

## TESTING HYPOTHESES CONCERNING THE PHENOTYPIC PLASTICITY OF ESCAPE PERFORMANCE IN FISH OF THE FAMILY COTTIDAE

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*Accepted 6 November 1997; published on WWW 14 January 1998*

### Summary

A knowledge of the natural history of two species of marine Cottidae was used to construct a set of complex *a priori* hypotheses to test the idea that seasonal temperature acclimation conferred a fitness advantage and to examine whether acclimation responses were constant through development. The species studied were the short-horn sculpin (*Myoxocephalus scorpius* L.) and the long-spined sea scorpion (*Taurulus bubalis* Euphr.). Fast-start escape performance was measured as a proxy of fitness, and maximum body-length-specific speed ( $\hat{U}_{\max}$ ) and acceleration ( $\hat{A}_{\max}$ ), and angular velocity ( $\omega_{\max}$ ) and cumulative turning angle (CTA) were quantified. Fish were acclimated to 5, 15 and 20 °C and filmed using high-speed cinematography at 0.8, 5.0, 15.0 and 20.0 °C. The following hypotheses were tested: (1) improvements in escape performance following acclimation to high temperature in adult short-horn sculpin that live offshore would involve a trade-off in performance at low temperature, (2) the escape response of the sea scorpion, which experiences large daily temperature variation, would exhibit low thermal sensitivity and be unchanged by acclimation between 5 and 15 °C, and (3) in short-horn sculpin, the ability to acclimate maximum speed thermally is acquired during ontogeny in parallel with the offshore migration of the late juvenile stage. At 20.0 °C, in adult short-horn sculpin,  $\hat{U}_{\max}$  and  $\hat{A}_{\max}$  were 110% and 55% higher, respectively, in 15 °C- than in 5 °C-acclimated fish. No evidence was obtained for improved fast-start performance at 0.8 °C or 5.0 °C

following cold acclimation. In the long-spined sea scorpion, acclimation to 5 and 15 °C did not improve  $\hat{U}_{\max}$  or  $\hat{A}_{\max}$  compared with fish acutely exposed to these temperatures, although acclimation to 5 °C increased  $\omega_{\max}$  ( $P=0.005$ ). When tested over the most extreme thermal range found in the field, all variables were improved at a test temperature of 0.8 °C in 5 °C- compared with 15 °C-acclimated sea scorpion. Acclimation therefore appeared to be beneficial in some instances in both species. How this affects relative fitness is uncertain. The scaling of  $\hat{U}_{\max}$  with acclimation to 5 and 15 °C was examined in both species over the test range 5.0–15.0 °C. Temperature acclimation did not affect scaling relationships of  $\hat{U}_{\max}$  in long-spined sea scorpion ranging in total body length ( $L$ ) from 45 to 160 mm. At a test temperature of 15.0 °C, the scaling of  $\hat{U}_{\max}$  for short-horn sculpin ranging in total body length from 43 to 270 mm changed from  $aL^{-0.98}$  in 5 °C-acclimated fish to  $aL^{-0.50}$  (where  $a$  is the proportionality coefficient in the regression equation) in 15 °C-acclimated fish ( $P<0.01$ ). In short-horn sculpin, therefore, the ability to modify escape performance with temperature acclimation was found to vary during ontogeny, potentially paralleling a migration from a more variable to a more stable thermal environment.

Key words: temperature acclimation, fast-starts, escape response, hypotheses, beneficial, fitness, short-horn sculpin, *Myoxocephalus scorpius*, long-spined sea scorpion, *Taurulus bubalis*.

### Introduction

Ectothermic organisms in the temperate zone exhibit phenotypic plasticity in response to changes in environmental factors such as temperature, producing what has been termed a norm of reaction over an environmental gradient (Scheiner, 1993). Seasonal temperature fluctuations have long been known to produce physiological compensatory changes, known as acclimation responses, in a diversity of rate functions and in a wide variety of species (see Bullock, 1955, for an early review). Fry and Hart (1948) carried out a classic study

examining the effect of thermal acclimation on fish swimming speed. Following acclimation to different temperatures for several weeks, maximum sustained cruising speed of goldfish (*Carassius auratus* L.) was measured at a range of temperatures. Acclimation extended the thermal range of activity and there was a shift in the optimum temperature for performance, between high- and low-temperature acclimation. Increases in speed at low temperature following cold acclimation were at the expense of swimming performance at

high temperature, compared with warm-acclimated fish. Following cold acclimation, the mechanisms underlying altered swimming performance include, *inter alia*, hypertrophy of red muscle fibres (Johnston and Lucking, 1978; Sidell, 1980), increases in mitochondrial volume density (Johnston and Maitland, 1980; Egginton and Sidell, 1989), altered expression of myosin light chain (MLC) isoforms (Crockford and Johnston, 1990; Langfeld *et al.* 1991; Hirayama *et al.* 1997) and of myosin heavy chain (MHC) isoforms (Gerlach *et al.* 1990; Hwang *et al.* 1991; Johnson and Bennett, 1995; Imai *et al.* 1997) and associated increases in fast muscle myofibrillar ATPase activity (Johnston *et al.* 1975; Heap *et al.* 1986; Crockford and Johnston, 1990).

Not all temperate species of fish exhibit thermal acclimation. In fact, fish from habitats with large daily temperature fluctuations may exhibit reduced, or no, thermal acclimation. In the killifish (*Fundulus heteroclitus*), rapid daily fluctuations in water temperature of salt marsh habitats have been associated with a lack of acclimation in myofibrillar ATPase activity over the temperature range 5–25 °C (Sidell *et al.* 1983). Over a broader test range, 10–35 °C, ATPase activity showed some acclimation, although reduced in comparison with that of goldfish. This was reflected in a similarly reduced acclimation response in escape performance (Johnson and Bennett, 1995). It has been suggested that acclimation modifications are avoided if the contractile complex has a low thermal sensitivity to acute temperature fluctuations experienced by the organism (Sidell *et al.* 1983). Furthermore, the high degree of short-term temperature variation may mask any stable cues for acclimation.

During ontogeny, seasonal shifts in temperature and/or changes in habitat can profoundly alter the thermal optimum for physiological processes in ectotherms. For example, the thermal optimum for growth increases during ontogeny in larval plaice (*Pleuronectes platessa*) (Hovenkamp and Witte, 1991) and winter flounder (*Pseudopleuronectes americanus*) (Buckley, 1982). In the dragonfly (*Libellula pulchella*), maturation is associated with a more defined thermal sensitivity and an increase in optimum thoracic temperature and upper lethal temperature (Marden, 1995), paralleling the increase in thoracic temperature associated with the more active lifestyle of adult stages (Marden *et al.* 1996).

The evolutionary significance of acclimation has been less rigorously examined than have the mechanisms involved (Huey and Berrigan, 1996). Precht (1958) classified acclimation responses according to the difference in physiological rate between the previous and current thermal regime. If the rate was the same, then compensation was perfect; if the rate changed initially on introduction to the new temperature, but did not change thereafter, there was no compensation. Compensation could also be partial, excess or inverse. Despite a cautionary note by Fisher (1958), that 'when acclimations occur it does not follow that these necessarily have obvious survival value', it has often been assumed that acclimation responses are adaptive (Hazel and Prosser, 1974) and that fitness is improved following a period of acclimation.

This has become known as the 'beneficial acclimation hypothesis' (Leroi *et al.* 1994; Huey and Berrigan, 1996) and, until recently, has been proffered as the primary evolutionary explanation of acclimation. However, it has received recent criticism by Huey and Berrigan (1996) since, in reality, *post-hoc* adaptive stories can be created for any of Precht's above-mentioned acclimation responses. According to Leroi *et al.* (1994), an acclimation response is only to be regarded as beneficial if it enhances differential reproduction, also known as Darwinian fitness. Furthermore, when specifically tested, the assumption has often been rejected (Leroi *et al.* 1994; Zamudio *et al.* 1995; Bennett and Lenski, 1997). Huey and Berrigan (1996) suggest that an examination of the evolutionary significance of patterns of acclimation should use more rigorous *a priori* hypotheses based on the natural history of the species. Using this rationale, we wished to test a set of hypotheses concerning thermal acclimation in two fish species of the family Cottidae.

Marine Cottidae are partial residents of the littoral zone (Gibson, 1969). Both short-horn sculpin (*Myoxocephalus scorpius* L.) and long-spined sea scorpion (*Taurulus bubalis* Euphr.) are benthic, marine teleosts of the temperate zone. In the north-east Atlantic, short-horn sculpin have a distribution between 45 and 78 °N, whilst long-spined sea scorpion occupy a somewhat lower clime, between 40 and 68 °N (Unesco, 1986) (Fig. 1). Around the British Isles, adult short-horn sculpin are caught offshore (Foster, 1969; King *et al.* 1983) between approximately 30 and 50 m (Unesco, 1986). In contrast, juvenile sculpin and all stages of long-spined sea scorpion are found in rock pools and in the shallow sublittoral zone (Foster, 1969; King and Fives, 1983; Unesco, 1986). Surface water temperatures in St Andrews Bay, Scotland, range from 3–5 °C in the winter to 15–18 °C in the summer (J. Murdoch, unpublished observations). Both sea scorpion and juvenile sculpin also experience acute changes in temperature, particularly in the intertidal zone. In the same bay, rock pools where these species are found have mean water temperatures in spring ranging from 5.7 to 12.5 °C over a 24 h period (G. Temple, unpublished observations). Pool temperatures have been found to be even more variable during the summer months (Morris and Taylor, 1983).

The short-horn sculpin and long-spined sea scorpion are 'ambush predators' and engage in very little steady swimming. Despite other forms of predator avoidance, such as camouflage and spines, both species commonly employ fast-starts for escape. Measurement of performance has been proposed as a practical method of yielding information on fitness and physiological compensation (Huey and Stevenson, 1979; Arnold, 1983). Several studies have shown or suggested a positive correlation between burst speed and survival, which can have a perceptible influence on fitness. Watkins (1996) found burst swimming speed of anuran tadpoles to be an important determinant of surviving attacks by garter snake predators. Thus, he thought it likely that selection acted directly on burst speed. Andraso (1997) suggested that the apparent equal success of an unarmoured morph of the brook

stickleback (*Culaea inconstans*) in a polymorphic population of two morphs, armoured and unarmoured, was due to the absence of the protective pelvic girdle, therefore enabling a superior escape performance. Swain (1992) actually measured a correlation between escape performance and survival in stickleback (*Gasterosteus aculeatus*) larvae. Superior burst swimming performance by certain vertebral phenotypes was matched by increases in the frequencies of those phenotypes in the field.

