



Influence of spawning on swimming performance and muscle contractile properties in the short-horn sculpin

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The total distance travelled during the first two kinematic stages of the escape response of short-horn sculpin was significantly greater in post spawning fish (0.41 L) than in gravid fish (0.23 L). The maximum velocity of the snout during the C-bend was significantly higher ($5.6 L s^{-1}$) in postspawning fish than in gravid fish ($3.8 L s^{-1}$). To investigate some of the mechanisms underlying changes in swimming performance, the contractile properties of fast muscle fibres were determined in fish of similar body length. The rate of tetanic force relaxation (time from last stimulus to 50% peak force) was 34% faster in gravid than in postspawning fish. Maximum contraction velocity, determined by the slack-test method, was significantly higher in gravid than in postspawning fish ($6.8 v. 5.9$ muscle lengths s^{-1}). In contrast, both maximum isometric stress and power output (determined from the force-velocity relationship) were >50% higher in fibres from postspawning than from gravid fish, even though myofibrillar protein and water contents were similar ($120 mg g^{-1}$ wet mass and 86% of body mass, respectively). The results show that swimming performance and the contractile properties of fast muscle fibres vary with the reproductive cycle in short-horn sculpin acclimated to the same photoperiodic and temperature regime.

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Key words: contractile properties; locomotion; myotomal muscle; *Myoxocephalus scorpius*; reproduction; temperature.

INTRODUCTION

Fish use fast-starts involving the recruitment of the entire mass of white muscle fibres to escape predators and catch prey (Domenici & Blake, 1997). Fast-start performance is modified by acclimation temperature in goldfish *Carassius auratus* L. (Johnson & Bennett, 1995), short-horn sculpin *Myoxocephalus scorpius* L. (Beddow *et al.* 1995; Temple & Johnston, 1998), and long spine sea scorpion *Taurulus bubalis* Euphrasen; (Temple & Johnston, 1998). For example, in adult short-horn sculpin the maximum velocity attained during escape responses at 20° C was 110% greater in fish acclimated to 15° C than to 5° C (Temple & Johnston, 1998).

Studies using isolated fast muscle fibres have shown that the maximum stress and shortening velocity are significantly higher in 15° C- than in 5° C-acclimated short-horn sculpin (Beddow & Johnston, 1995). Johnston *et al.* (1995) measured the power output of fast muscle fibres under conditions simulating predation fast-starts on shrimps *Crangon crangon* using a modification of the work loop technique developed by Josephson (1985). Isolated muscle fibres from 5° C- and 15° C-acclimated fish developed 12 and 23 W kg^{-1} (wet muscle mass),

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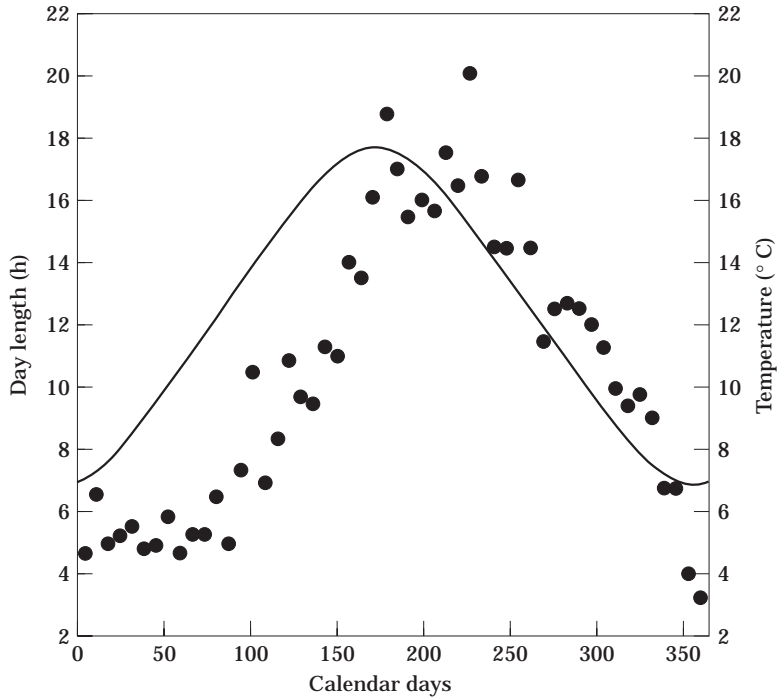


FIG. 1. The relationship between calendar days (starting at 1 January) and day length/sea temperature in St Andrews, Scotland. The curved line and the closed symbols represent day length and weekly mean sea temperatures, respectively, measured in 1995. The straight line represents the spawning season for short-horn sculpin during 1995.

respectively, during the initial C-bend of the fast-start at 15°C . This increase in muscle power output with temperature acclimation was associated with a 33% increase in maximum swimming velocity and an increase in prey capture success rate from 23 to 74%.

