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Effect of development rate on the swimming, escape responses, and morphology of yolk-sac stage larval American plaice, *Hippoglossoides platessoides*

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Abstract To examine the impact of development rate on swimming performance, escape response, and morphology, yolk-sac larvae of American plaice (*Hippoglossoides platessoides*, Fabricius) were reared at two temperatures (5 and 10 °C). Videomicroscopy and silhouette collimation videography were used to examine swimming, escape behaviour, and morphology (standard length, finfold area, and yolk-sac area) of individual larvae. Larvae were examined from 0 d post hatch (dph) to 14 dph for the 5 °C treatment group and from 0 to 6 dph for the 10 °C treatment group (3 August to 17 August 1996). Since larvae were not fed, yolk-sac reserves were essentially exhausted by 14 and 6 dph for the 5 and 10 °C treatment groups, respectively. To control for the effect of testing temperature on behaviour, larvae from each temperature treatment were tested at both 5 and 10 °C. Testing temperature had an effect on some swimming parameters but not on escape response. Swimming performance, escape response, and morphology varied with age, while only morphology and escape response varied with development rate. Morphology and swimming performance, and morphology and escape response were found to be correlated as determined by canonical correlation. This study suggests that both types of swimming behaviours should be examined when developing models of the impacts of predation on the early life history of larval fish.

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Introduction

Recruitment and subsequent year-class strength in marine fish have been attributed to survival experienced during larval stages (Hjort 1914; May 1974; Cushing 1975). Predation is considered to be the major agent of mortality during the yolk-sac stage (Batty 1989; Blaxter and Fuiman 1990; Paradis et al. 1996), while starvation may become important only after the transition to exogenous feeding (Litvak and Leggett 1992). Understanding mechanisms of survival during these stages is key to understanding recruitment (Bailey and Houde 1989). Both biological and environmental factors have been shown to influence predation on fish larvae. These factors include size (Fuiman and Gamble 1989; Litvak and Leggett 1992; Pepin et al. 1992; Paradis et al. 1996), age (Litvak and Leggett 1992), level of starvation (Gamble and Hay 1989), density (Fuiman and Gamble 1989), ontogeny (Fuiman 1994), temperature (Fuiman and Batty 1994; Elliot and Leggett 1996) and development rate (Houde 1987). Changes in rates of larval mortality, regardless of the particular agent, can have large effects on juvenile recruitment and adult population levels (Leggett and DeBlois 1994).

Two prevalent views of larval fish predation and starvation are that it is better to be bigger (the bigger is better hypothesis) and it is better to develop faster (the stage duration hypothesis; Houde 1987). There have been a number of tests of the bigger is better hypothesis suggesting that it is not always better to be a bigger larva (e.g. Litvak and Leggett 1992; Pepin et al. 1992; Cowan et al. 1996). However, there have been few tests of the stage duration hypothesis in relation to predation and starvation.

To better understand the effect of development rate on the probability of a larva being eaten, the predation event may be divided into its component probabilities. Vulnerability to predation is considered to be the product of three independent probabilities – encounter, attack, and capture (O'Brien 1979). The

probability of encounter is directly influenced by development and behaviour of larvae. For example, larvae become more conspicuous with increased size, activity, and pigmentation, increasing the chances of being detected by a visual predator (Fuiman 1989). Larger larvae have a greater muscle mass which correlates with increased swimming performance (Webb and Weihs 1986), increased swimming speed, time spent swimming, and distance travelled. This increases the probability of encountering visual or non-visual predators (Bailey and Houde 1989). Increased size may also increase probability of attack, as a larger prey item may be a preferred target (Litvak and Leggett 1992). This potential increase in encounter and attack probabilities may make larvae more vulnerable to predation. However, the accompanying decrease in probability of capture (increase in escape capability) may overcome the increase in encounter and attack probabilities, making larger larvae less vulnerable to predation. This system is dynamic; changes in any combination of these probabilities may favour different growth strategies.

Strong predation pressures result in selection for anti-predator defences (Fuiman 1989; Williams et al. 1996). One well studied anti-predator defence is the escape response (Eaton et al. 1977; Webb and Corolla 1981; Blaxter and Batty 1985; Eaton and DiDomenico 1986; Batty 1989; Williams and Brown 1992; Williams et al. 1996). The escape response generally begins with a series of movements comprising a c-start, in which a larva rapidly contracts musculature on alternating sides of the body. The result is a displacement of the larva, often several body lengths from its original position (Eaton et al. 1977). Following the initial displacement, a period of burst swimming removes the individual from the predator's visual field (Webb and Corolla 1981).

The rate of neural development influences escape response timing (Eaton et al. 1977; Taylor and McPhail 1985; Blaxter 1986). Development of musculature and fins (or finfolds) regulates swimming acceleration and velocity (Webb and Weihs 1986). Therefore, rate of development can influence the outcome of an escape response. In addition to a larva's own scope for growth, fluctuations in environmental conditions, such as temperature, can heavily influence larval developmental rate and subsequent larval morphology (Chambers and Leggett 1987; Houde 1987; Elliot and Leggett 1996). Thus, changes in an individual's environmental history would be expected to influence their predator avoidance capabilities and therefore their vulnerability to predation.