In our study, the following predictions were made, using the escape response as a correlate of fitness: (1) in the short-horn sculpin, warm acclimation improves escape performance at high temperature, at the expense of performance at low temperature, compared with cold-acclimated fish, (2) escape performance in the intertidal long-spined sea scorpion exhibits reduced thermal sensitivity and therefore does not vary with acclimation temperature over the average range of seasonal temperatures, and (3) in short-horn sculpin, the ability to acclimate maximum speed thermally is acquired during ontogeny in parallel with the change in thermal habitat due to offshore migration of late juveniles.

## Materials and methods

### Fish

All fish were collected between March 1995 and November 1996. Adult short-horn sculpin (*Myoxocephalus scorpius* L.) were caught using trawls and creels off the Fife coast and Isle of Cumbrae, Scotland. Long-spined sea scorpion (*Taurulus bubalis* Euphr.) and juvenile short-horn sculpin were collected on rocky shores around St Andrews, Scotland, using hand nets. In one series of experiments, adult fish of a discrete size class (sculpin,  $157.2 \pm 2.7$  mm total length,  $L$ ,  $59.2 \pm 3.4$  g wet mass,  $N=38$ ; sea scorpion,  $L=116.0 \pm 1.2$  mm,  $27.0 \pm 1.5$  g wet mass,  $N=35$ ; values are means  $\pm$  S.E.M.) were acclimated to one of three temperatures. During the winter months (November to March), fish were caught and acclimated to  $5 \pm 0.5$  °C for a minimum of 6 weeks in a recirculating seawater aquarium. Fish were kept under a 10 h:14 h light:dark photoperiod regime. Similarly, during the summer months (June to September) fish were caught and acclimated to 15 or  $20 \pm 0.5$  °C and kept under a 13 h:11 h light:dark photoperiod regime. In both cases, fish were fed twice a week on prawns and squid.

To examine the scaling of temperature acclimation responses, fish of a range of lengths (sculpin,  $L=43$ – $270$  mm; sea scorpion,  $L=45$ – $160$  mm) were either caught in winter and acclimated to  $5 \pm 0.5$  °C or caught in summer and acclimated to  $15 \pm 0.5$  °C. Acclimation was again carried out for a minimum of 6 weeks, under the photoperiod and feeding regimes described above. This investigation included the discrete size class of fish described above that were acclimated to 5 or  $15 \pm 0.5$  °C.

### Measurement of fast-start performance

Following thermal acclimation to 5, 15 or  $20 \pm 0.5$  °C, fish were transferred to a 2.0 m  $\times$  0.6 m  $\times$  0.25 m static filming arena at their acclimation temperature. Escape responses were

elicited by tactile stimulation. A 4 mm diameter rod was presented randomly from either side of the caudal fin of a stationary fish. Filming was carried out using a high-speed ciné camera (NAC, Japan) operating at 500 frames  $s^{-1}$ , via a mirror set at 45 ° above the tank. Lighting from below the tank enabled sharp silhouettes of the fish to be recorded on film. Adult short-horn sculpin ( $L=157.2 \pm 2.7$  mm) and long-spined sea scorpion ( $L=116.0 \pm 1.2$  mm) acclimated to 5 and  $15 \pm 0.5$  °C, were filmed at 0.8, 5.0, 15.0 and 20.0 °C. Fish acclimated to  $20 \pm 0.5$  °C were only filmed at their acclimation temperature.

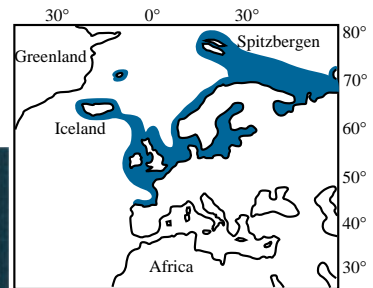
The scaling of fast-start performance was assessed in fish acclimated to 5 and  $15 \pm 0.5$  °C, and tested acutely at 5.0 and 15.0 °C, in a reciprocal experimental design.

### Kinematic analysis

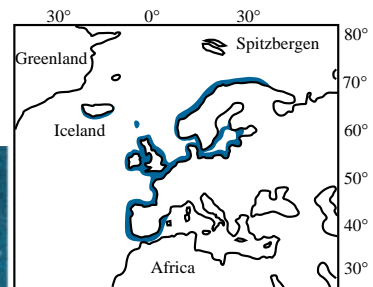
Prior to analysis, it was necessary to calculate the position of the mid-point (centre of mass) of the fish relative to the snout. A long pin with a weighted string attached was placed transversely through the head of a straight, frozen fish. The pin was balanced on two parallel bars. The fish, inclined at an angle of approximately 45 ° to the horizontal, was photographed. The pin was then removed and placed through the body beneath the first dorsal fin, and the fish was photographed again. On superimposing one photograph on top of the other, the point at which the strings crossed was found and used as the mid-point. This procedure was carried out on three fish of varying sizes for each species.

After processing, the high-speed films were digitized using MOVIAS-NAC Corp. software. Escape sequences from adult fish of the discrete size classes were analysed by digitizing ten points down the centre of the fish (the approximate position of the spine; J. Wakeling, unpublished results) from snout to tail tip. This was done for every fourth frame (8 ms) owing to the large number of points being digitized. Thirty frames were digitized before the start of the response and after the end of the second half-tailbeat, guaranteeing that the start and end of the response were included and avoiding the problems associated with smoothing the first few and last few frames (Harper and Blake, 1989). The end of the second half-tailbeat was taken as the maximum angular displacement of the snout to the left or right before myotomal contraction caused bending on the contralateral side of the fish at the start of the third half-tailbeat or glide (Figs 1C, 2). The  $x$  and  $y$  position data were smoothed using piecewise cubic regressions (Mathmatica, Wolfram Research Inc. USA). Length-specific maximum velocity ( $\hat{U}_{\max}$ ,  $s^{-1}$ ) and length-specific maximum acceleration ( $\hat{A}_{\max}$ ,  $s^{-2}$ ) of the centre of mass were calculated from the first and second differentials of each cubic regression. The correct smooth width was established by plotting values of  $\hat{U}_{\max}$  calculated using a number of different widths. As the smooth width was increased from three, values for  $\hat{U}_{\max}$  showed an initial decrease, because of the digitizing errors being smoothed.  $\hat{U}_{\max}$  then reached a plateau and finally decreased a second time owing to oversmoothing using smooth widths that were too wide. The correct smooth width was thus taken from the plateau region of graphs of  $\hat{U}_{\max}$  versus smooth width. This resulted in 13-point

A Short-horn sculpin (*Myoxocephalus scorpius* L.)



B Long-spined sea scorpion (*Taurulus bubalis* Euphr.)



C



Fig. 1. (A) A short-horn sculpin (*Myoxocephalus scorpius* L.) and its geographical distribution in the north-east Atlantic. (B) A long-spined sea scorpion (*Taurulus bubalis* Euphr.) and its geographic distribution in the north-east Atlantic. Scale bars, 20 mm. (C) Selected frames from a high-speed video recording showing the first two half-tailbeats and the gliding stage of an escape response typical of both the short-horn sculpin and the long-spined sea scorpion.

smoothing for short-horn sculpin and 9-point smoothing for long-spined sea scorpion. The fastest escape sequence from each fish was selected for further analysis. The cumulative turning angle (CTA, in degrees), defined as the sum of the absolute angles of turning of the snout relative to the mid-point throughout the first and second half-tailbeats (Fig. 2), and the maximum angular velocity ( $\omega_{\max}$ ,  $\text{rad s}^{-1}$ ) were determined in a similar way to the position and velocity. The CTA was only calculated for sequences in which escape responses consisted of two complete half-tailbeats.

To examine the scaling of swimming velocity, a second method was used to calculate  $\hat{U}_{\max}$ . This involved digitizing the mid-point of the fish frame-by-frame using an overhead projector overlay on which the distance of the midpoint from the snout had been marked. This method was used because only maximum velocities and the response durations (the time to the end of the second half-tailbeat) were calculated from these sequences; the first method used above enabled further calculations not discussed here. Once again, digitizing was started before the fish first moved and after the end of the second half-tailbeat. The  $x$  and  $y$  position data were smoothed using cubic piecewise regressions (LabView, National Instruments, USA) and  $\hat{U}_{\max}$  was calculated. The width of each piece was set to ensure that the standard error of all points from their smoothed positions matched the standard error of digitizing. This resulted in smoothing ranging from 21 points for large fish to 15 points for small fish. The fastest escape response from each fish was again selected. Maximum absolute velocity was also derived from each response. Only sequences in which two half-tailbeats were completed were used to measure the response duration.

To check that the two methods of calculating  $\hat{U}_{\max}$  were comparable, fast-starts from the discretely sized adult fish which had been acclimated to 5 and 15 °C and swum at 5.0 and 15.0 °C were analysed using both methods and compared using a one-way analysis of variance (ANOVA); there was found to be no significant difference between values from the two procedures ( $P > 0.2$ ).

### Statistics

A two-way general linear model (GLM) ANOVA was used

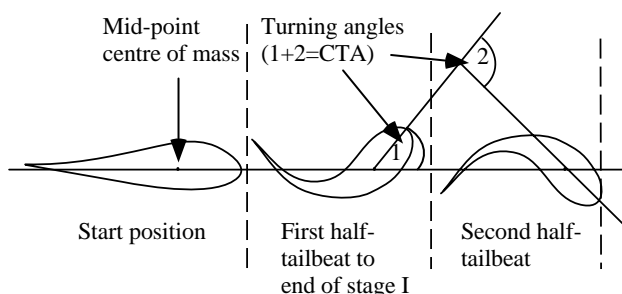


Fig. 2. Schematic diagram of a typical escape response. The cumulative turning angle (CTA) is the sum of the absolute values of degrees turned during the first and second half-tailbeats. The response duration is taken as the time to the end of the second half-tailbeat.

to examine the effects of acclimation temperature (5 and 15 °C) and acute (test) temperature on  $\hat{U}_{\max}$ ,  $\hat{A}_{\max}$ ,  $\omega_{\max}$  and CTA. A one-way ANOVA was used to examine the effects of acclimation temperature at all four test temperatures. If fish were unable to swim (scored as zero), Mann–Whitney  $U$ -tests were used to test for differences between acclimation to 5 and 15 °C at that particular test temperature. All statistical tests were computed using Minitab software (Minitab Inc., USA).