Short-horn sculpin eggs increase in diameter during the 3 months prior to spawning (Ennis, 1970; King *et al.*, 1983). In St Andrews Bay the short-horn sculpin spawns during February and March, when sea temperature is at a seasonal low of $4\text{--}5^{\circ}\text{C}$ (Fig. 1), and the eggs and larvae develop as sea temperature rises during spring and early summer. Altringham & Johnston (1988a) noticed a decrease in the physical condition of white myotomal muscle as spawning approached. Stimulation of the motor nerve always resulted in overshooting action potentials in the white muscle outside the spawning season, whereas in gravid females junction potentials were often observed in the absence of action potentials, resulting in a failure to elicit a twitch response. Muscle resting potentials were lower in gravid than postspawning females.

At temperate latitudes some fish reduce their food consumption in winter at the same time that energy reserves are mobilized from the liver and muscle to build up the gonadal tissue (Love, 1980). Prolonged starvation lasting several months results in changes in metabolic enzyme profiles (Moon & Johnston, 1980; Black & Love, 1986), an increase in proteolytic enzyme activities (Beardall &

Johnston, 1985), the mobilization of myofibrillar proteins and increased water content in white muscle (Johnston & Goldspink, 1973; Love, 1980).

The aim of the present study was to compare fast-start performance between gravid and postspawning short-horn sculpin acclimated to the same photo-periodic and temperature regime. The results showed that gravid females had a significantly lower maximum swimming velocity. A number of possible mechanisms could have been responsible for the decrease in swimming performance in gravid fish, including changes in the contractile properties of the muscle due to muscle disuse atrophy or starvation, a decreased ratio of skeletal muscle mass to body mass and/or alterations in the patterns of drag or bending during locomotion due to the increased girth of the fish. A comparison of the contractile properties of muscle in gravid and postspawning fish was made and the possible mechanisms underlying altered locomotory and muscular performance during spawning are discussed.

MATERIALS AND METHODS

ANIMALS

Female short-horn sculpin were caught in lobster creels or by trawling in the Firth of Forth/St Andrews Bay by local fishermen during January and June in both 1995 and 1996. Fish caught in 1995 were used to investigate the effects of reproductive state on the contractile properties of muscle. Fish caught in 1996 were used to investigate the effects of reproductive state on swimming performance. Weekly mean sea temperatures measured in St Andrews Bay during 1995 ranged from 5–6° C in January to 14–19° C in June (Fig. 1). Fish caught in January (gravid) and June (postspawning) were held in re-circulating aquaria at 5–6° C (15 D : 9 L) for 1–4 weeks and 6 weeks, respectively. Fish were fed twice a week on chopped whitebait or live shrimps. The spawning date was recorded for all fish kept in the aquarium to ensure that gravid fish chosen for experimental studies were close to spawning.

MORPHOMETRICS

Total body length and mass were determined for all fish used in this study. The mass of the ovaries and the maximum girth (measured posterior to the operculum using a piece of string) were determined for all fish used for studies of the contractile properties of muscle.

SWIMMING PERFORMANCE

Gravid ($n=4$) and postspawning ($n=6$) females were filmed in a static tank ($2.0 \times 0.62 \times 0.26$ m: length \times width \times depth) of circulating sea water maintained at 5 ± 0.5 ° C. The tank had a perspex base and was lit from underneath by five 70-W fluorescent strip lights.

Escape responses were elicited by touching the tail of the fish with a metal rod presented from behind the fish at a 30–45° angle to the midline of the tail. The resultant escape response was filmed in silhouette from above via a mirror angled at 45° to a high-speed video camera (NAC, Japan) operating at 200 frames s^{-1} . The field of view of the camera was between 2.5×1.8 (width \times height)– 5×3.5 fish body lengths. Two to six escape responses were elicited for each fish with 30–60 min between each response. The mean number of escape response trials was not significantly different ($P>0.7$) between gravid (3.0 ± 0.4 trials) and postspawning (3.3 ± 0.7 trials) fish.

The position of the snout and the centre of mass of each fish was digitized for each field of video film using a motion analysis system (MOVIAS, NAC). An object of known length was digitized on the first frame of each escape response sequence to act as a distance calibration. Errors in digitizing the snout and centre of mass caused less than