In the present study we test the hypothesis that development rate, manipulated through rearing temperature, affects swimming performance, escape response, and morphology of American plaice (*Hippoglossoides platessoides*) yolk-sac larvae. In an attempt to partition the effect of rearing temperature and test temperature, we also test the hypothesis that test temperature affects swimming performance and escape response.

Materials and methods

Eggs and larvae

Fertilized American plaice (*Hippoglossoides platessoides*, Fabricius) eggs were shipped from the Department of Fisheries and Oceans in Halifax, Nova Scotia (23 July 1996), in bags containing salt water (30 ppt, 4 °C). Upon arrival, the fertilised eggs (embryos) were acclimated to laboratory conditions through the addition of 150 ml of prepared salt water (Forty Fathoms sea salts in distilled water, 30 ppt, 4 °C) to each bag. Embryos were disinfected in a solution of UV-sterilised salt water (30 ppt, 4 °C) containing 0.4 ml gluteraldehyde l^{-1} (Douillett and Holt 1994). They were then rinsed three times with UV sterilised salt water (30 ppt, 4 °C), and placed into glass, egg incubating trays (Marinex, 14 cm × 24 cm) containing 800 ml of an incubating solution of 0.02 mg ml^{-1} streptomycin and 25 IU ml^{-1} penicillin in UV-sterilised salt water (30 ppt, 4 °C; Smigielski 1979). Incubating trays were then placed in a water table maintained at 5 °C (±0.2 °C). Each day, non-viable embryos (discoloured and resting on bottom) were removed. We increased salinity to 34 ppt and maintained water quality by replacing half of the incubating solution daily with a more saline solution (36 ppt, 5 °C).

Larvae began to hatch 11 d after stocking and continued to do so for 3 d. Each day, newly hatched larvae were gently pipetted from the egg incubating trays, counted, and placed into larval rearing trays (Marinex, 14 cm × 24 cm) containing 800 ml of salt water (34 ppt, 5 °C) at a density of 100 per tray. Howell and Caldwell (1984) suggested that there was a positive relationship between development rate and temperature for American plaice larvae reared at temperatures of 2 to 10 °C. Based on this work we chose temperatures of 5 and 10 °C to create two developmental cohorts (slow and fast). Thus, at hatch, half of the larval rearing trays were placed in the 5 °C (±0.2) water table and the remaining were placed into a water table at 10 °C (±0.2). Dead larvae were removed, and half of the water within each tray was siphoned and replaced daily (34 ppt). Larvae were studied until just before death due to starvation. Trials were conducted on larvae reared at 5 °C on 0, 3, 4, 6, 8, 10, 12, and 14 dph and for larvae reared at 10 °C on 0, 3, 4, and 6 dph.

Experimental protocol

In this study we examined responses of individual larvae using a silhouette collimation videographic system, similar to that of Litvak and Leggett (1992), to record swimming performance and escape responses of each larva. Larvae used on each experimental day were acclimated to the appropriate experimental temperature for 2 h. An individual larva was then placed in a 10.2 cm diameter, glass-bottomed, PVC experimental arena with a water depth of approximately 5 cm and allowed an additional 2 min acclimation period. The arena sat inside a partially filled 90-litre aquarium on a supporting table. A large Fresnel lens (46 cm diameter, focal length ca. 60 cm) placed below the aquarium was used to collimate light rays. A video camera (Sony DXC-1821) was located directly above the aquarium. The camera was connected to an S-VHS videocassette recorder (Sony SVO-9500MD) operating at 30 frames s^{-1}. A time–date generator (Panasonic WJ-810) was used to imprint the time, to 1/100th of a second, on the video image. By using one camera, swimming and escape behaviour could only be captured in two dimensions, which may have underestimated distances travelled and velocities. However, the experimental arenas allowed relatively little vertical movement of the larvae due to the shallow depth and therefore any errors were relatively small.

After a 2 min acclimation period, swimming behaviour of each larva was videotaped for 2 min. Recording was continued while a blunt probe (2 mm diameter) was used to simulate a contact predator. We used this approach because larvae of other fish species are known to respond earliest to a contact predator (Blaxter and Batty 1985; Eaton and DiDomenico 1986). An escape response was

deemed suitable for analysis if the larva's activity over the entire response event was in view. A maximum of three attempts were made on each larva to avoid habituation.

Once a suitable escape response was recorded, the larva was removed from the arena with a wide-mouth pipette and placed in a 100 ml beaker with an anaesthetising solution of MS-222 (0.8 mg l^{-1}) of the same temperature as the arena. The anaesthetised larva was then videotaped with a dissecting microscope (Olympus SZ6045) outfitted with a black and white camera (Panasonic WV-BD400) and S-VHS videocassette recorder (Sony SVO-9500MD) in order to later measure morphological parameters using image analysis.

To evaluate the effect of testing temperature, two types of trials were conducted. Half of the selected larvae were tested at their rearing temperature and the remaining larvae were tested at the alternate rearing temperature after slowly adjusting the temperature over a 2 h period. This short acclimation period of 2 h is commonly used in studies involving marine fish larvae since they develop very quickly (Batty et al. 1993; Fuiman and Batty 1997). Ten larvae were examined within each rearing temperature/test temperature group on each experimental day.