To examine the scaling of  $\hat{U}_{\max}$ ,  $U_{\max}$  (absolute swimming velocity,  $\text{m s}^{-1}$ ) and response duration with temperature acclimation, linear regressions ( $y = aL^b$ , where  $L$  is total body length) for the following groups, 5@5.0, 5@15.0, 15@5.0 and 15@15.0 (acclimation temperature at test temperature, °C), were calculated on log-transformed data using least-squares regression analysis. The significance of all regression lines was assessed by means of the ANOVA  $F$ -statistic. To test for differences in acclimation ability between short-horn sculpin from the two different habitats, a two-way GLM analysis of covariance (ANCOVA) (Minitab Inc., USA) was used, with habitat in which the fish were caught and temperature group as between-subject factors and length as a covariate. Furthermore, ANCOVA was used to test for differences in regression lines. Slopes (exponent  $b$ ) were compared using the first stage of ANCOVA. Tukey multiple-comparison tests were employed to determine between which groups any differences lay. Where no differences were found, the second stage of ANCOVA was used to test for differences in regression elevations (intercepts, proportionality coefficient  $a$ ). Tukey tests were again used to determine where differences lay. These tests were carried out following Zar (1996).

### Results

Escape responses fitted the description of Weihs (1973) and were typical 'C-starts'. Initial contraction of the trunk muscles on one side of the fish (stage 1) bent the fish into a curved shape. Following this was a propulsive stroke or strokes (stage 2), consisting of one or more tailbeats, and then a final gliding or steady swimming stage (stage 3) (Figs 1C, 2).

#### Configuration of temperature acclimation responses

The results for the eight two-way GLM ANOVAs are given in Table 1. Test temperature had a significant effect on  $\hat{U}_{\max}$  and  $\hat{A}_{\max}$  in both short-horn sculpin ( $\hat{U}_{\max}$   $F_{(3,59)} = 8.38$ ,  $P < 0.0005$ ;  $\hat{A}_{\max}$   $F_{(3,59)} = 6.76$ ,  $P = 0.001$ ) and long-spined sea scorpion ( $\hat{U}_{\max}$   $F_{(3,58)} = 36.05$ ,  $P < 0.0005$ ;  $\hat{A}_{\max}$   $F_{(3,58)} = 21.07$ ,  $P < 0.0005$ ) (Fig. 3). In the short-horn sculpin, the adjusted mean  $\hat{U}_{\max}$  and  $\hat{A}_{\max}$  for both acclimation groups increased by 54.8% and 53.1%, respectively, between 0.8 and 15.0 °C. In long-spined sea scorpion, these values increased by 177.8% and 161.6%, respectively, over the same temperature range. Test temperature had a significant effect on  $\omega_{\max}$  ( $F_{(3,58)} = 5.55$ ,  $P = 0.002$ ) and CTA ( $F_{(3,54)} = 4.75$ ,  $P = 0.005$ ) in long-spined sea scorpion, but not in short-horn sculpin ( $P = 0.441$  for  $\omega_{\max}$ ,  $P = 0.393$  for CTA) (Table 1; Fig. 4). In sea scorpion, the mean

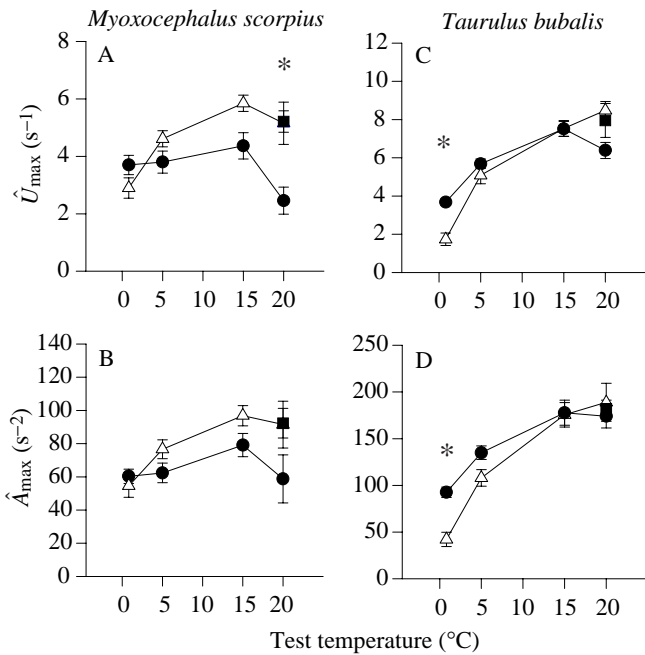


Fig. 3. Maximum length-specific velocity  $\hat{U}_{max}$  (A and C) and maximum length-specific acceleration  $\hat{A}_{max}$  (B and D) attained during the first two half-tailbeats of escape responses in short-horn sculpin (A,B) and long-spined sea scorpion (C,D). Acclimation temperatures were 5 °C (●), 15 °C (△) and 20 °C (■). Values represent means  $\pm$  S.E.M. Asterisks indicate significant differences between acclimation groups at a particular test temperature (short-horn sculpin, Tukey multiple-comparison test; long-spined sea scorpion, Mann–Whitney  $U$ -tests). For short-horn sculpin groups, 5@0.8, 5@5.0, 5@15.0, 5@20.0, 20@20.0, 15@20.0, 15@15.0, 15@5.0, 15@0.8 (acclimation temperature at test temperature, °C),  $N=9, 9, 9, 5, 11, 5, 10, 11, 9$ , respectively. For long-spined sea scorpion  $N=7, 12, 8, 7, 8, 8, 13, 9, 2$ , respectively. Sculpin were  $157.2 \pm 2.6$  mm in total body length  $L$ ,  $N=38$ ; for sea scorpion,  $L=116.0 \pm 1.2$  mm,  $N=35$ .

value of  $\omega_{max}$  increased by 136.6% and the mean value of CTA by 81.3% between test temperatures of 0.8 and 5.0 °C.

Acclimation temperatures of 5 and 15 °C had a significant effect on  $\hat{U}_{max}$  ( $F_{(1,59)}=13.52$ ,  $P=0.001$ ) and  $\hat{A}_{max}$  ( $F_{(1,59)}=7.68$ ,  $P=0.007$ ) in short-horn sculpin (two-way ANOVA) (Table 1; Fig. 3A,B). However, there was only a significant interaction between test and acclimation temperature for  $\hat{U}_{max}$  ( $F_{(3,59)}=6.02$ ,  $P=0.001$ ) (Fig. 3A). For 5 °C-acclimated short-horn sculpin, mean  $\hat{U}_{max}$  was  $3.7 \text{ s}^{-1}$  at 0.8 °C and  $3.8 \text{ s}^{-1}$  at 5.0 °C, giving a  $Q_{10}$  of 1.07 (Table 2). In contrast, the  $Q_{10}$  for the 15 °C-acclimated group was 3.02, with mean  $\hat{U}_{max}$  increasing from  $2.9 \text{ s}^{-1}$  at 0.8 °C to  $4.6 \text{ s}^{-1}$  at 5.0 °C. Similar differences in  $Q_{10}$  were found for  $\hat{A}_{max}$  (Table 2). However, Tukey tests revealed no significant differences in  $\hat{U}_{max}$  or  $\hat{A}_{max}$  at 0.8 or 5.0 °C between the two acclimation groups (Fig. 3A,B). In contrast, at 20.0 °C, the 20 °C- and 15 °C-acclimated fish had a significantly higher  $\hat{U}_{max}$  ( $5.2 \text{ s}^{-1}$ ) than the 5 °C-acclimated fish ( $2.5 \text{ s}^{-1}$ ) ( $P<0.001$  for the 20 °C- and 15 °C-acclimated fish,  $P<0.05$  for the 5 °C-acclimated fish; Tukey multiple-comparison test) (Fig. 3A). At this test temperature, only 20% of 5 °C-acclimated

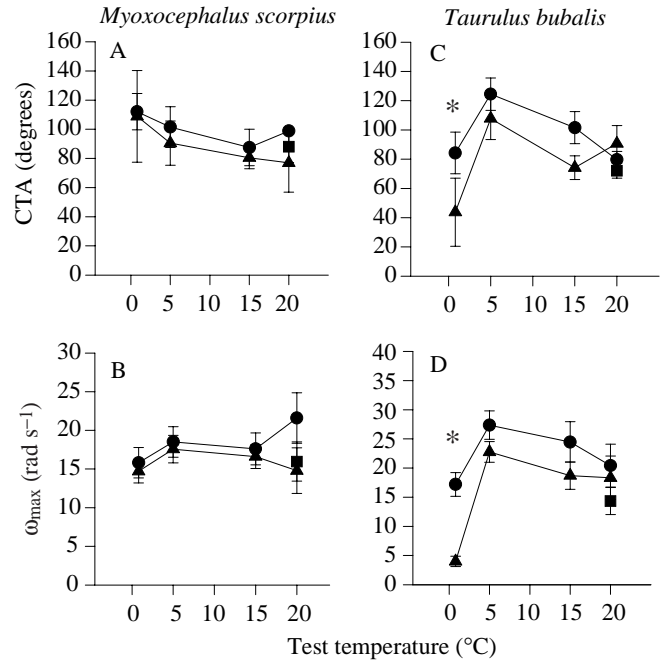


Fig. 4. Cumulative turning angle CTA (A and C) and maximum angular velocity  $\omega_{max}$  (B and D) attained during escape responses in short-horn sculpin (A,B) and long-spined sea scorpion (C,D). Acclimation temperatures were 5 °C (●), 15 °C (▲) and 20 °C (■). Values represent means  $\pm$  S.E.M. Asterisks indicate significant differences between the 5 and 15 °C acclimation groups at a particular test temperature (short-horn sculpin, Tukey multiple-comparison test; long-spined sea scorpion, Mann–Whitney  $U$ -tests). For short-horn sculpin groups, 5@0.8, 5@5.0, 5@15.0, 5@20.0, 20@20.0, 15@20.0, 15@15.0, 15@5.0, 15@0.8 (acclimation temperature at test temperature, °C) for CTA,  $N=8, 8, 8, 1, 10, 5, 10, 9, 3$ , respectively, and for  $\omega_{max}$ ,  $N=9, 9, 9, 5, 11, 5, 10, 11, 9$ , respectively. For long-spined sea scorpion for CTA,  $N=7, 12, 8, 5, 8, 8, 13, 7, 2$ , respectively, and for  $\omega_{max}$ ,  $N=7, 12, 8, 7, 8, 8, 13, 9, 2$ , respectively. Sculpin were  $157.2 \pm 2.6$  mm in total body length  $L$ ,  $N=38$ ; for sea scorpion,  $L=116.0 \pm 1.2$  mm,  $N=35$ .

fish completed two half-tailbeats compared with 90.9% of the 20 °C-acclimated fish and 80.0% of 15 °C-acclimated short-horn sculpin. At a test temperature of 15.0 °C, the 15 °C-acclimated sculpin had a mean  $\hat{U}_{max}$  of  $5.8 \text{ s}^{-1}$  compared with  $4.4 \text{ s}^{-1}$  for the 5 °C-acclimated fish ( $P<0.1$ , Tukey multiple-comparison test). In this species, CTA and  $\omega_{max}$  were not influenced by acclimation temperature ( $P=0.441$ ,  $P=0.097$ , respectively, two-way ANOVA) (Table 1; Fig. 4A,B).