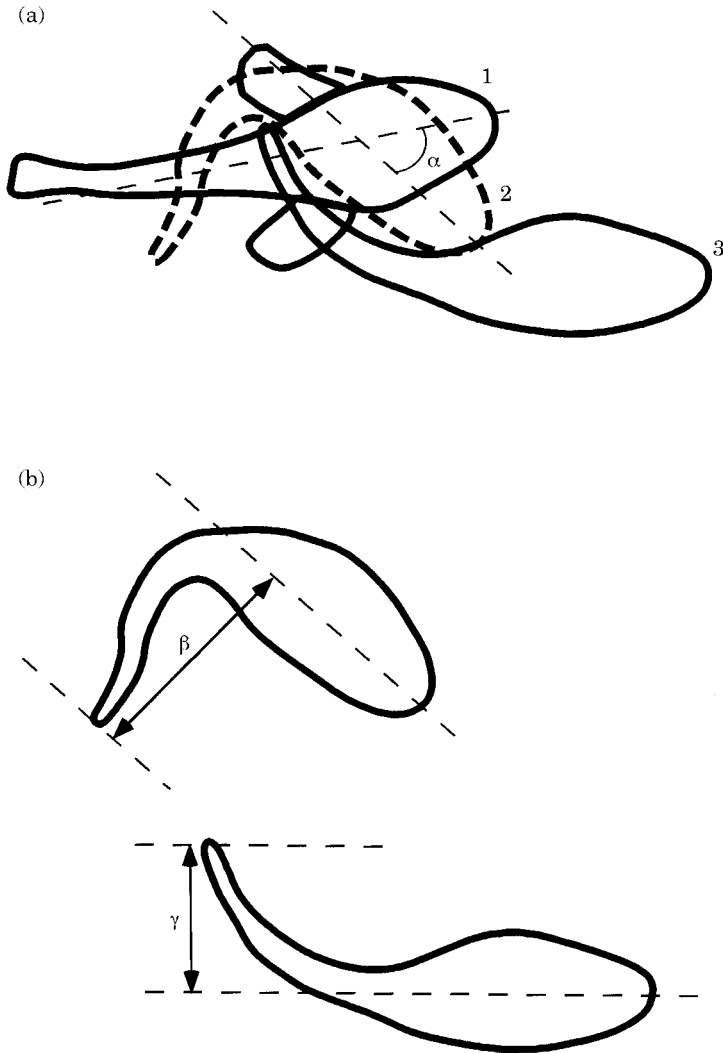


FIG. 2. Body outlines of short-horn sculpin during the escape response. (a) (1) the start of stage 1, (2) the end of stage 1 (C-bend) and (3) the end of stage 2 (contralateral contraction) as defined in the methods. Angle α represents the turning angle used during stage 1. (b) Distance β + distance γ = total tail beat amplitude during the contralateral contraction. Straight lines were drawn through either the snout and centre of mass or the tip of the tail parallel to the line through the snout and centre of mass.

a $\pm 1.5\%$ error in both the x and y position co-ordinates. The smoothed position data for both the snout and the centre of mass were interpolated from the best-fit least-squares cubic regression line that was fitted to that data point and the adjacent 20 points (Wakeling & Johnston, 1998). The smoothed x and y co-ordinate position data were used to calculate velocity for both x and y directions for each timepoint. The swimming velocity (U) of the fish was then calculated for each timepoint as the resultant velocity of the velocities in the x and y direction. The response with the fastest velocity of the centre of mass was used for further analysis.

Fish outlines were traced at the beginning of the escape response, the end of the C-bend and the end of the contralateral contraction (Fig. 2). The end of the C-bend was taken

to be the time when the fish had completed the first half tail-beat (most extreme tail position: position 2 in Fig. 2). The end of the contralateral contraction was determined as the time when the fish had reached its most extreme tail position during the contralateral contraction (position 3 in Fig. 2). The turning angle was calculated using MOVIAS by drawing a line from the snout to the centre of mass of the fish and measuring the angle of rotation during the C-bend [α in Fig. 2(a)]. The tail-beat amplitude was determined as the total lateral distance the tail had moved during the C-bend (β) or the contralateral contraction ($\beta + \gamma$) [Fig. 2(b)]. In two of the four maximum escape responses recorded in gravid fish, the most extreme tail position at the end of the contralateral contraction corresponded to a straightening of the tail and not a full half tail-beat. In these two responses the angle γ was negative. However, the end of the contralateral contraction was still determined as the most extreme tail position. The position of the centre of mass was determined for both gravid and postspawning fish as described previously by Temple & Johnston (1998). The centre of mass for a representative gravid fish was shifted posteriorly along the body length when compared with a typical postspawning fish, 0.38 body lengths (L) compared with 0.32 L , respectively. Therefore, the centre of mass in the two groups of fish is not in exactly the same anatomical position. The biological relevance of this difference is unclear. Predators are thought to aim at a region approximating the centre of mass of the fish (0.3–0.5 L from the snout; Webb & Skadsen, 1980).