Data acquisition

OPTIMAS v5.1a (Optimas Corporation, Seattle, Washington) was used to analyse images. For swimming behaviour, the larva's position within the arena was digitised once per second for 60 s. Co-ordinates for the anterior-most point of the larva were determined. The distance travelled by the larva was calculated using the formula for Euclidean distance. The mean swimming speed, maximum swimming speed, and mean swimming bout distance (a "bout" was defined as an event of continuous swimming) over the entire sampling period were calculated.

To determine escape behaviour of each larva, the first escape response in which the entire event was clearly captured on videotape was analysed. A frame-by-frame analysis over the escape response duration (30 frames s^{-1}) was conducted. The first frame measured was the frame just prior to contact of the larva by the probe. The second and third frames were contact of the probe and larva. Fourth and subsequent frames were of the response. Co-ordinates for the anterior-most point of the larva were determined. An APL (STSC*APLPLUS v7.1) program (Litvak unpublished) was used to calculate mean escape speed, maximum escape speed, and total escape displacement of each larva (Euclidean distance from start to end co-ordinates of escape response).

Three morphometric parameters were recorded for each larva using image analysis: standard length (measured as a polyline connecting the anterior-most point of the upper jaw to the posterior-most point of the notochord), finfold area, and yolk-sac area.

Statistical analysis

All data were analysed using SAS v6.12 (SAS Institute Inc., Cary, North Carolina). Data were tested for normality using PROC UNIVARIATE (SAS Institute 1990) and for homogeneity of variances using an F_{max}-test (Sokal and Rohlf 1981). All data were $\log_{10}$-transformed in order to meet assumptions of univariate and multivariate normality. A series of three-way ANOVAs (SAS Institute 1990, PROC GLM) were used to evaluate the effect of testing temperature, rearing temperature, and age on swimming and escape response parameters. The ANOVAs were first run as saturated models, then re-run with non-significant interactions and main effects sequentially deleted (Keppel 1982). When an interaction was detected, the three-way ANOVAs were decomposed in order to test biologically relevant effects; two-way ANOVAs were run on test temperature and rearing temperature at each age, and a series of one-way ANOVAs was run on age for each testing temperature/rearing temperature group. When an interaction was detected in a two-way ANOVA, it was decomposed into the interacting components and re-run. A series of two-way ANOVAs was run on morphological parameters (testing temperature was not included as a factor). Their interpretation was similar to that for the swimming and escape response ANOVAs.

Levels of α for interactions in all ANOVAs were set at 0.20, following Winer's (1971) suggestion that the significance of interactions in ANOVAs should be interpreted conservatively. A posteriori comparisons between groups in ANOVA models were performed with a least-squares means multiple comparison test using sequential Bonferroni adjustments (Rice 1990).

To investigate the morphological correlates of swimming performance and escape response, canonical correlations (SAS Institute 1990, PROC CANCORR) were performed between morphological parameters, and both swimming performance and escape response parameters. All data were used in the canonical correlation analysis (0, 3, 4, 6, 8, 10, 12, and 14 dph for larvae reared at 5 °C, and 0, 3, 4, and 6 dph for larvae reared at 10 °C). Correlations in the loading matrices of the canonical correlations with values of <0.3 where not interpreted since they explained $<10\%$ of the model variance (Tabachnick and Fidell 1989).

Results

Larval mortalities were low ($\sim$10% d^{-1}) in both experimental treatments until yolk-sac absorption approached. Time to 50% yolk-sac absorption (50% of larvae had adsorbed their yolk-sac) was 6 dph at 10 °C and 12 dph at 5 °C. At the time of 50% yolk-sac absorption, mortalities increased greatly to $\sim$90% d^{-1}.

Morphology

Standard length was affected only by age ($df = 3, 101$; $F = 11.45$; $p < 0.001$), with larval length increasing from 0 to 3 dph after which it remained constant (Fig. 1). For finfold area, a significant interaction was found between rearing temperature and age ($df = 3, 97$; $F = 2.48$; $p = 0.066$). One-way ANOVAs were therefore run on rearing temperature across ages, and on age across rearing temperatures. Rearing temperature had a significant effect on finfold area at 4 dph ($df = 1, 29$; $F = 9.82$; $p = 0.004$) and at 6 dph ($df = 1, 24$; $F = 21.01$; $p < 0.001$), with larvae reared at 5 °C having larger finfolds than larvae reared at 10 °C (Fig. 1). Age also affected finfold area for larvae reared at both 5 °C ($df = 3, 49$; $F = 8.67$; $p < 0.001$) and 10 °C ($df = 3, 48$; $F = 3.88$; $p = 0.015$), with finfold area peaking at 4 and 3 dph, respectively.