In long-spined sea scorpion, two-way ANOVA revealed that acclimation temperature had no significant effect on  $\hat{U}_{max}$ ,  $\hat{A}_{max}$  or CTA ( $P=0.751$ ,  $P=0.136$ ,  $P=0.068$ , respectively) (Table 1; Figs 3C,D, 4C), but did significantly affect  $\omega_{max}$  ( $F_{(1,58)}=8.37$ ,  $P=0.005$ ), with higher values for 5 °C-acclimated fish (Fig. 4D). There was a significant interaction between acclimation and test temperature in  $\hat{U}_{max}$  ( $F_{(3,58)}=5.87$ ,  $P=0.001$ ) (Fig. 3C). Only two out of five fish acclimated to 15 °C were able to swim at 0.8 °C, although all individuals recovered fully following a rise in temperature. When zero was

Table 1. Analysis of variance for maximum velocity, maximum acceleration, maximum angular velocity and cumulative turning angle

Variable	Source	<i>Myoxocephalus scorpius</i>			<i>Taurulus bubalis</i>		
		d.f.	F-ratio	P	d.f.	F-ratio	P
$\hat{U}_{max}$ (s <sup>-1</sup> )	A	1	13.52	0.001	1	0.10	0.751
	B	3	8.38	0.0005	3	36.05	0.0005
	A×B	3	6.02	0.001	3	5.87	0.001
	Error	59			58		
$\hat{A}_{max}$ (s <sup>-2</sup> )	A	1	7.68	0.007	1	2.29	0.136
	B	3	6.76	0.001	3	21.07	0.0005
	A×B	3	2.04	0.118	3	1.60	0.199
	Error	59			58		
CTA (degrees)	A	1	0.61	0.441	1	3.46	0.068
	B	3	1.02	0.393	3	4.75	0.005
	A×B	3	0.06	0.982	3	1.13	0.344
	Error	43			54		
$\omega_{max}$ (rad s <sup>-1</sup> )	A	1	2.84	0.097	1	8.37	0.005
	B	3	0.91	0.441	3	5.55	0.002
	A×B	3	0.73	0.539	3	0.76	0.521
	Error	59			58		

A, acclimation temperature; B, test temperature; d.f., degrees of freedom;  $\hat{U}_{max}$ , maximum length-specific velocity;  $\hat{A}_{max}$ , maximum length-specific acceleration; CTA, cumulative turning angle;  $\omega_{max}$ , maximum angular velocity.

scored for fish that did not swim, Mann–Whitney *U*-tests revealed significantly higher values for all variables for 5 °C- than for 15 °C-acclimated fish at 0.8 °C ( $P<0.05$ ) (Figs 3C,D, 4C,D). Using mean values for fish that swam,  $\hat{U}_{max}$  was 3.7 s<sup>-1</sup> at 0.8 °C and 5.7 s<sup>-1</sup> at 5.0 °C in 5 °C-acclimated fish ( $Q_{10}=2.83$ ) (Table 2). For 15 °C-acclimated sea scorpion,  $\hat{U}_{max}$  was 1.7 s<sup>-1</sup> at 0.8 °C and 5.1 s<sup>-1</sup> at 5.0 °C ( $Q_{10}=12.88$ ) (Table 2). At the other end of the temperature range, no variable showed any significant difference between acclimation temperatures ( $P>0.05$ ).

Scaling of temperature acclimation responses

In both species of fish, maximum length-specific velocity of the midpoint decreased and response duration (in ms) increased with increasing total body length at all temperature regimes (5@5.0, 5@15.0, 15@15.0, 15@5.0 °C; acclimation temperature at test temperature) ( $P<0.05$ , *F*-statistic) (Fig. 5; Table 3). Absolute velocity ( $U_{max}$ , m s<sup>-1</sup>) increased with increasing total fish length for all groups of short-horn sculpin ( $P<0.01$ , *F*-statistic) apart from those acclimated to 5 °C and swum at 15.0 °C ( $P=0.865$ , *F*-statistic; slope exponent *b* of 0.02) (Table 3). In the long-spined sea scorpion,  $U_{max}$  increased significantly with total fish length for all groups ( $P<0.05$ , *F*-statistic) except those acclimated to 5 °C and swum at 5 °C ( $P=0.34$ , *F*-statistic; slope exponent *b*=0.11) (Table 3).

Using  $\hat{U}_{max}$  of short-horn sculpin as the response variable, the two-way GLM ANCOVA revealed that there was a significant interaction between habitat and temperature group ( $F_{(3,104)}=4.41$ ,  $P=0.006$ ), thus indicating differences in the acclimation ability of sculpin from the two localities (Table 4).  $\hat{U}_{max}$  scaled to a common slope exponent of  $-0.55$

for 5 °C-acclimated fish at a test temperature of 5.0 °C and for 15 °C-acclimated fish at test temperatures of 15.0 °C and 5.0 °C. In the latter acclimation group, values of the proportionality coefficient *a* were significantly different, with log<sub>10</sub>  $\hat{U}_{max}$  being 26.1 % higher in fish swimming at their acclimation temperature (test temperature) of 15.0 °C than at an acute temperature of 5.0 °C ( $P<0.05$ , Tukey multiple-comparison test) (Fig. 5C; Table 5). However, escape performance was not significantly different between acclimation groups at a test temperature of 5.0 °C (Fig. 6C; Table 6). The scaling relationship for  $\hat{U}_{max}$  changed to  $aL^{-0.98}$ , when 5 °C-acclimated short-horn sculpin were swum at an acute temperature of 15.0 °C (Figs 5A, 6A). This caused the scaling relationships (exponent *b*) to differ significantly

Table 2.  $Q_{10}$  values for 5 °C- and 15 °C-acclimated fish over the temperature ranges 0.8–15.0 °C and 0.8–5.0 °C

Variable	Acclimation temperature (°C)			
	<i>Myoxocephalus scorpius</i>		<i>Taurulus bubalis</i>	
	5.0	15.0	5.0	15.0
	Temperature range 0.8–15.0 °C			
$\hat{U}_{max}$ (s <sup>-1</sup> )	1.13	1.64	1.65	2.81
$\hat{A}_{max}$ (s <sup>-2</sup> )	1.21	1.50	1.58	5.20
	Temperature range 0.8–5.0 °C			
$\hat{U}_{max}$ (s <sup>-1</sup> )	1.07	3.02	2.83	12.88
$\hat{A}_{max}$ (s <sup>-2</sup> )	1.08	2.24	2.45	9.37

See Table 1 for an explanation of abbreviations.

Table 3. Regression data for the scaling of maximum length-specific velocity, maximum absolute velocity and response duration with temperature

Variable	Temperature	<i>Myoxocephalus scorpius</i>				<i>Taurulus bubalis</i>			
		<i>b</i>	<i>a</i>	<i>r</i> <sup>2</sup>	<i>P</i>	<i>b</i>	<i>a</i>	<i>r</i> <sup>2</sup>	<i>P</i>
$\dot{U}_{\max}$ (s <sup>-1</sup> )	5@5.0 °C	-0.66	2.05	0.63	<0.01	-0.89	2.56	0.68	<0.01
	5@15.0 °C	-0.98	2.79	0.75	<0.01	-0.79	2.51	0.81	<0.01
	15@15.0 °C	-0.50	1.87	0.76	<0.01	-0.71	2.34	0.66	<0.01
	15@5.0 °C	-0.49	1.70	0.42	<0.01	-0.70	2.15	0.47	<0.01
$U_{\max}$ (m s <sup>-1</sup> )	5@5.0 °C	0.34	-0.96	0.30	<0.01	0.11	-0.44	0.03	=0.34
	5@15.0 °C	0.02	-0.22	0.001	=0.87	0.21	-0.49	0.22	=0.02
	15@15.0 °C	0.50	-1.13	0.74	<0.01	0.29	-0.66	0.26	<0.01
	15@5.0 °C	0.51	-1.30	0.43	<0.01	0.31	-0.85	0.15	=0.05
Response duration (ms)	5@5.0 °C	0.45	1.17	0.54	<0.01	0.35	1.35	0.30	<0.01
	5@15.0 °C	0.63	0.64	0.57	<0.01	0.25	1.34	0.18	=0.04
	15@15.0 °C	0.33	1.24	0.35	<0.01	0.27	1.28	0.26	<0.01
	15@5.0 °C	0.30	1.51	0.19	=0.02	0.26	1.54	0.28	<0.01

Temperature denotes acclimation temperature at test temperature.

Values are given for the slope (exponent *b*), intercept (proportionality coefficient *a*), coefficient of determination (*r*<sup>2</sup>) and significance of regression (*P*).

$U_{\max}$ , maximum absolute velocity; all other abbreviations are defined in Table 1.

( $P < 0.01$ , Tukey multiple-comparison test) between fish swum at an acute temperature of 15.0 °C and those swimming at their acclimation temperature of 15.0 °C (Fig. 6A; Table 6). The first part of this study revealed that  $\dot{U}_{\max}$  at 15.0 °C was higher in adult fish acclimated to 15 °C than in those acclimated to 5 °C. However, the scaling of  $\dot{U}_{\max}$  changed with temperature such that the regression lines for these two groups converged for the small size range of fish, indicating that this was not true for the smaller fish;  $\dot{U}_{\max}$  at 15.0 °C was not higher in sculpin acclimated to 15 °C compared with those acclimated to 5 °C.

Using response duration as the response variable, the two-way GLM ANCOVA revealed there to be no significant interaction between habitat and temperature group. Thus, there was no significant change in the scaling of response duration in short-horn sculpin with acclimation temperature, the common slope exponent being 0.41. However, there was significant regression elevation (Table 5;  $P < 0.01$ , Tukey multiple-comparison test), with fish swimming at 15.0 °C having an approximately 9% shorter response duration than

fish swimming at 5.0 °C irrespective of previous acclimation temperature. This was also the pattern found in long-spined sea scorpion. Response duration scaled to a common slope exponent of 0.28, with fish swimming at 15.0 °C having significantly shorter response durations (by approximately 11%) than fish swimming at 5.0 °C, irrespective of thermal acclimation temperature ( $P < 0.01$ , Tukey multiple-comparison test). Furthermore,  $\dot{U}_{\max}$  of sea scorpion scaled to a common slope exponent of -0.76. Regardless of acclimation temperature, fish swimming at 15.0 °C had a significantly higher  $\dot{U}_{\max}$  than fish swimming at 5.0 °C (by approximately 18%) (Tables 5, 6; Figs 5B,D, 6B,D).