CONTRACTILE PROPERTIES OF MUSCLE

Preparation of muscle fibre bundles

Gravid ($n=8$) and postspawning ($n=5$) fish were stunned by a blow to the head and pithed to destroy the central nervous system. An incision was made from the anus to the pectoral gill arch and the anterior abdominal myotomes (0.35 L from the snout) were removed. Small bundles of five to 22 fibres were dissected from anterior abdominal myotomes in Ringer solution maintained at 4° C. The Ringer solution contained (in mmol l⁻¹): NaCl, 143; sodium pyruvate, 10; KCl, 2.6; MgCl₂, 1.0; NaHCO₃, 6.18; NaH₂PO₄, 3.2; CaCl₂, 2.6; HEPES-sodium salt, 3.2; HEPES, 0.97 (James *et al.*, 1998). An aluminium foil T-shaped clip was folded over the myoseptum at each end of the muscle fibre bundle. The preparation was then transferred to a flow-through chamber of Ringer maintained at 5.0 ± 0.5° C. The foil clips were used to attach the preparation to a force transducer at one end (AME 801, SenSonor, Norway; sensitivity of 0.5 mN V⁻¹) and a servo arm at the other (Servomotor: MFE model R4-077, Emerson Electronics, U.K.). Stimuli were delivered by a stimulator (S48, Grass Instruments, Massachusetts, U.S.A.) via parallel platinum wire electrodes placed either side of the muscle fibre preparation. Muscle length changes and electrical stimuli were controlled by an in-house electronics system. Muscle force, length and electrical stimulation data were collected and analysed using a digital oscilloscope (1602, Gould Electronics, Ilford, U.K.).

Measurement of isometric properties

Muscle fibre preparations were held at constant length. Stimulus amplitude (gravid 10.0–14.0 V; postspawning 13.0–18.5 V), pulse width (gravid 1.1–1.4 ms; postspawning 1.3–1.7 ms) and fibre length were adjusted to maximize twitch force. The fibre length for maximal twitch force was termed l_0 and corresponded to sarcomere lengths between 2.20 and 2.30 μm , as measured by laser diffraction. Stimulation frequency was adjusted to maximize tetanus height (70–90 Hz). A 7–10-min recovery period was allowed between each tetanic contraction. Time to peak twitch force, time from stimulus to 90% twitch force relaxation, time from stimulus to 50% peak tetanic force and time from last stimulus to 50% tetanic relaxation were all measured. Preparations were then used to determine maximum unloaded shortening velocity and/or the force–velocity relationship.

Measurement of unloaded shortening velocity

Unloaded contraction velocity (V_0) was determined using the slack-test method (Edman, 1979). Preparations were stimulated to produce maximal tetanic force and

subjected to a rapid shortening step (of <2 ms duration) of sufficient magnitude to reduce the force generated to zero. After 200 ms the muscle was re-extended to its original length (l_0). This protocol was repeated six to nine times using different magnitudes of length step with 6–10 min recovery time allowed between each run. The time taken from the onset of the rapid shortening step to the beginning of force development was plotted against the magnitude of the length step. V_0 of the fibre bundle was then calculated as the slope of the first order polynomial line fitted to the data by least squares regression (Edman, 1979).

Measurement of the force-velocity relationship

Each muscle preparation was stimulated under isometric conditions to produce a tetanus. When maximum force was achieved the muscle preparation was subjected to an initial rapid shortening step (of <2 ms duration) to reduce force to a new level and then a shortening step of a lower constant velocity to maintain constant force for 8–20 ms (Altringham & Johnston, 1988*b*). Muscle length was then returned to l_0 . This protocol was repeated 13 to 21 times with each muscle fibre preparation, maintaining force at different levels between 0.012 and 0.95 P/P_0 . The muscle preparation was allowed to recover for 5 min between each contraction. A best-fit hyperbolic-linear curve (Marsh & Bennett, 1986) was fitted to each set of force-velocity (P - V) data using the software package Regression (Blackwell Scientific-Software, Oxford, U.K.). The hyperbolic-linear equation used to fit the curve was of the form:

$$V = \frac{B(1 - P/P_0)}{(A + P/P_0)} + C(1 - P/P_0)$$

where A is a dimensionless constant, B and C are constants with dimensions of velocity.

Muscle power output was calculated as the product of force and velocity and the maximum power output was assumed to be the highest calculated value.

The curvature of the P - V relationship was estimated using the power ratio (Marsh & Bennett, 1986):

$$\frac{\dot{W}_{\max}}{V_{\max} \times P_0}$$

where P_0 is the maximum isometric force, \dot{W}_{\max} and V_{\max} are the maximum power output and the maximum shortening velocity, respectively, determined from the force-velocity data.

DETERMINATION OF FIBRE BUNDLE CROSS-SECTIONAL AREA AND MASS

On completion of the *in vitro* experiments each muscle preparation was frozen rapidly in isopentane cooled to -159°C by liquid nitrogen. Transverse sections, 10 μm thick, were cut and stained for myosin ATPase activity at pH 9.4 using the method of Johnston *et al.* (1974). A microscope drawing arm was used to draw the outline of each fibre in cross-section. The cross-sectional area of the muscle fibre bundle was then determined via a digital planimeter interfaced to a microcomputer using Videoplan software (Kontron, Eching, Germany). Wet muscle mass was calculated using the measurements of muscle length and cross-sectional area assuming a density of 1060 kg m^{-3} (Méndez & Keys, 1960). Relative muscle power output was calculated as watts kg^{-1} wet muscle mass.