A significant first order interaction was found between rearing temperature and age for yolk-sac area ($df = 3, 97$; $F = 4.74$; $p = 0.004$). One-way ANOVAs were therefore run on rearing temperature across ages, and on age across rearing temperatures. Rearing temperature had a significant effect on yolk-sac area at 3 dph ($df = 1, 30$; $F = 62.03$; $p < 0.001$) and at 4 dph ($df = 1, 29$; $F = 9.40$; $p = 0.005$), with larvae reared at 5 °C having larger yolk-sacs than larvae reared at 10 °C (Fig. 1). Age also affected yolk-sac area for larvae reared at 5 °C ($df = 3, 49$; $F = 35.86$; $p < 0.001$) and 10 °C ($df = 3, 48$; $F = 41.17$; $p < 0.001$), with yolk-sac area decreasing exponentially with age (Fig. 1).

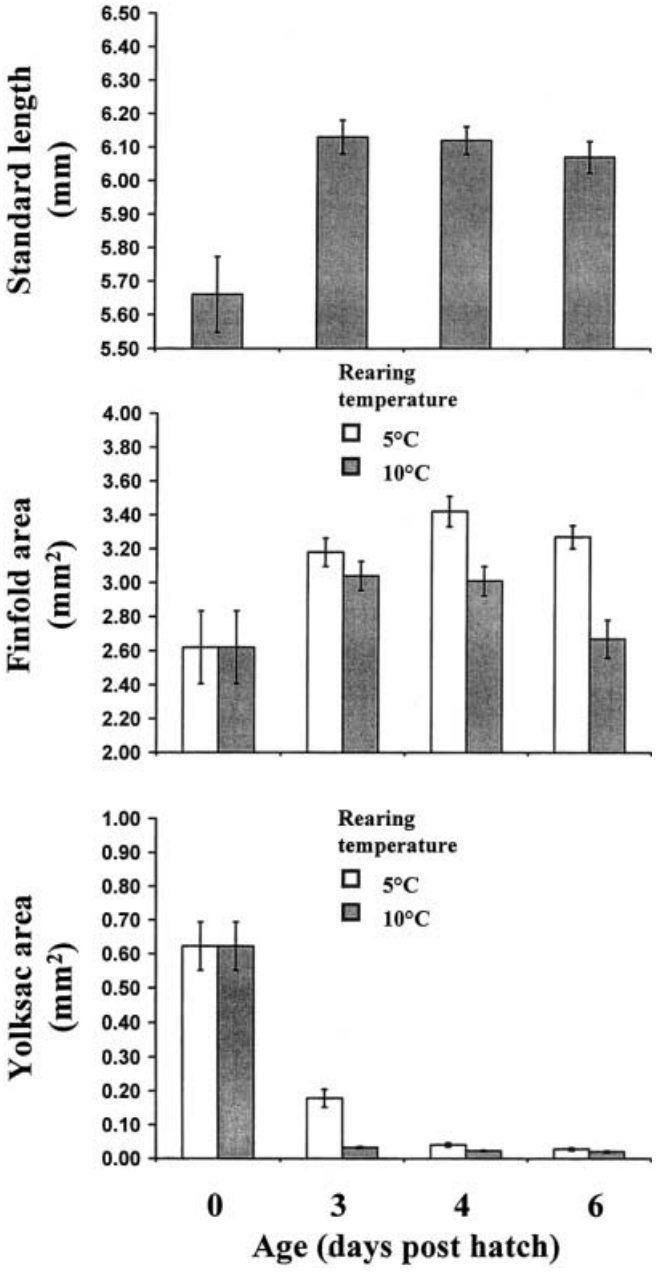

Fig. 1 *Hippoglossoides platessoides*. Morphological parameters of larvae reared and tested at 5 and 10 °C over 6 d. Rearing and testing temperatures had no effect on standard length so all data were pooled. Test temperature had no effect on finfold area or yolk-sac area so data for the two test temperatures were pooled. Error bars represent ±1 standard error

Swimming

Mean swimming speed

There was a significant first order interaction between rearing temperature and test temperature ($df = 1, 98$; $F = 5.24$, $p = 0.024$) in the three-way ANOVA. Two-way ANOVAs were therefore conducted for each rearing temperature and test temperature across each age. At 3 dph, larvae tested at 10 °C had higher mean swimming speeds than did larvae tested at 5 °C ($df = 1, 28$; $F = 4.98$; $p = 0.034$; Fig. 2). At 6 dph there was a significant first order interaction between rearing temperature and test temperature ($df = 1, 22$; $F = 11.05$; $p = 0.003$). One-way ANOVAs were therefore run on rearing temperature at each test temperature, and on test temperature at each rearing temperature at 6 dph.

