science.

LITERATURE CITED

Batty RS (1994) The effect of temperature on the vertical distribution of larval herring (*Clupea harengus* L.). J Exp Mar Biol Ecol 177:269–276

Brett JR (1971) Energetic responses of salmon to temperature. A study of some thermal relations in physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). Am Zool 11:99–113

Catalán IA, Vollset KW, Morales-Nin B, Folkvord A (2011) The effect of temperature gradients and stomach fullness on the vertical distribution of larval herring in experimental columns. J Exp Mar Biol Ecol 404:26–32

Clay TW, Bollens SM, Bochdansky AB, Ignoffo TR (2004) The effects of thin layers on the vertical distribution of larval Pacific herring, *Clupea pallasi.* J Exp Mar Biol Ecol 305:171–189

Colton AR, Hurst TP (2010) Behavioral responses to light gradients, olfactory cues, and prey in larvae of two North Pacific gadids (*Gadus macrocephalus* and *Theragra chalcogramma*). Environ Biol Fishes 88:39–49

Davis MW, Olla BL (1994) Ontogenetic shift in geotaxis for walleye pollock, *Theragra chalcogramma* free embryos and larvae: potential role in controlling vertical distribution. Environ Biol Fishes 39:313–318

Ehrlich KF, Muszynski G (1982) Effects of temperature on interactions of physiological and behavioural capacities of larval California grunion: adaptions to the planktonic environment. J Exp Mar Biol Ecol 60:223–244

Ellertsen B, Solemdal P, Strømme T, Tilseth S, Westgård T, Moksness E (1980) Some biological aspects of cod larvae (*Gadus morhua* L.). Fiskeridir Skr Ser HavUnders 17: 29–47

Fiksen Ø, Jørgensen C (2011) Model of optimal behaviour in fish larvae predicts that food availability determines survival, but not growth. Mar Ecol Prog Ser 432:207–219

Fiksen Ø, Jørgensen C, Kristiansen T, Vikebø F, Huse G (2007) Linking behavioural ecology and oceanography: larval behaviour determines growth, mortality and dispersal. Mar Ecol Prog Ser 347:195–205

Folkvord A (2005) Comparison of size-at-age of larval Atlantic cod (*Gadus morhua*) from different populations based on size- and temperature-dependent growth models. Can J Fish Aquat Sci 62:1037–1052

Houston AI, McNamara JM (1999) Models of adaptive behaviour: an approach based on state. Cambridge University Press, Cambridge

Houston AI, McNamara JM, Hutchinson JMC (1993) General results concerning the trade-off between gaining energy and avoiding predation. Philos Trans R Soc Lond B 341:375–397

Huntley M, Brooks ER (1982) Effects of age and food availability on diel vertical migration of *Calanus pacificus.* Mar Biol 71:23–31

Hurst TP, Cooper DW, Scheingross JS, Seale EM, Laurel BJ, Spencer ML (2009) Effects of ontogeny, temperature, and light on vertical movements of larval Pacific cod (*Gadus macrocephalus*). Fish Oceanogr 18:301–311

Huwer B, Clemmesen C, Grønkjær P, Köster FW (2011) Vertical distribution and growth performance of Baltic cod larvae — field evidence for starvation-induced recruitment regulation during the larval stage? Prog Oceanogr 91:382–396

Jordaan A, Brown JA (2003) The risk of running on empty: the influence of age on starvation and gut fullness in larval Atlantic cod (*Gadus morhua*). Can J Fish Aquat Sci 60:1289–1298

Kingsford MJ, Leis JM, Shanks A, Lindeman KC, Morgan SG, Pineda J (2002) Sensory environments, larval abilities and local self-recruitment. Bull Mar Sci 70:309–340

Kiørboe T (2011) How zooplankton feed: mechanisms, traits and trade-offs. Biol Rev 86:311–339

Kristiansen T, Fiksen Ø, Folkvord A (2007) Modelling feeding, growth, and habitat selection in larval Atlantic cod (*Gadus morhua*): observations and model predictions in a macrocosm environment. Can J Fish Aquat Sci 64: 136–151

Kristiansen T, Jørgensen C, Lough RG, Vikebø F, Fiksen Ø (2009) Modeling rule-based behavior: habitat selection and the growth-survival trade-off in larval cod. Behav Ecol 20:490–500

Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. Can J Zool 68:619–640

Loose CJ, Dawidowicz P (1994) Trade-offs in diel vertical migration by zooplankton: the costs of predator avoidance. Ecology 75:2255–2263

Lough RG, Potter DC (1993) Vertical distribution patterns and diel migrations of larval and juvenile haddock *Melanogrammus aeglefinus* and Atlantic cod *Gadus morhua* on Georges Bank. Fish Bull 91:281–303

May RC (1971) Effects of delayed initial feeding on larvae of the grunion, *Leuresthes tenuis* (Ayres). Fish Bull 69: 411–425

McNamara JM, Houston AI (1987) Starvation and predation as factors limiting population size. Ecology 68:1515–1519

Mullin MM (1993) Webs and scales: physical and ecological processes in marine fish recruitment. University of Washington, Seattle, WA

Munk P (1995) Foraging behaviour of larval cod (*Gadus morhua*) influenced by prey density and hunger. Mar Biol 122:205–212

Neverman D, Wurtsbaugh WA (1994) The thermoregulatory function of diel vertical migration for a juvenile fish, *Cottus extensus.* Oecologia 98:247–256

Olla BL, Davis MW (1990) Effects of physical factors on the vertical distribution of larval walleye pollock *Theragra chalcogramma* under controlled laboratory conditions. Mar Ecol Prog Ser 63:105–112

Otterlei E, Nyhammer G, Folkvord A, Stefansson SO (1999) Temperature- and size-dependent growth of larval and early juvenile Atlantic cod (*Gadus morhua*): a comparative study of Norwegian coastal cod and northeast Arctic cod. Can J Fish Aquat Sci 56:2099–2111

Pearre S (2003) Eat and run? The hunger/satiation hypothesis in vertical migration: history, evidence and consequences. Biol Rev Camb Philos Soc 78:1–79

Pepin P, Dower JF, Davidson FJM (2003) A spatially explicit study of prey−predator interactions in larval fish: assessing the influence of food and predator abundance on larval growth and survival. Fish Oceanogr 12:19–33

Skajaa K, Fernö A, Folkvord A (2003) Swimming, feeding and predator avoidance in cod larvae (*Gadus morhua* L.): trade-offs between hunger and predation risk. In: Browman HI, Skiftesvik AB (eds) The big fish bang. Proceedings of the 26th Annual Larval Fish Conference Institute of Marine Research, Bergen, p 105–121

Skiftesvik AB (1994) Impact of physical environment on the behaviour of cod larvae. ICES Mar Sci Symp 198: 646–653

Smolinský R, Gvozdik L (2009) The ontogenetic shift in thermoregulatory behaviour of newt larvae: testing the 'enemy-free temperatures' hypothesis. J Zool (Lond) 279: 180–186

Sogard SM, Olla BL (1996) Food deprivation affects vertical distribution and activity of a marine fish in a thermal gradient: potential energy-conserving mechanisms. Mar Ecol Prog Ser 133:43–55

Somero GN (2002) Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. Integr Comp Biol 42:780–789

Swiergiel AH, Ingram DL (1986) Effect of diet and temperature acclimation on thermoregulatory behavior in piglets. Physiol Behav 36:637–642

Vikebø F, Jørgensen C, Kristiansen T, Fiksen Ø (2007) Drift, growth, and survival of larval Northeast Arctic cod with simple rules of behaviour. Mar Ecol Prog Ser 347:207–219

Vollset KW, Fiksen Ø, Folkvord A (2009a) Vertical distribution of larval cod (*Gadus morhua*) in experimental temperature gradients. J Exp Mar Biol Ecol 379:16–32

Vollset KW, Seljeset O, Fiksen Ø, Folkvord A (2009b) A common garden experiment with larval Northeast Arctic and Norwegian coastal cod cohorts in replicated mesocosms. Deep-Sea Res II 56:1984–1991

Zar JH (1999) Biostatistical analysis. Prentice Hall, Upper Saddle River, NJ

Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer, New York, NY

Editorial responsibility: Kenneth Sherman,
Narragansett, Rhode Island, USA

Submitted: April 10, 2012; Accepted: October 18, 2012
Proofs received from author(s): February 3, 2013