MEASUREMENT OF PROTEIN AND WATER CONTENT

The anterior abdominal myotomes remaining from the muscle fibre bundle dissection were used for the analysis of muscle protein and water content. Between 200 and 500 mg of anterior abdominal muscle were placed in an oven at 60°C and re-weighed at intervals until the mass remained constant. The water content of the muscle fibres was then determined as a percentage of the total wet muscle mass. A further 350–800 mg of muscle were frozen rapidly in liquid nitrogen and stored at -20°C prior to analysis of

myofibrillar protein content. The total myofibrillar fraction was extracted and washed as described by Crockford & Johnston (1995) and the protein concentration determined by the method of Maddy & Spooner (1970).

STATISTICS

Unless otherwise stated all values represent mean or mean \pm standard error. Each set of results for gravid and postspawning fish was analysed statistically using a two sided unpaired Student's *t*-test. An *F*-test was used to check that both populations (i.e. gravid and postspawning) in each set of results had equal variances. The Mann-Whitney *U*-test was then used to reanalyse data sets where the populations were found to have unequal variances (ovaries mass, V_0 , hyperbolic linear equation constants *B* and *C*).

RESULTS

All fish spawned within 27 days of each other. Fish used in locomotory studies spawned 2 to 7 days after completion of swimming experiments. Therefore, it was concluded that all gravid fish were close to spawning when used for locomotory or *in vitro* muscle contractile studies.

MORPHOMETRICS

The postspawning fish used for swimming studies were significantly longer than the gravid fish ($P < 0.001$) (Fig. 3), however there was no significant difference in body mass ($P = 0.11$; Table I). Although both groups of fish used for studies on the contractile properties of muscle had the same total body length ($P = 0.48$) the gravid fish had an 83% larger body mass ($P < 0.001$; Fig. 3). When gravid fish were compared with postspawning fish they had a larger mass of ovaries ($P < 0.0001$), 39% of body mass compared with 1.0% of body mass, respectively, and a 62% larger body girth ($P < 0.0001$; Table I).

SWIMMING PERFORMANCE

The C-bend duration and total duration of the first two kinematic stages (C-bend and contralateral contraction) were not significantly different ($P > 0.6$) between postspawning fish and gravid fish (Table II). The total distance covered by the snout during the C-bend was not significantly different ($P > 0.4$) between gravid and postspawning fish. In contrast, the distance covered by the centre of mass during the first two kinematic stages was 78% higher ($P < 0.01$) in postspawning than in gravid fish (Table II). The mean maximum velocity (U_{\max} in body lengths s^{-1}) of the snout during the C-bend was 50% ($P < 0.05$) higher in postspawning than in gravid fish (Fig. 4, Table II). However, there was no significant difference in the mean U_{\max} of the centre of mass during the first two kinematic stages achieved by gravid and postspawning fish. No significant differences ($P > 0.3$) were found between gravid and postspawning fish in the tail-beat amplitude or the turning angle used during the C-bend (Table II). However, the tail-beat amplitude used during the contralateral contraction was a significant 60% greater ($P < 0.01$) in the postspawning fish (Table II).

CONTRACTILE PROPERTIES

The muscle fibre length for maximum isometric twitch height (l_0) was not significantly different ($P = 0.14$) between gravid fish (9.13 ± 1.46 mm) and postspawning fish (7.72 ± 1.67 mm). However, when l_0 was expressed as a