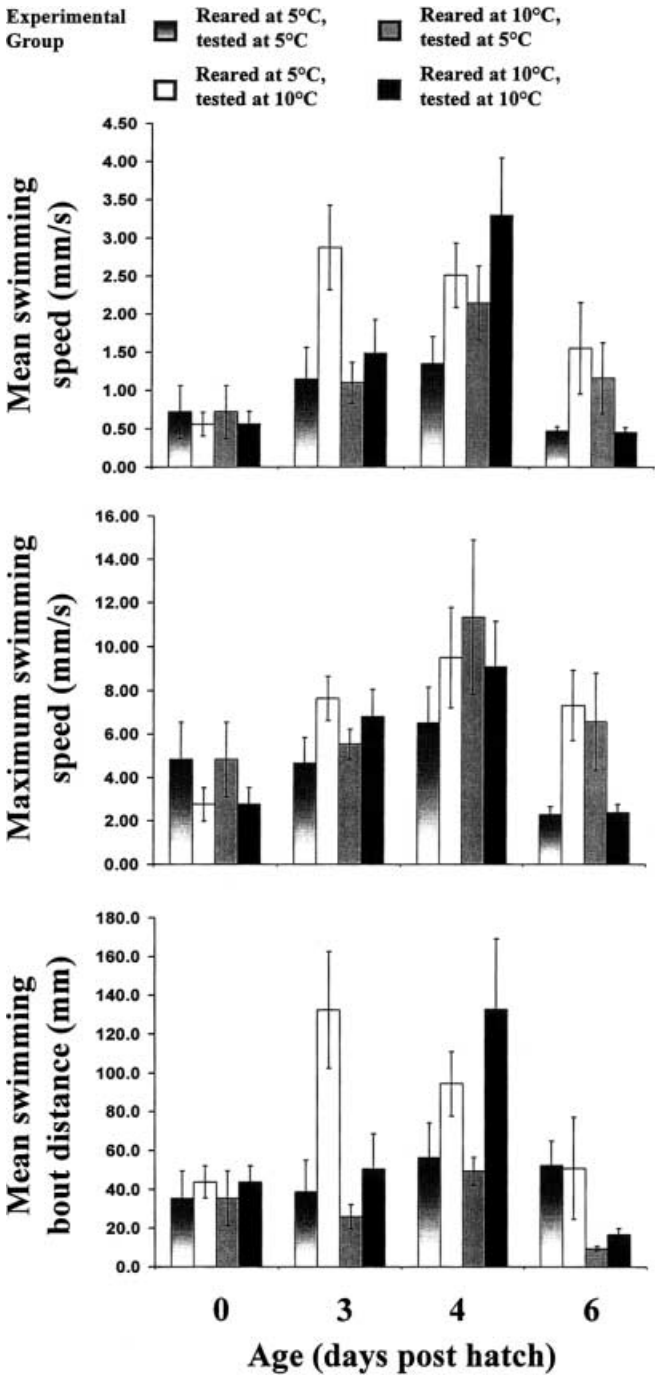

Fig. 2 *Hippoglossoides platessoides*. Swimming parameters of larvae reared and tested at 5 and 10 °C over 6 d. Error bars represent ±1 standard error

Rearing temperature was found to affect mean swimming speed at each test temperature. At 6 dph, when larvae were tested at 5 °C, larvae reared at 10 °C swam faster than did larvae reared at 5 °C ($df = 1, 10$; $F = 5.95$; $p = 0.035$; Fig. 2). The opposite effect was seen for larvae tested at 10 °C, where larvae reared at 5 °C swam faster than those reared at 10 °C ($df = 1, 12$; $F = 6.22$; $p = 0.028$; Fig. 2). The effect of test temperature differed across rearing temperatures at 6 dph. When larvae were reared at 5 °C, those tested at 10 °C swam faster than those tested at 5 °C ($df = 1, 12$; $F = 6.05$; $p = 0.030$; Fig. 2). However, when larvae were reared at 10 °C, those tested at 5 °C swam faster than those tested at 10 °C ($df = 1, 10$; $F = 6.22$; $p = 0.032$; Fig. 2). Age had a significant effect only on those larvae tested at 10 °C. This effect was similar for larvae reared at 5 °C ($df = 3, 22$; $F = 3.80$; $p = 0.025$) and 10 °C ($df = 3, 24$; $F = 7.45$; $p = 0.001$), with swimming speeds increasing after hatch to a relative peak at 3 and 4 dph after which speeds decreased at 6 dph (Fig. 2).

Maximum swimming speed

A significant second order interaction was found between rearing temperature, test temperature and age in the three-way ANOVA ($df = 3, 89$; $F = 1.98$; $p = 0.123$). Two-way ANOVAs were therefore conducted for each rearing temperature and test temperature across age. At 6 dph there was a significant first order interaction between rearing temperature and test temperature ($df = 1, 22$; $F = 18.80$; $p < 0.001$). One-way ANOVAs were therefore run on rearing temperature at each test temperature, and on test temperature at each rearing temperature at 6 dph. Rearing temperature was found to affect maximum swimming speed at each test temperature. When larvae were tested at 5 °C, larvae reared at 10 °C swam faster than those reared at 5 °C ($df = 1, 10$; $F = 7.10$; $p = 0.024$). When larvae were tested at 10 °C, those larvae reared at 5 °C swam faster ($df = 1, 12$; $F = 12.49$; $p = 0.004$; Fig. 2). A similar effect was also found for test temperature, where effects differed across rearing temperatures at 6 dph. When larvae were reared at 5 °C, larvae tested at 10 °C swam faster than those tested at 5 °C ($df = 1, 12$; $F = 13.52$; $p = 0.003$). When larvae were reared at 10 °C, those larvae tested at 5 °C swam faster ($df = 1, 10$; $F = 6.48$; $p = 0.029$; Fig. 2). The effect of age was only seen in those larvae tested at 10 °C. This effect was similar for larvae reared at 5 °C ($df = 3, 20$; $F = 6.49$; $p = 0.003$) and 10 °C ($df = 3, 24$; $F = 6.46$; $p = 0.002$), where maximum swimming speeds reached a relative peak at 3 or 4 dph after which they decreased at 6 dph (Fig. 2).