#### Summary of results

At a test temperature of 15.0 °C, short-horn sculpin achieved maximum length-specific velocities and accelerations during the escape response that were 34% and 22% higher, respectively, in 15 °C-acclimated than in 5 °C-acclimated fish. At a test temperature of 20.0 °C, these values increased to 110% and 55%, respectively. However, at cold temperatures of 5.0

Table 4. Analysis of covariance for maximum length-specific velocity of short-horn sculpin (size range 43–270 mm total length)

Source	d.f.	Sequential sum of squares	Adjusted sum of squares	Adjusted mean square	<i>F</i> -ratio	<i>P</i>
Length	1	337.008	58.486	58.486	35.36	0.000
Temperature group	3	83.178	87.540	29.180	17.64	0.000
Habitat	1	3.424	5.316	5.316	3.21	0.076
Temperature × habitat	3	21.906	21.906	7.302	4.41	0.006
Error	104	172.008	172.008	1.654		
Total	112	617.523				

A, temperature group; B, habitat; d.f., degrees of freedom.



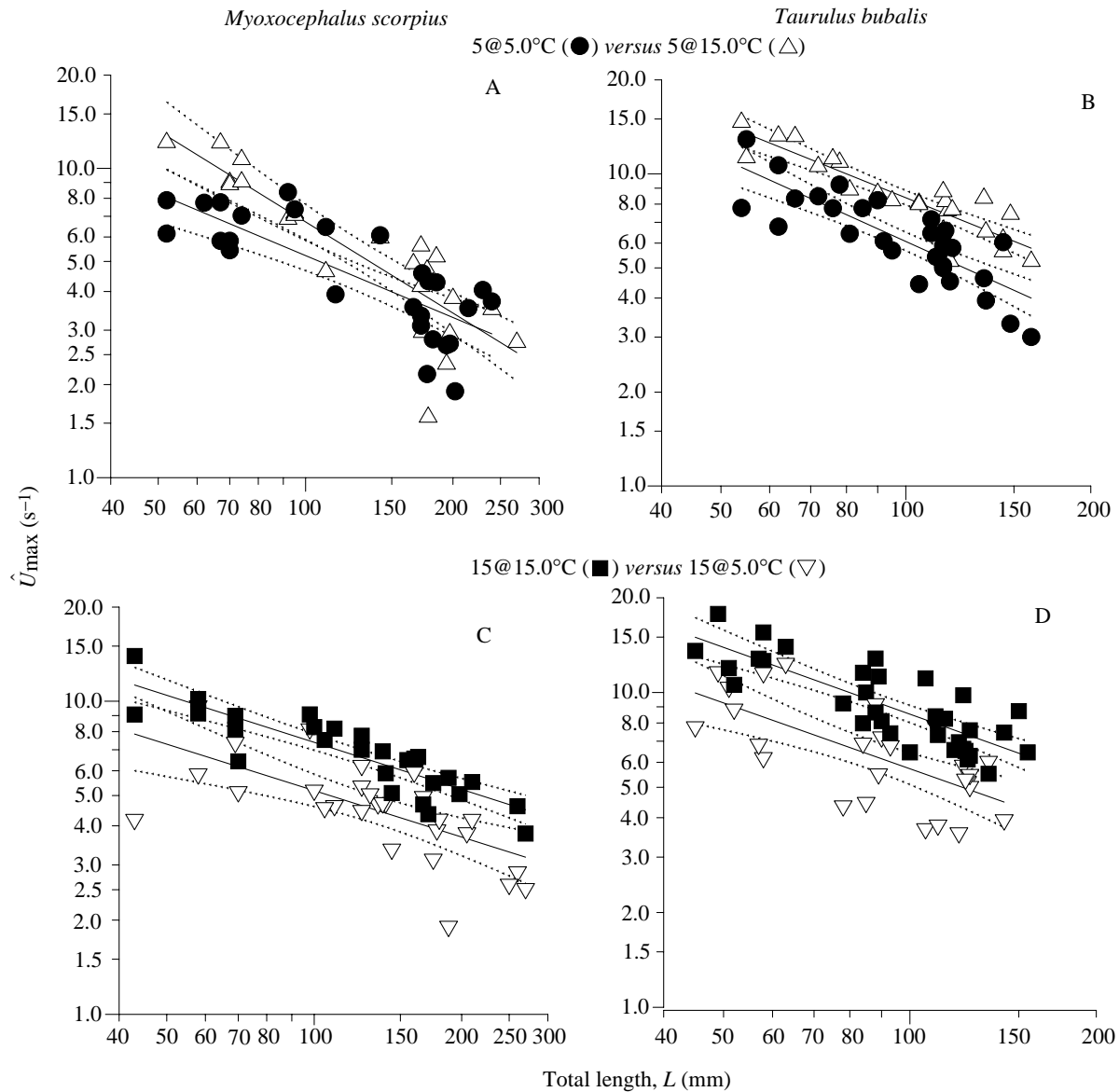


Fig. 5. The effect of test temperature on maximum length-specific velocity  $\hat{U}_{\max}$  attained during the first two half-tailbeats of escape responses for short-horn sculpin (A,C), size range 43–270 mm in total body length  $L$ , and long-spined sea scorpion (B,D),  $L=45$ –160 mm. 95% confidence intervals appear as dotted lines. Legends are acclimation temperature at test temperature. A and B are for fish acclimated to 5°C; C and D are for fish acclimated to 15°C.

and 0.8°C, 5°C-acclimated fish did not have significantly improved escape performance over 15°C-acclimated fish.

Over the test range 5.0–15.0°C, the long-spined sea scorpion only showed acclimation responses in maximum angular velocity, this being greater in 5°C- than in 15°C-acclimated fish. Over the extended temperature range 0.8–20.0°C, long-spined sea scorpion exhibited acclimation responses in all variables at low temperature (0.8°C). For example, maximum length-specific velocity and acceleration were 111% and 120% greater, respectively, in cold- than in warm-acclimated fish. At 20.0°C, there was no significant difference in any variable between acclimation temperatures.

In short-horn sculpin, the scaling of maximum length-specific velocity at 15°C was significantly different between

the two acclimation groups. Juvenile short-horn sculpin found on the shore and all stages of long-spined sea scorpion showed increased velocities upon acute exposure to 15.0°C, whereas adult short-horn sculpin showed enhanced velocity only following a period of thermal acclimation to 15°C.

## Discussion

### Testing of hypotheses

#### Hypothesis 1

Our first hypothesis stated that acclimation in short-horn sculpin improves escape performance at high temperature, but at the expense of performance at low temperature, compared with cold-acclimated fish. Consistent with this prediction, we

Table 5. Regression analysis of the effects of acute temperature on the scaling of maximum length-specific velocity and response duration for the groups 5@5.0, 5@15.0, 15@15.0 and 15@5.0 °C (acclimation temperature at test temperature)

Variable	Acute temperature effects			
	<i>Myoxocephalus scorpius</i>		<i>Taurulus bubalis</i>	
	5@5.0 vs 5@15.0 °C	15@15.0 vs 15@5.0 °C	5@5.0 vs 5@15.0 °C	15@15.0 vs 15@5.0 °C
$\hat{U}_{\max}$ (s <sup>-1</sup> )	–	$P<0.01$ (15@15.0 °C)	$P<0.01$ (5@15.0 °C)	$P<0.01$ (15@15.0 °C)
Response duration (ms)	$P<0.01$ (5@5.0 °C)	$P<0.01$ (15@5.0 °C)	$P<0.01$ (5@5.0 °C)	$P<0.01$ (15@5.0 °C)

All regressions were significant ( $P<0.05$ , ANOVA).

All results refer to differences in slope elevations (stage 2 ANCOVA) with the most elevated groups in parentheses.

For  $\hat{U}_{\max}$  of short-horn sculpin, this was assessed between groups 15@15.0 and 15@5.0 °C.

The regression for 5@15 °C in *M. scorpius* was responsible for significant differences in stage 1 ANCOVA and was therefore omitted from second stage ANCOVA analysis.

$\hat{U}_{\max}$ , maximum length-specific velocity.

found that acclimation to 15 °C and 20 °C clearly improved the swimming speed and acceleration at 15.0 and 20.0 °C over those of 5 °C-acclimated fish acutely exposed to high temperature. Our results echo those of Beddow *et al.* (1995), who examined the effect of acclimation to a summer temperature of 15 °C on the prey capture response in the same species. In their study, maximum velocity at 15 °C was 33 % higher in summer- than in winter-acclimated sculpin. This phenomenon is reflected in the power output of fast muscle fibres. Johnson and Johnston (1991) used the work loop technique to examine the maximum power output of fast muscle fibres performing oscillatory work in summer- and winter-acclimated short-horn sculpin. At 15 °C, summer-acclimated fish produced three times the power output of winter-acclimated fish. Similarly, Johnston *et al.* (1995) used the work loop technique under conditions simulating the first tailbeat of a fast-start. At 15 °C, power output increased from 6.3 W kg<sup>-1</sup> in 5 °C-acclimated fish to 23.8 W kg<sup>-1</sup> in 15 °C-

acclimated fish. Examination of the power output from the force–velocity ( $P$ – $V$ ) relationship, which gives a good estimate of the maximum instantaneous power output (Josephson, 1993), revealed that power was approximately six times higher in 15 °C- than in 5 °C-acclimated short-horn sculpin at 15 °C (Beddow and Johnston, 1995).

Contrary to our hypothesis, however, we found that acclimation to high temperature did not significantly compromise escape performance at low temperature in short-horn sculpin (Fig. 3A,B). Work loop studies using imposed sinusoidal length changes about resting muscle fibre length found that maximum power output at 4 °C showed little variation between summer- and winter-caught short-horn sculpin (Johnson and Johnston, 1991). The relaxation rates of fast muscle fibres in the short-horn sculpin were also found to be independent of acclimation temperature (Beddow and Johnston, 1995). However, these authors found that the  $P$ – $V$  relationship was less curved at 5 °C in fast muscle fibres from 5 °C- than from 15 °C-acclimated short-

Table 6. Regression analysis of the effects of acclimation temperature on the scaling of maximum length-specific velocity and response duration for the groups 5@5.0, 5@15.0, 15@15.0 and 15@5.0 °C (acclimation temperature at test temperature)

Variable	Effect of acclimation temperature			
	<i>Myoxocephalus scorpius</i>		<i>Taurulus bubalis</i>	
	Improvement in performance after acclimation to 15 °C (5@15.0 vs 15@15.0 °C)	Improvement in performance after acclimation to 5 °C (15@5.0 vs 5@5.0 °C)	Improvement in performance after acclimation to 15 °C (5@15.0 vs 15@15.0 °C)	Improvement in performance after acclimation to 5 °C (15@5.0 vs 5@5.0 °C)
$\hat{U}_{\max}$ (s <sup>-1</sup> )	Significant difference in slope exponent $b$ (stage 1 ANCOVA, $P<0.01$ )	NS	NS	NS
Response duration (ms)	NS	NS	NS	NS

NS, not significant;  $\hat{U}_{\max}$ , maximum length-specific velocity.