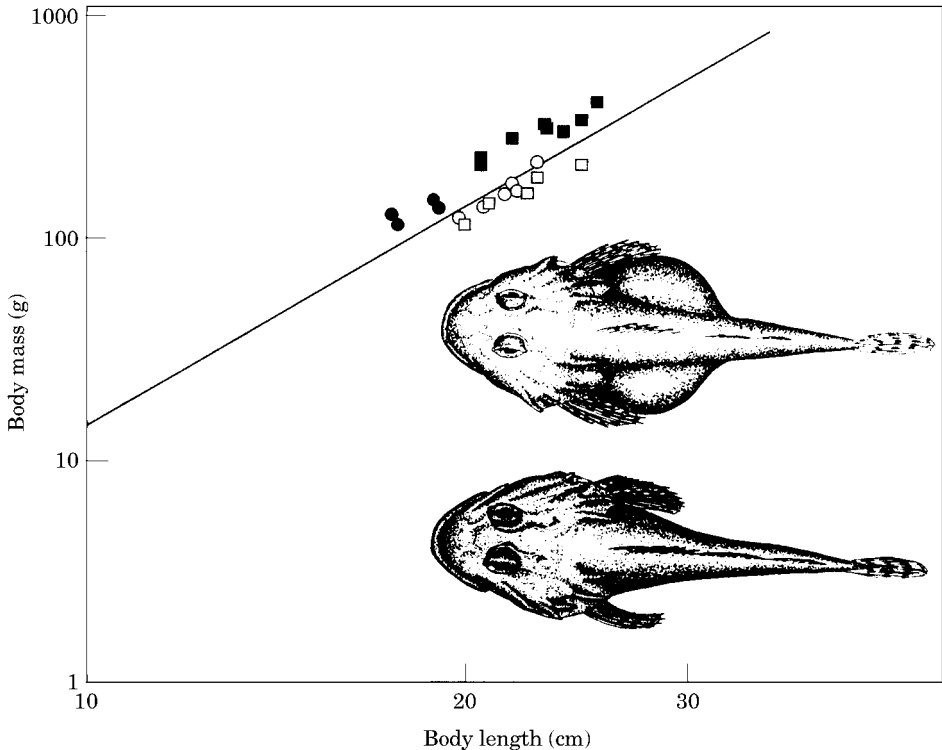


FIG. 3. The relationship between body length and body mass of fish used in the present study. Filled and open symbols represent gravid and postspawning fish, respectively. Squares and circles represent fish used in swimming and *in vitro* experiments respectively. The solid line represents the scaling relationship of body mass against length determined by James *et al.* (1998). The inset drawings represent the dorsal view of gravid and postspawning short-horn sculpin.

proportion of fish body length (to account for differences in body length between the fish used in each study) there was a significant difference ($P < 0.05$) between gravid and postspawning fish, with mean values of 0.043 and 0.035, respectively. There was no significant difference ($P > 0.29$) in the sarcomere length for maximum twitch height, 2.24 ± 0.01 and 2.27 ± 0.01 μm in gravid and postspawning fish, respectively. Twitch activation (time from stimulus to peak twitch force) and relaxation (time from stimulus to 90% twitch force relaxation) times were significantly shorter ($P < 0.05$) in gravid fish, by 14.7 and 18.0%, respectively [Fig. 5(a), Table III]. Tetanus activation time (time from first stimulus to half tetanus peak force) was not significantly different between the two sets of fish ($P = 0.12$; Table III). In contrast tetanus relaxation time (time from last stimulus to half tetanus force relaxation) was 33.8% shorter in gravid fish ($P < 0.05$). The maximum isometric stress produced by muscle preparations from postspawning fish was 54% higher than in gravid fish ($P < 0.001$; Table III).

V_0 was 14.6% faster ($P < 0.05$) in postspawning fish than in gravid fish, however there was no significant difference ($P = 0.28$) in V_{max} between the two groups (Table III). There were no significant differences ($P > 0.1$) found in constants A , B or C (from the hyperbolic linear equation) or in the power ratio values (Table III) between the groups of fish.