Mean swimming bout distance

A significant second order interaction was found between rearing temperature, test temperature, and age in the three-way ANOVA ($df = 3, 88$; $F = 2.36$; $p = 0.077$). Two-way ANOVAs were therefore conducted for each rearing temperature and test temperature across age. At 3 dph there was a significant first order interaction between rearing temperature and test temperature ($df = 1, 28$; $F = 2.96$; $p < 0.096$). One-way ANOVAs were therefore run on rearing temperature at each test temperature, and on test temperature at each rearing temperature at 3 dph. A significant effect of test temperature was found at 3 dph for larvae reared at 5 °C, with larvae tested at 10 °C having longer swimming bouts than larvae tested at 5 °C ($df = 1, 14$; $F = 7.52$; $p = 0.016$; Fig. 2). A similar effect of test temperature was also found at 4 dph, with larvae tested at 10 °C having longer swimming bouts than larvae tested at 5 °C ($df = 1, 26$; $F = 5.72$; $p = 0.024$; Fig. 2). Rearing temperature also affected swimming bout distance at 6 dph, where larvae reared at 5 °C had longer swimming bouts than larvae reared at 10 °C ($df = 1, 22$; $F = 8.64$; $p = 0.008$; Fig. 2). Age only affected larvae reared at 10 °C; swimming bout distance of larvae tested at 5 °C ($df = 3, 20$; $F = 6.49$; $p = 0.003$) and 10 °C ($df = 3, 24$; $F = 6.46$; $p = 0.002$) peaked at 4 dph and decreased thereafter (Fig. 2).

Escape response

Rearing temperature affected only mean escape speed. Larvae reared at 5 °C exhibited faster escape responses than larvae reared at 10 °C ($df = 1, 100$; $F = 12.01$; $p = 0.001$; Fig. 3). Test temperature did not affect escape behaviour. Age affected both mean escape speed ($df = 3, 100$; $F = 9.96$; $p < 0.001$) and total escape displacement ($df = 3, 101$; $F = 7.14$; $p < 0.001$). Mean escape speeds were at a relative maximum at hatch, after which they decreased. Total escape displacement reached a relative peak at 3 and 4 dph and decreased at 6 dph (Fig. 3).

Morphological correlates of performance

Swimming

The canonical correlation between morphological and swimming parameters showed the two sets to be correlated on the first and only significant eigenvector ($p = 0.0406$), which accounted for 66% of the variance in the model. Standard length and finfold area were positively correlated with swimming parameters. Yolk-sac area was not interpreted because the loading was < 0.30 (see "Materials and methods"; Table 1). Mean swimming speed and finfold area had the highest loadings on this first eigenvector, indicating they explained most of the variance in the model (Table 1).

Escape response

The canonical correlation between morphological and escape response parameters showed the two sets to be