All regressions were significant ( $P<0.05$ , ANOVA). Unless stated otherwise, all results refer to differences in slope elevations (stage 2 ANCOVA).

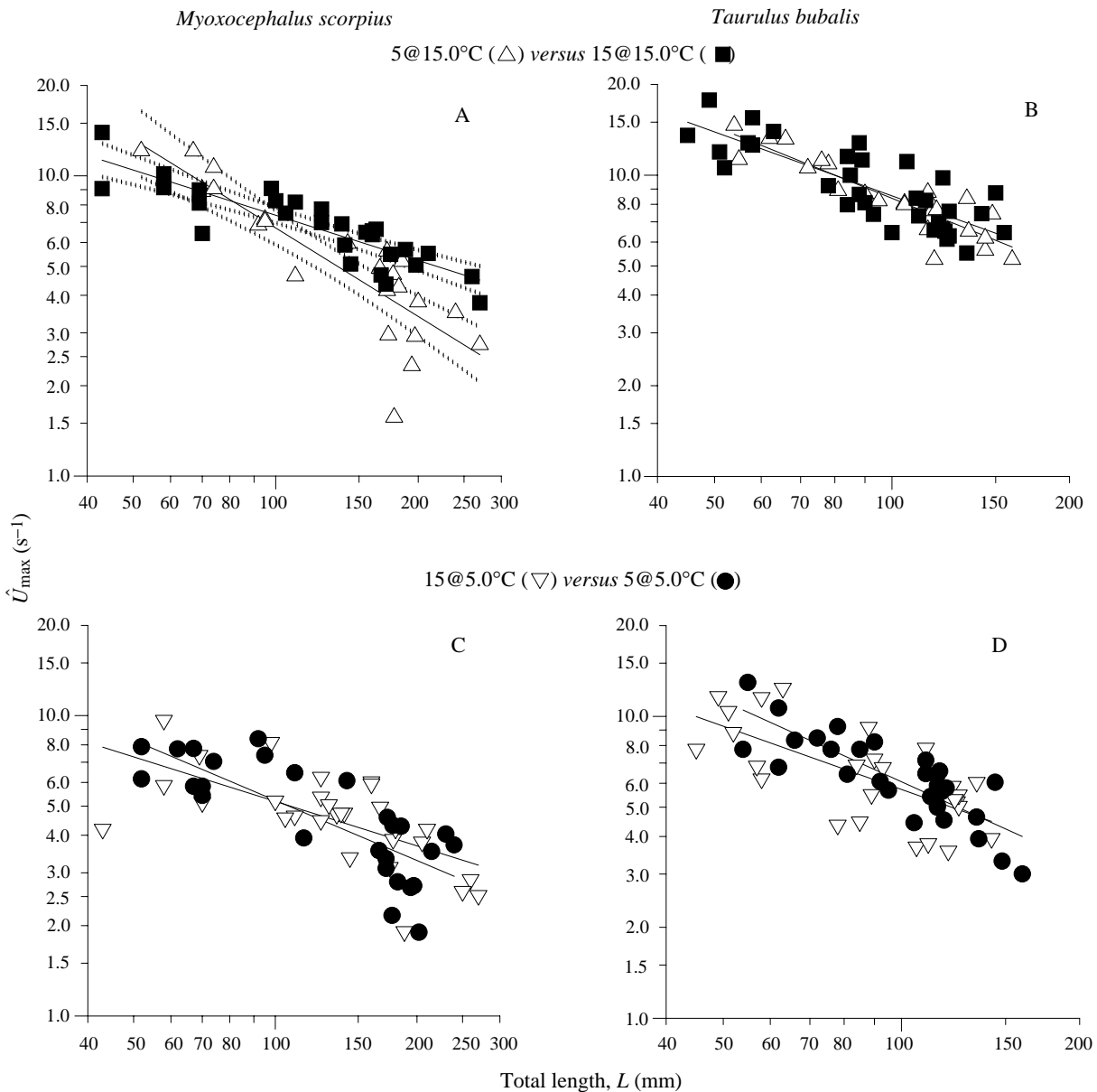


Fig. 6. The effect of acclimation temperature on maximum length-specific velocity  $\hat{U}_{\max}$  attained during the first two half-tailbeats of escape responses for short-horn sculpin (A,C), size range 43–270 mm in total body length  $L$ , and long-spined sea scorpion (B,D),  $L=45$ –160 mm. The data for each group are the same as in Fig. 5. 95% confidence intervals appear as dotted lines in A. Legends are acclimation temperature at test temperature. A and B are for fish tested at 15°C; C and D are for fish tested at 5°C.

horn sculpin. After normalising the curves for maximum force ( $P_0$ ) and velocity ( $V_{\max}$ ), this change in curvature produced a 40% increase in relative power output in 5°C-acclimated fish. It was concluded that the contractile properties of fibres are modified at low, as well as at high, temperatures.  $Q_{10}$  values in the present study did indicate a less thermally sensitive performance by 5°C- than by 15°C-acclimated fish. Between 0.8 and 5.0°C,  $\hat{U}_{\max}$  and  $\hat{A}_{\max}$  in the 5°C-acclimated fish were virtually unchanged, whereas  $Q_{10}$  values were 3.02 and 2.24, respectively, in the 15°C-acclimated fish (Table 2).

On the basis of these results, we can only accept part of our first hypothesis. Seasonal changes in fast-start performance

occurred predominantly at high temperature. Following warm acclimation, escape performance was improved. The emphasis of the hypothesis was on the trade-off in performance between high- and low-temperature acclimation. At low temperatures, acclimation was not detrimental but it did not improve performance and therefore there was no trade-off. Consequently, we must reject this part of the prediction. Likewise, in terms of the beneficial acclimation hypothesis, only warm acclimation was beneficial (as regards fast-start performance). Bennett and Lenski (1997) similarly found acclimation in *Escherichia coli* to be beneficial in some cases but not in others. In their experiments, fitness was measured

directly; in ours, we can only speculate that improved fast-start performance with warm acclimation may enhance individual survival and ultimately fitness.

### Hypothesis 2

Our second hypothesis concerned the inability of long-spined sea scorpion to acclimate over the average range of seasonal seawater temperatures (test temperatures 5.0–15.0 °C). The rationale was that temperature sensitivity is lower in organisms subjected to a high degree of short-term temperature variation, and that this variation would not provide a stable cue for acclimation. An increase in temperature accelerates most processes (Schmidt-Nielsen, 1990) and this did occur with the escape response, but it was unaffected by previous thermal history. At test temperatures of 5.0 and 15.0 °C, the values for  $\hat{U}_{\max}$  and  $\hat{A}_{\max}$  were not significantly different between acclimation groups (Fig. 3C,D). Furthermore, the study on the scaling of escape performance revealed there to be no significant differences in  $\hat{U}_{\max}$  and response duration between acclimation groups;  $\hat{U}_{\max}$  was always lower and response duration always longer at 5.0 than at 15.0 °C (Figs 5B,D, 6B,D; Tables 5, 6). Therefore, no effect of acclimation at these two test temperatures was found in these particular kinematic variables.

Acclimation temperature did affect  $\omega_{\max}$ , which is calculated from angle and time components and therefore reflects the CTA and response duration. As response durations were not significantly different between the two main acclimation temperatures,  $\omega_{\max}$  consequently mirrors CTA (although this was not significantly affected by acclimation temperature,  $P=0.068$ ), both being greater in 5 °C- than in 15 °C-acclimated sea scorpion over the test range of 5.0–15.0 °C (Fig. 4C,D). The functional significance of this is not known. However, greater body curvature during fast-starts has been found in fish from cold habitats compared with those from warm habitats (J. Wakeling, unpublished results). In the present study, the fact that one measure of fast-start performance changed with acclimation between 5.0 and 15.0 °C encourages a rejection of our second hypothesis. However, the existence of acclimation in this sole variable could still be an indication of a reduced acclimation response in long-spined sea scorpion over this temperature range.

With regards to test temperature, CTA was greater at a test temperature of 5.0 °C than at 15.0 °C in both acclimation groups (Fig. 4C). A greater degree of turning has been associated with submaximal responses (Domenici and Blake, 1991). Therefore, the high CTA may be a result of the lower values of  $\hat{U}_{\max}$  and  $\hat{A}_{\max}$  at test temperatures of 5.0 °C compared with 15.0 °C. In addition, long-spined sea scorpion at 5.0 °C may undertake more turning throughout the first two half-tailbeats as an avoidance mechanism, used to confuse predators, when higher values of velocity and acceleration are not attainable.

Over the entire thermal test range, acclimation responses were evident in  $\hat{U}_{\max}$ ,  $\hat{A}_{\max}$ , CTA and  $\omega_{\max}$  of adult long-spined sea scorpion, with 5 °C-acclimated fish having significantly

greater values at 0.8 °C than 15 °C-acclimated fish. In the strict sense, 'beneficial acclimation' has been defined as conferring an advantage at the temperature that produced the phenotypic response (Leroi *et al.* 1994). However, acclimation at 5 °C was clearly advantageous to the fish at 0.8 °C, because only 40 % of 15 °C-acclimated sea scorpion could swim at this temperature. We therefore believe cold acclimation to be beneficial in this species. There was little evidence for a trade-off in performance optimum temperature with thermal acclimation. This might have been manifest at higher test temperatures. However, higher temperatures would have been above those normally experienced by these fish and would therefore have been ecologically irrelevant. Therefore, in contrast to the short-horn sculpin, performance curves crossed at high temperature in the sea scorpion because seasonal changes in swimming performance were principally manifest at low temperature.

It is possible that acclimation ability is retained in this species, despite diurnal temperature fluctuations, because the seasonal differences in water temperatures are so large. The general seasonal change in temperatures may be sufficient to cue acclimation, although other factors are also known to have some control. For example, photoperiod is known to influence acclimation in some species (Kleckner and Sidell, 1985; Kolok, 1991). In the pupfish (*Cyprinodon neuadensis amargosae*), a eurythermal desert stream fish, acclimation has been achieved using a cycling thermal regime. The fish adapted their metabolic rate to both high and low daily temperatures, increasing their thermal tolerance by 2 °C (Feldmeth *et al.* 1974).