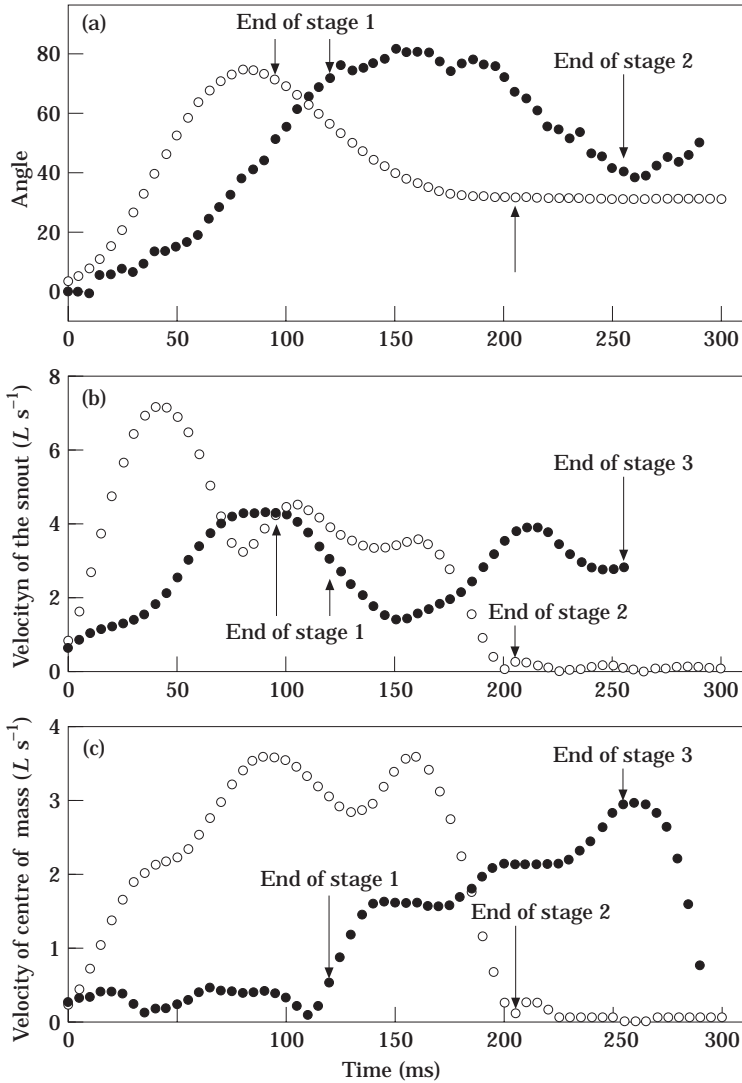


FIG. 4. Typical time-related changes in kinematic parameters of escape responses. (a) Turning angle, (b) velocity of the snout, (c) velocity of the centre of mass. Open and closed symbols are representative smoothed data for postspawning (21.5 cm) and gravid (17.7 cm) fish, respectively.

When force and velocity data were normalized to cross-sectional area and muscle length, respectively, fibre preparations from both groups maintained similar stresses at high shortening velocities [Fig. 5(b)]. However, at lower shortening velocities, muscle preparations from postspawning fish maintained higher stresses. Each muscle preparation from postspawning fish produced higher power output across the range of normalized forces (P/P_0) than each preparation from gravid fish [Fig. 5(c)]. On average fast muscle fibres from postspawning fish produced a 58% higher maximum power output than muscle fibres from gravid fish ($P < 0.01$; Table III).

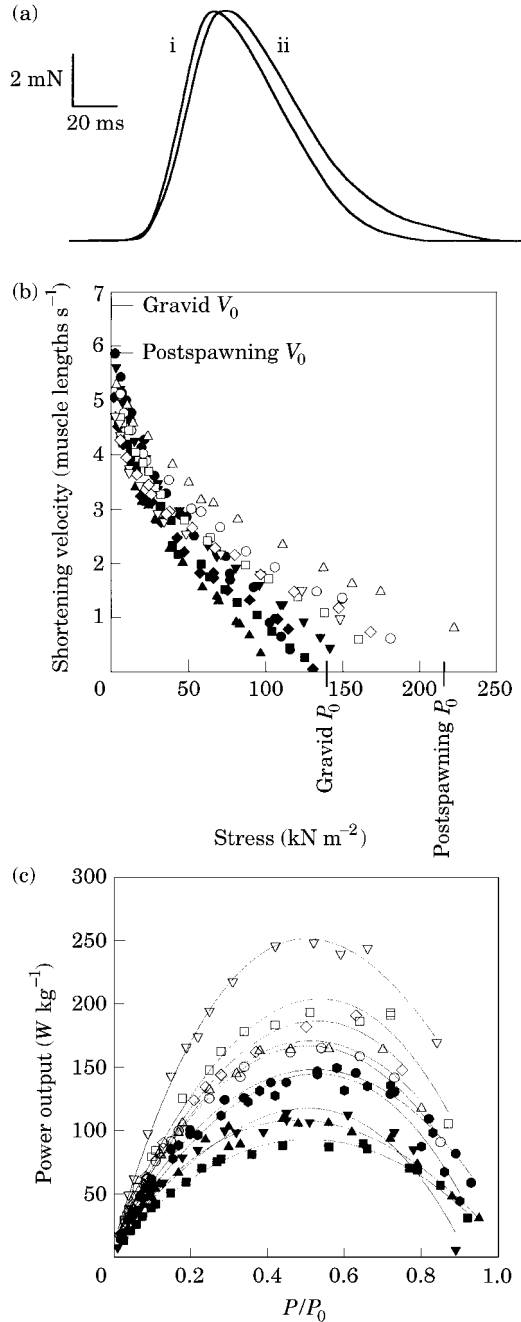


FIG. 5. Contractile properties in gravid and postspawning fish determined by isometric, isovelocity and slack-test experiments. (a) Typical twitches produced by fast muscle fibre preparations from (i) gravid and (ii) postspawning fish. (b) The relationship between shortening velocity and stress for anterior abdominal muscle preparations isolated from gravid (closed symbols) and postspawning fish (open symbols). The mean values for V_0 (maximum unloaded shortening velocity) and P_0 (maximum isometric force) are shown. (c) The relationship between mass specific power output and relative force for muscle preparations from gravid (closed symbols) and postspawning fish (open symbols), where P =force produced and P_0 =maximum isometric force.

TABLE I. Mass of ovaries, girth, water and myofibrillar protein content in gravid ($n=8$) and postspawning ($n=5$) short-horn sculpin *Myoxocephalus scorpius*

	Gravid		Postspawning	
	Mean	S.E.	Mean	S.E.
Ovaries mass (g)	115	12.2	1.63***	0.39
Girth (cm)	20.1	0.52	12.4***	0.68
Water content (% muscle mass)	85.8	0.36	85.5	0.77
Protein content (mg g^{-1} muscle mass)	116	3.49	125	3.75

Statistical significance is denoted by: *** $P<0.001$.