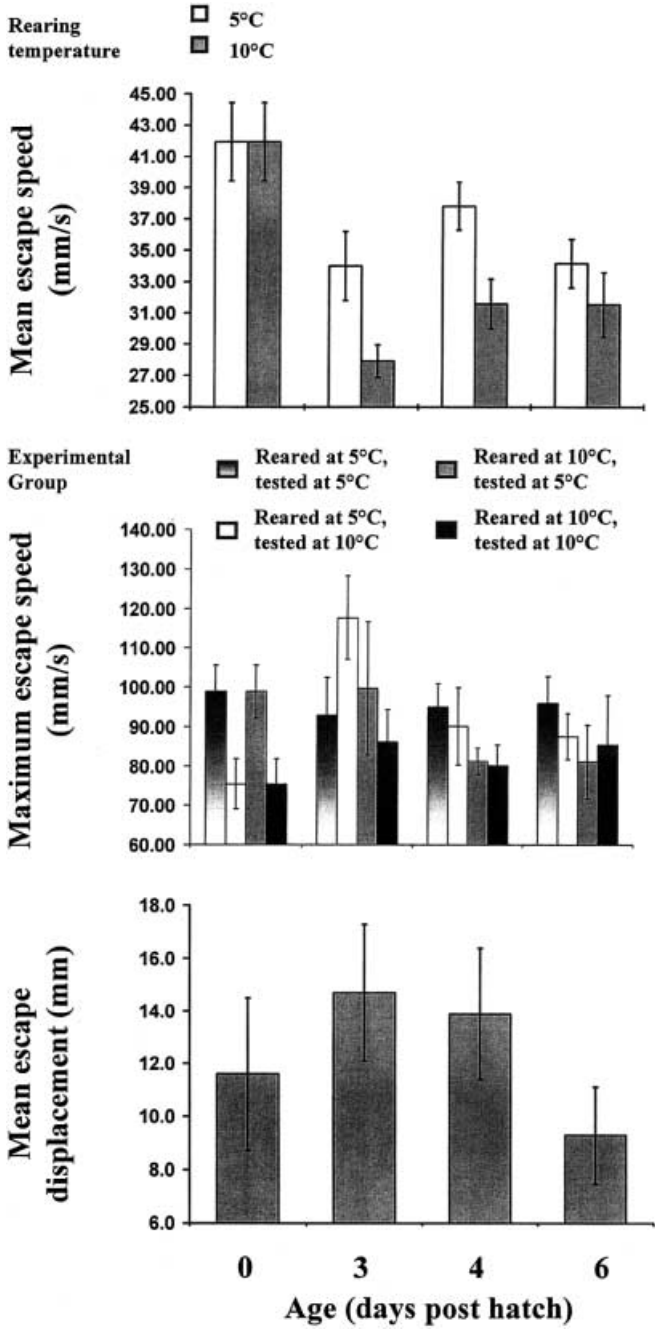

Fig. 3 *Hippoglossoides platessoides*. Escape response parameters of larvae reared and tested at 5 and 10 °C over 6 d. Test temperature had no effect on mean escape speed so data were pooled. Neither rearing temperature nor testing temperature affected mean escape displacement so data for all four test groups were pooled. Error bars represent ± 1 standard error

strongly correlated on the first and only significant eigenvector ($p = 0.0017$), which explained 86% of the model variance. Finfold area and maximum escape speed had loadings < 0.30 and were not interpreted (Table 1). Of the remaining variables, yolk-sac area was positively correlated with mean escape speed and total displacement of escape, while standard length was negatively correlated with the escape response parameters (Table 1). The highest loadings were found for mean escape speed and yolk-sac area, indicating that they explained most of the model variance.

Discussion

Temperature had a significant effect on finfold area and yolk-sac size at age. Larvae reared in the higher temperature treatment increased their finfold area more and exhausted their yolk-sac reserves earlier. This suggests that we did create two developmental cohorts through the manipulation of rearing temperature.

Development rate, as manipulated through rearing temperature, was found to affect swimming performance but its effect was not consistent. While the effect on the length of swimming bouts was strong, the effect on swimming speeds depended on the testing temperature as was demonstrated by the significant rearing temperature × test temperature interaction at 6 dph for mean and maximum swimming speeds.

The lack of a clear effect of rearing temperature on swimming is somewhat counter-intuitive, since temperature is known to affect growth (Chambers and Leggett 1987; Houde 1987; Elliot and Leggett 1996). Red muscle fibres are presumed to be responsible for cruising activity in adult fish and similar muscle fibres are known to be present in fish larvae (El-Fiky et al. 1987). The total number of these aerobic red muscle fibres has been shown to increase with cold acclimation (Johnston and Lucking 1978). While this has not been examined in American plaice, juvenile turbot (*Scophthalmus maximus*) acclimated to lower temperatures showed an increase in the proportion of red muscle fibres (Calvo and Johnston 1992). Similar results were also found in larval whitefish (*Coregonus lavaretus*; Hanel et al. 1996).

This phenomenon, which is often referred to as thermal compensation of locomotion (Hazel 1993), is a mechanism whereby fish acclimated to different temperatures exhibit similar cruising speeds due to compensatory muscle growth. This compensatory mechanism appears to be important during the yolk-sac stage, but may not be later in a fish's life history. A number of studies on the effects of temperature on muscle development of larval herring found evidence of compensatory muscle growth in very early larvae (Vieira and Johnston 1992) but no such phenomenon in later larvae (Johnston et al. 1997, 1998). In our experiments, the lack of a rearing temperature effect on the swimming of larvae tested at the same rearing temperature may be due to a thermal compensation of locomotion.

The effect of testing temperature on swimming was much stronger than that of rearing temperature (development rate). Where an effect of testing temperature was found, with one exception (6 dph 10 °C), larvae tested at a higher temperature increased their performance approximately three to four times over their performance at a lower temperature. Larvae at 6 dph, reared at 10 °C

Table 1 *Hippoglossoides platessoides*. Loading matrix from canonical correlations between morphological, and swimming and escape response parameters of larvae reared for 14 d at 5 °C and for 6 d at 10 °C (*SL* standard length; *FA* finfold area; *YA* yolk-sac area; *MSS* mean swimming speed; *MXS* maximum swimming speed; *MBD* mean swimming bout distance; *MES* mean escape speed; *MXE* maximum escape speed; *TED* total displacement of escape)

	Swimming parameters			Escape response	
	Variable sets	Variable loadings		Variable sets	Variable loadings
Morphology	SL	0.4619	Morphology	SL	−0.4048
	FA	0.9615		FA	0.0062
	YA	0.1067		YA	0.9441
Swimming	MSS	0.9134	Escape response	MES	0.7948
	MXS	0.8980		MXE	0.1201
	MBD	0.5583		TED	0.6414

and tested at 5 °C, actually increased their swimming speeds. We cannot presently explain this unexpected result. It did occur at a time of high mortality due to yolk-sac absorption when the larvae may have increased activity in an attempt to find food. Alternatively, it may reflect an acute lowering of metabolic rate in cold water, decreasing the energetic demands of growth and thus freeing energy reserves for swimming activity.