### Hypothesis 3

Our third hypothesis was accepted over the temperature range tested. In short-horn sculpin, the ability to acclimate maximum speed  $\hat{U}_{\max}$  thermally was acquired during ontogeny. Sculpin exhibited differential responses in reaction to temperature change depending on acclimation temperature and stage of development. In contrast to adults found offshore, juveniles from the intertidal and shallow subtidal zones increased  $\hat{U}_{\max}$  upon acute exposure to 15.0 °C (Fig. 5A) but did not increase  $\hat{U}_{\max}$  any further following acclimation to 15 °C (Fig. 6A). At a test temperature of 5.0 °C, all sizes of sculpin failed to improve  $\hat{U}_{\max}$  following acclimation to 5 °C (Fig. 6C). All stages of long-spined sea scorpion lacked compensation in  $\hat{U}_{\max}$  following acclimation to 5 or 15 °C. We conclude that thermal sensitivity in the short-horn sculpin changes during ontogeny, such that the potential to exhibit acclimation responses to these temperatures is acquired concomitant with a migration to deeper, more thermally stable but seasonally fluctuating, water mass.

### Scaling of burst swimming speed

Maximum length-specific velocities decreased with increasing total fish length in both species of Cottidae, with absolute velocities increasing with length for the majority of temperature groups. Bainbridge (1958) measured swimming

speed in dace (*Leuciscus leuciscus*), trout (*Salmo irideus*) and goldfish (*Carassius auratus*) and found that absolute speed increased with length at any particular tailbeat frequency. Size similarly affected maximum absolute speed during escape responses in rainbow trout (*Salmo gairdneri*) (Webb, 1976) and turbot (*Scophthalmus maximus*) larvae (Gibson and Johnston, 1995), but not in angelfish (*Pterophyllum eimekei*) (Domenici and Blake, 1993). On examination of the literature, Wardle and He (1988) found that maximum velocity scaled to an average  $aL^{0.85}$ . In turbot larvae, Gibson and Johnston (1995) found that the maximum length-specific velocity scaled to  $aL^{-0.69}$ . Maximum length-specific velocity scaled to an average  $aL^{-0.55}$  in short-horn sculpin (all tests except 5@15.0 °C) and to  $aL^{-0.76}$  in long-spined sea scorpion. Response durations were found to increase with size, as was also found in rainbow trout (Webb, 1976) and angelfish fast-starts (Domenici and Blake, 1993). Archer and Johnston (1989) found that length-specific tailbeat amplitude in the Antarctic fish *Notothenia coriiceps* was also dependent on size and decreased with increasing fish length.

Changes in swimming performance with increasing size can be related to increases in myotomal area, mass and length, and fibre length (Webb, 1978; Webb and Johnstrude, 1988; Archer *et al.* 1990). Isotonic contraction (Wardle, 1975) and isometric twitch contraction times of fast muscle fibres increase with size (Archer *et al.* 1990), in part reflecting tailbeat duration and, hence, maximum speed (Wardle, 1975). James *et al.* (1998) have recently examined scaling in the short-horn sculpin and found myofibrillar ATPase activity of fast muscle to decline with length, a situation found in a number of marine teleosts (Witthames and Greer-Walker, 1982). However, this was not related to changes in MHC or MLC ratios with increasing size, but accompanied changes in Troponin I isoforms (James *et al.* 1998).

Swimming performance also alters with size because of the change in the ratio of inertial to viscous forces (Reynold's number, *Re*) during growth. However, *Re* is most relevant to larvae, for which viscous forces dominate swimming performance. Although the viscous hydrodynamic regime has recently been found to extend to a higher *Re* than previously thought (Fuiman and Batty, 1997), the fish used in our study were juveniles whose swimming performance would have been dominated by inertial forces.

#### *Evolutionary significance of temperature acclimation responses*

Phenotypic plasticity has evolutionary significance only if it enhances fitness (Leroi *et al.* 1994). The most compelling studies of the beneficial acclimation hypothesis have used organisms with short generation times, such as bacteria (Leroi *et al.* 1994; Bennett and Lenski, 1997), for which fitness could be assessed directly. In order to study any organisms with longer generation times, correlates usually have to be used. In the present study, we did not measure fitness directly but used the escape response as a correlate. Warm acclimation was found to be beneficial and to improve fast-start performance in

adult short-horn sculpin, whereas cold acclimation appeared to offer no benefit. In the long-spined sea scorpion, these results were reversed. If we had measured fitness *per se* and obtained similar results, we would be encouraged to 'reject the generality of the beneficial acclimation assumption' (Leroi *et al.* 1994), since acclimation did not always confer an advantage in either species.

The influence of fast-start performance on fitness, however, is not known in these species, and perhaps we should question the assumption that this performance parameter is a good indicator of fitness. The short-horn sculpin extends to a high latitude and is a cold-tolerant species which excretes a polypeptide antifreeze from the liver during the winter months (Fletcher *et al.* 1989). This perhaps indicates an obvious adaptation to its environment, but not one that is manifest in a significantly improved escape performance following cold acclimation. For cold temperatures to restrict locomotor capacity is not uncommon (see Bennett, 1990, for a review). A combination of lower predation pressure in the winter and a preferential investment in cold tolerance rather than fast-start capability may perhaps explain why locomotor and muscle contractile compensations (Johnson and Johnston, 1991) are lacking at low temperature in this species. Only further studies including field experiments could adequately address this question.

It should be noted that these experiments were conducted at different times of the year that corresponded to a particular acclimation group. This was to avoid disrupting the natural seasonal rhythms of the fish too greatly and thereby to enhance our ability to detect acclimation responses if present. Although gravid fish were not used, our results may have been influenced by some factor other than temperature, such as hormonal status. However, in a previous study, Beddow and Johnston (1995) found fast muscle contractile properties in short-horn sculpin to be similar between 15 °C- and 5 °C-laboratory-acclimated short-horn sculpin and summer- and winter-caught fish, respectively. We therefore believe that the thermal acclimation conditions used in the present study are relevant to the field situation.

We conclude that escape performance was significantly altered by acclimation temperature in both the short-horn sculpin and the long-spined sea scorpion. Walsh *et al.* (1997) examined the temperature tolerance of a number of different species and populations of freshwater sculpin in order to explain habitat distributions. Stream-dwelling species and populations were expected to be more stenothermal than those inhabiting thermally labile springs. Although they found this in a number of cases, they did not find support for the theory that temperature alone accounts for the distribution patterns observed. In the present study, we found acclimation responses in both species of marine Cottidae that differed from predicted patterns. However, there were interspecific and intraspecific differences in these patterns which we believe may reflect local niche distributions, although other factors are undoubtedly important.

This work was supported by the Natural Environment

Research Council. The authors wish to thank Dr James Wakeling for the kinematic programmes and Nicholas Cole for the photographs.

### References

- ANDRASO, G. M. (1997). A comparison of startle response in two morphs of the brook stickleback (*Culaea inconstans*): Further evidence for a trade-off between defensive morphology and swimming ability. *Evol. Ecol.* **11**, 83–90.
- ARCHER, S. D., ALTRINGHAM, J. D. AND JOHNSTON, I. A. (1990). Scaling effects on the neuromuscular system, twitch kinetics and morphometrics of the cod, *Gadus morhua*. *Mar. Behav. Physiol.* **17**, 137–146.
- ARCHER, S. D. AND JOHNSTON, I. A. (1989). Kinematics of labriform and subcarangiform swimming in the Antarctic fish *Notothenia neglecta*. *J. exp. Biol.* **143**, 195–210.
- ARNOLD, S. J. (1983). Morphology, performance and fitness. *Am. Zool.* **23**, 347–361.
- BAINBRIDGE, R. (1958). The speed of swimming of fish as related to size and to the frequency of the tail beat. *Exp. Biol.* **53**, 109–133.
- BEDDOW, T. A. AND JOHNSTON, I. A. (1995). Plasticity of muscle contractile properties following temperature acclimation in the marine fish *Myoxocephalus scorpius*. *J. exp. Biol.* **198**, 193–201.
- BEDDOW, T. A., VAN LEEUWEN, J. L. AND JOHNSTON, I. A. (1995). Swimming kinematics of fast starts are altered by temperature acclimation in the marine fish *Myoxocephalus scorpius*. *J. exp. Biol.* **198**, 203–208.
- BENNETT, A. F. (1990). Thermal dependence of locomotor capacity. *Am. J. Physiol.* **259**, R253–R258.
- BENNETT, A. F. AND LENSKI, R. E. (1997). Evolutionary adaptations to temperature. VI. Phenotypic acclimation and its evolution in *Escherichia coli*. *Evolution* **51**, 36–44.
- BUCKLEY, L. J. (1982). Effects of temperature on growth and biochemical composition of larval winter flounder *Pseudopleuronectes americanus*. *Mar. Ecol. Prog. Ser.* **8**, 181–186.
- BULLOCK, T. H. (1955). Compensation for temperature in the metabolism and activity of poikilotherms. *Biol. Rev.* **30**, 311–342.
- CROCKFORD, T. AND JOHNSTON, I. A. (1990). Temperature acclimation and the expression of contractile protein isoforms in the skeletal muscles of the common carp (*Cyprinus carpio* L.). *J. comp. Physiol.* **160B**, 23–30.
- DOMENICI, P. AND BLAKE, R. W. (1991). The kinematics and performance of the escape response in the angelfish (*Pterophyllum eimekei*). *J. exp. Biol.* **156**, 187–205.
- DOMENICI, P. AND BLAKE, R. W. (1993). The effect of size on the kinematics and performance of angelfish (*Pterophyllum eimekei*) escape responses. *Can. J. Zool.* **71**, 2319–2326.
- EGGINTON, S. AND SIDELL, B. D. (1989). Thermal acclimation induces adaptive changes in subcellular structure of fish skeletal muscle. *Am. J. Physiol.* **256**, R1–R9.
- FELDMETH, C. R., STONE, E. A. AND BROWN, J. H. (1974). An increased scope for thermal tolerance upon acclimating pupfish (*Cyprinodon*) to cycling temperatures. *J. comp. Physiol.* **89**, 39–44.
- FISHER, K. C. (1958). An approach to the organ and cellular physiology of adaptation to temperature in fish and small mammals. In *Physiological Adaptation* (ed. C. L. Prosser), pp. 3–49. Washington, DC: American Physiological Society.
- FLETCHER, G. L., KING, M. J., KAO, M. H. AND SHEARS, M. A. (1989). Antifreeze proteins in the urine of marine fish. *Fish Physiol. Biochem.* **6**, 121–127.
- FOSTER, M. A. (1969). Ionic and osmotic regulation in three species of *Cottus* (Cottidae, Teleost). *Comp. Biochem. Physiol.* **30**, 751–759.
- FRY, F. E. J. AND HART, J. S. (1948). Cruising speed of goldfish in relation to water temperature. *J. Fish. Res. Bd Can.* **7**, 169–175.
- FUIMAN, L. A. AND BATTY, R. S. (1997). What a drag it is getting cold: partitioning the physical and physiological effects of temperature on fish swimming. *J. exp. Biol.* **200**, 1745–1755.
- GERLACH, G.-F., TURAY, L., MALIK, K. T. A., LIDA, J., SCUTT, A. AND GOLDSPIK, G. (1990). Mechanisms of temperature acclimation in the carp: a molecular biology approach. *Am. J. Physiol.* **259**, R237–R244.
- GIBSON, R. N. (1969). The biology and behaviour of littoral fish. *Oceanogr. mar. Biol. A. Rev.* **7**, 367–410.
- GIBSON, S. AND JOHNSTON, I. A. (1995). Scaling relationships, individual variation and the influence of temperature on maximum swimming speed in early settled stages of the turbot *Scophthalmus maximus*. *Mar. Biol.* **121**, 401–408.
- HARPER, D. G. AND BLAKE, R. W. (1989). On the error involved in high-speed film used to evaluate maximum acceleration of fish. *Can. J. Zool.* **67**, 1929–1936.
- HAZEL, J. R. AND PROSSER, C. L. (1974). Molecular mechanisms of temperature compensation in poikilotherms. *Physiol. Rev.* **54**, 620–677.
- HEAP, S. P., WATT, P. W. AND GOLDSPIK, G. (1986). Myofibrillar ATPase activity in the carp (*Cyprinus carpio*): interactions between starvation and environmental temperature. *J. exp. Biol.* **123**, 373–382.
- HIRAYAMA, Y., KANO, S., NAKAYA, M. AND WATABE, S. (1997). The two essential light chains of carp fast skeletal myosin, LC1 and LC3, are encoded by distinct genes and change their molar ratio following temperature acclimation. *J. exp. Biol.* **200**, 693–701.
- HOVENKAMP, F. AND WITTE, J. I. J. (1991). Growth, otolith growth and RNA/DNA ratios of larval plaice *Pleuronectes platessa* in the North Sea 1987–1989. *Mar. Ecol. Prog. Ser.* **70**, 105–116.
- HUEY, R. B. AND BERRIGAN, D. A. (1996). Testing evolutionary hypotheses of acclimation. In *Animals and Temperature Phenotypic and Evolutionary Adaptation* (ed. I. A. Johnston and A. F. Bennett), pp. 205–237. Cambridge: Cambridge University Press.
- HUEY, R. B. AND STEVENSON, R. D. (1979). Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. *Am. Zool.* **19**, 357–366.
- HWANG, G. C., OCHIAI, Y., WATABE, S. AND HASHIMOTO, K. (1991). Changes in the carp myosin subfragment-1 induced by temperature acclimation. *J. comp. Physiol.* **161B**, 141–146.
- IMAI, J.-I., HIRAYAMA, Y., KIKUCHI, K., KATINUMA, M. AND WATABE, S. (1997). cDNA cloning of myosin heavy chain isoforms from carp fast skeletal muscle and their gene expression associated with temperature acclimation. *J. exp. Biol.* **200**, 27–34.
- JAMES, R. S., COLE, N. J., DAVIES, M. L. F. AND JOHNSTON, I. A. (1998). Scaling of intrinsic properties and myofibrillar composition of fast-muscle fibres in the short-horn sculpin (*Myoxocephalus scorpius*). *J. exp. Biol.* (in press).
- JOHNSON, T. P. AND BENNETT, A. F. (1995). The thermal acclimation of burst swimming escape performance in fish: an integrated study of molecular and cellular physiology and organismal performance. *J. exp. Biol.* **198**, 2165–2175.
- JOHNSON, T. P. AND JOHNSTON, I. A. (1991). Power output of fish

- muscle fibres performing oscillatory work: effects of acute and seasonal temperature change. *J. exp. Biol.* **157**, 409–423.
- JOHNSTON, I. A., DAVISON, W. AND GOLDSPIK, G. (1975). Adaptations of  $Mg^{2+}$ -activated myofibrillar ATPase activity induced by temperature acclimation. *FEBS Lett.* **50**, 293–295.
- JOHNSTON, I. A. AND LUCKING, M. (1978). Temperature induced variation in the distribution of different types of muscle fibres in the goldfish (*Carassius auratus*). *J. comp. Physiol.* **124**, 111–116.
- JOHNSTON, I. A. AND MAITLAND, B. (1980). Temperature acclimation in crucian carp: a morphometric study of muscle fibre ultrastructure. *J. Fish Biol.* **17**, 113–125.
- JOHNSTON, I. A., VAN LEEUWEN, J. L., DAVIES, M. L. F. AND BEDDOW, T. (1995). How fish power predation fast-starts. *J. exp. Biol.* **198**, 1851–1861.
- JOSEPHSON, R. K. (1993). Contraction dynamics and power output of skeletal muscle. *A. Rev. Physiol.* **55**, 527–546.
- KING, P. A. AND FIVES, J. M. (1983). Littoral and benthic investigations on the west coast of Ireland. XVI. The biology of the long-spined sea scorpion *Taurulus bubalis* (Euphrases, 1786) in the Galway Bay area. *Proc. R. Irish Acad.* **83B**, 215–239.
- KING, P. A., FIVES, J. M. AND DUNNE, J. (1983). Littoral and benthic investigations on the west coast of Ireland. XVIII. The biology of the short-spined sea scorpion *Myoxocephalus scorpius* (L.) in the Galway Bay area. *Proc. R. Irish Acad.* **83B**, 325–334.
- KLECKNER, N. W. AND SIDELL, B. D. (1985). Comparisons of maximal activities of enzymes from tissues of thermally-acclimated and naturally-acclimatized chain pickerel (*Esox niger*). *Physiol. Zool.* **58**, 18–28.
- KOLOK, A. S. (1991). Photoperiod alters the critical swimming speed of juvenile largemouth bass, *Micropterus salmoides*, acclimated to cold water. *Copeia* **4**, 1085–1090.
- LANGFELD, K. S., CROCKFORD, T. AND JOHNSTON, I. A. (1991). Temperature acclimation in the common carp: force-velocity characteristics and myosin subunit composition of slow muscle fibres. *J. exp. Biol.* **155**, 291–304.
- LEROI, A. M., BENNETT, A. F. AND LENSKI, R. E. (1994). Temperature acclimation and competitive fitness: an experimental test of the beneficial acclimation assumption. *Proc. natn. Acad. Sci. U.S.A.* **91**, 1917–1921.
- MARDEN, J. H. (1995). Large-scale changes in thermal sensitivity of flight performance during adult maturation in a dragonfly. *J. exp. Biol.* **198**, 2095–2102.
- MARDEN, J. H., KRAMER, M. G. AND FRISCH, J. (1996). Age-related variation in body temperature, thermoregulation and activity in a thermally polymorphic dragonfly. *J. exp. Biol.* **199**, 529–535.
- MORRIS, S. AND TAYLOR, A. C. (1983). Diurnal and seasonal variation in physico-chemical conditions within intertidal rock pools. *Est. coast. Shelf Sci.* **17**, 339–355.
- PRECHT, H. (1958). Concepts of the temperature adaptation of unchanging reaction systems of cold-blooded animals. In *Physiological Adaptation* (ed. C. L. Prosser), pp. 50–78. Washington, DC: American Physiological Society.
- SCHNEIDER, S. M. (1993). Genetics and evolution of phenotypic plasticity. *A. Rev. Ecol. Syst.* **24**, 35–68.
- SCHMIDT-NIELSEN, K. (1990). *Animal Physiology Adaptation and Environment*. Cambridge: Cambridge University Press.
- SIDELL, B. D. (1980). Response of goldfish (*Carassius auratus* L.) to acclimation temperature: alterations in biochemistry and proportions of different fibre types. *Physiol. Zool.* **53**, 98–107.
- SIDELL, B. D., JOHNSTON, I. A., MOERLAND, T. S. AND GOLDSPIK, G. (1983). The eurythermal myofibrillar protein complex of the mummichog (*Fundulus heteroclitus*): adaptation to a fluctuating thermal environment. *J. comp. Physiol.* **153**, 167–173.
- SWAIN, D. P. (1992). The functional basis of natural selection for vertebral traits of larvae in the stickleback *Gasterosteus aculeatus*. *Evolution* **46**, 987–997.
- UNESCO (1986). *Fishes of the North-eastern Atlantic and the Mediterranean*, vol. 3 (ed. P. J. P. Whitehead, M.-L. Bauchot, J.-C. Hureau, J. Nielsen and E. Tortonese). Paris: Unesco.
- WALSH, S. J., HANEY, D. C. AND TIMMERMAN, C. M. (1997). Variation in thermal tolerance and routine metabolism among spring- and stream-dwelling freshwater sculpins (Teleostei: Cottidae) of the southeastern United States. *Ecol. freshw. Fish* **6**, 84–94.
- WARDLE, C. S. (1975). Limit of fish swimming speed. *Nature* **255**, 725–727.
- WARDLE, C. S. AND HE, P. (1988). Burst swimming speeds of mackerel, *Scomber scombrus* L. *J. Fish Biol.* **32**, 471–478.
- WATKINS, T. B. (1996). Predator-mediated selection on burst swimming performance in tadpoles of the Pacific tree frog, *Pseudacris regilla*. *Physiol. Zool.* **69**, 154–167.
- WEBB, P. W. (1976). The effect of size on the fast-start performance of rainbow trout (*Salmo gairdneri*) and a consideration of piscivorous predator-prey interactions. *J. exp. Biol.* **65**, 157–177.
- WEBB, P. W. (1978). Fast-start performance and body form in seven species of teleost fish. *J. exp. Biol.* **74**, 211–226.
- WEBB, P. W. AND JOHNSTRUDE, C. L. (1988). The effect of size on the mechanical properties of the myotomal-skeletal system of rainbow trout (*Salmo gairdneri*). *Fish Physiol. Biochem.* **5**, 163–171.
- WEIHS, D. (1973). The mechanism of rapid starting in slender fish. *Biorheology* **10**, 343–350.
- WITTHAMES, P. R. AND GREER-WALKER, M. (1982). The activity of myofibrillar and actomyosin ATPase in the skeletal muscle of some marine teleosts in relation to their length and age. *J. Fish Biol.* **20**, 471–478.
- ZAMUDIO, K. R., HUEY, R. B. AND CRILL, W. D. (1995). Bigger isn't always better: body size, developmental and parental temperature and territorial success in *Drosophila melanogaster*. *Anim. Behav.* **49**, 671–677.
- ZAR, J. H. (1996). *Biostatistical Analysis*. London: Prentice-Hall International. 662pp.