It is commonly accepted that the cruising speed (swimming speed) of adult fish is dependent on ambient (test) temperature (Fry 1971). Such results have been reported for swimming speed of northern anchovy (*Engraulis mordax*) larvae (Hunter 1981) and for general swimming activity of a number of Japanese fish larvae (Fukuhara 1990). Test temperature may also interact with larval size. For example, Fuiman and Batty (1997) found the swimming speeds of large Atlantic herring (*Clupea harengus*) larvae to be dependent on test temperature, while those of small herring larvae were not. While the objective of the present study was not to evaluate such an interaction, we found a test temperature effect only in the larger and older yolk-sac larvae (>3 dph).

Escape responses of American plaice larvae in this experiment were well developed at hatch, but decreased quickly with starvation. Yin and Blaxter (1987) found a distinct peak before the PNR (point-of-no-return) in mean escape speeds of a number of starved marine fish larvae. A similar peak was seen in the present study only for total distance of escape response. Escape speeds of American plaice larvae were similar to those of other species. Mean and maximum escape speeds were ~5.9 and 15.0 body lengths (BL) s^{-1}, respectively, close to the range of 6.5 to 7.2 BL s^{-1} and 13.0 to 14.9 BL s^{-1} reported for a number of other species (Yin and Blaxter 1987).

The escape responses of American plaice larvae in this experiment were affected by rearing temperature but not test temperature. The presence of a rearing temperature effect is most likely due to a more rapid starvation of larvae reared at 10 °C, whose escape performance was generally poorer than for larvae reared at 5 °C. Escape responses differed most between rearing temperature groups on the two days when yolk-sacs of larvae reared at 10 °C were smaller than those of larvae reared at 5 °C (3 and 4 dph). This is supported by the results of the canonical correlation analysis, which showed escape response performance was lower when yolk-sac size was smaller.

The lack of a testing temperature effect on escape responses differs from the results of most other studies on temperature and burst swimming in larval fishes (Fuiman 1986; Batty and Blaxter 1992; Batty et al. 1993). However, Morley (1996), in a study of foraging strikes of larval herring, found only a weak relationship between strike velocities and test temperature. Burst swimming in fish larvae, as occurs during an escape response, is presumed to occur due to recruitment of fast-twitch white muscle fibres (Akster et al. 1995). As with red muscle fibres, the power output of white muscle fibres is often dependent on test temperature (Blaxter 1992). However, this response is often species-specific, with some fish being able to maintain muscular power output in relatively cold water by utilising a greater number of white muscle fibres in the burst swimming event (Dabrowski 1986).

The canonical correlation between morphology and swimming demonstrated an important point that is often not addressed in studies involving larval fish locomotion. Standard length is typically the "standard" measure of fish growth (excluding weight) and this measure is often incorporated into larval fish swimming models. While these models may be successful in explaining a portion of the observed variability, the high loading of finfold area in the morphology/swimming canonical correlation suggests that similar models may benefit from inclusion of other morphological parameters such as finfold area.

It has been suggested that the larval finfold is used directly in escape locomotion by providing large amounts of thrust for fleeing from predators (Hunter 1984) or feeding (Hunter 1972). However, Webb and Weihs (1986) argued that the finfold's primary function is to act as a gas exchange area. The lack of a significant correlation between finfold area and escape performance in American plaice larvae does tend to question the importance of finfold area for escape responses.

Our results agree with Howell and Caldwell's (1984) suggestion that under conditions of low food availability, American plaice larvae with a low development rate may be at an advantage over larvae with a higher development rate. To better understand this result we should look to the individual components of the vulnerability to predation equation. For example, the similar swimming performance may lead to similar probabilities of encounter (Gerritsen and Strickler 1977), while the improved escape performance of those larvae with a relatively low development rate may lead to a reduced probability of capture. This is contrary to the prediction of the stage duration hypothesis. However, we did not assess probability of attack in our experiment. It is not known what effect the larger finfold areas of the slower developing larvae would have on this probability. While larger body sizes are sometimes associated with higher attack rates (Litvak and Leggett 1992), the finfold is essentially transparent. Future work will focus on defining the impact of development rate on the probability of attack. This information will be integrated into a model of predation and the stage duration hypothesis.

The present study demonstrates that two modes of larval swimming behaviour, routine swimming activity and burst swimming activity involved in escape, responded differently to the manipulations of rearing and test temperatures. This suggests that both types of swimming behaviours should be examined when developing models of predation. While the burst swimming in marine fish larvae has been well studied, routine swimming activity has not received the same attention (Blaxter 1992). Our results also suggest that thermal compensation of locomotion in marine fish larvae should be further investigated. While recent work has allowed the partitioning of physical and physiological effects of ambient temperature on larval swimming (Fuiman and Batty 1997), studies on the effects of temperature on the muscle physiology of fish larvae remain vital to the understanding of larval fish ecology (Johnston et al. 1997, 1998).

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