TABLE II. The kinematics of escape responses in gravid ($n=4$) and postspawning ($n=6$) short-horn sculpin *Myoxocephalus scorpius*

	Gravid		Postspawning	
	Mean	S.E.	Mean	S.E.
Total body length (cm)	18.3	0.40	21.4***	0.43
Total body mass (g)	129	6.9	158	13.2
C-bend duration (ms)	108	22.8	95.8	10.4
Total duration (ms)	213	40.9	191	19.2
Total distance travelled by the snout during the C-bend (body lengths)	0.28	0.06	0.35	0.06
Total distance travelled by the centre of mass (body lengths)	0.23	0.02	0.41**	0.04
Maximum velocity of the snout ($\text{body lengths s}^{-1}$)	3.75	0.28	5.64*	0.55
Maximum velocity of the centre of mass ($\text{body lengths s}^{-1}$)	2.55	0.26	3.38	0.32
Turning angle during C-bend ($^{\circ}$)	59.0	11.2	67.4	12.8
Tail-beat amplitude used during the C-bend (body lengths)	0.43	0.07	0.44	0.05
Tail-beat amplitude used during the contralateral contraction (body lengths)	0.40	0.07	0.63**	0.04

Statistical significance is denoted by: * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

PROTEIN AND WATER CONTENT

There were no significant differences in myofibrillar protein content ($P>0.1$), 120 mg g^{-1} wet muscle mass, and water content ($P>0.6$) 86%, between the two groups of fish (Table I).

DISCUSSION

SWIMMING PERFORMANCE

In the present study the maximum velocity of the snout during the C-bend was significantly greater in postspawning fish (Fig. 4, Table II). The postspawning

TABLE III. The effects of reproductive state on the contractile properties of muscle fibre bundles from the short-horn sculpin

	Gravid		Postspawning	
	Mean	S.E.	Mean	S.E.
Time from stimulus to peak twitch force (ms)	41.6	1.51	47.7*	2.23
Time from stimulus to 90% twitch force relaxation (ms)	107	4.42	126*	5.93
Time from first stimulus to half tetanus peak force (ms)	30.4	0.93	33.4	1.77
Time from last stimulus to half tetanus force relaxation (ms)	105	5.54	140*	12.1
V_0 (muscle lengths s^{-1})	6.75	0.33	5.89*	0.06
V_{max} (muscle lengths s^{-1})	5.84	0.26	5.51	0.11
Constant A	0.085	0.014	0.064	0.007
Constant B (muscle lengths s^{-1})	0.269	0.048	0.174	0.018
Constant C (muscle lengths s^{-1})	4.45	0.38	4.52	0.15
Maximum isometric stress ($kN m^{-2}$)	140	7.9	216***	11.5
Maximum power output ($W kg^{-1}$ wet muscle mass)	122	11.5	192**	15.2
Power ratio	0.155	0.006	0.161	0.003

$n=8$ observations for gravid fish, except V_0 and maximum isometric stress ($n=7$), V_{max} , constants A , B and C , maximum power output and the power ratio ($n=5$). $n=5$ observations for postspawning fish. Statistical significance is denoted by: * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

fish swam a significantly greater distance and achieved a 33% faster velocity than gravid fish during the contralateral contraction (Fig. 4, Table II). James & Johnston (1998) found that as body length increased in short-horn sculpin the maximum swimming velocity (in body lengths s^{-1}) of the snout and the centre of mass decreased. Therefore, it is unlikely that the differences in kinematic performance found in the present study were due to the gravid fish being shorter in body length than the postspawning fish.

The turning angle [Fig. 4(a)] and tail-beat amplitude used during the C-bend were not significantly different between gravid and postspawning fish (Table II). However, the tail-beat amplitude used during the contralateral contraction was significantly higher in postspawning than in gravid fish (Table II). The smaller contralateral tail-beat amplitude in gravid fish was due to two of the four gravid fish not completing a full half tail beat during the contralateral contraction, such that the value for γ was negative. Domenici & Blake (1991) found that angelfish *Pterophyllum eimekei* that performed an incomplete contralateral contraction produced a significantly lower swimming velocity than those with a complete contralateral contraction. In contrast in the present study if all 12 gravid fish escape responses were considered, only three had a negative value for γ and the mean snout velocity for the fish with a negative value for γ was $4.35 \pm 0.95 L s^{-1}$ not significantly different ($P>0.5$) than $3.88 \pm 0.30 L s^{-1}$ for the other fish. In these 12 responses there was also no significant difference ($P>0.3$) in the time taken to reach the most extreme tail position in the contralateral contraction between responses with a negative or a positive value for γ . Domenici & Blake

(1993) found that incomplete contralateral tail-beats increased in frequency with increased fish body size, allowing larger fish to achieve large turning angles despite their relatively limited flexibility around the centre of mass. In the present study the increased egg mass in gravid fish could cause a decrease in the flexibility of the fish around the centre of mass, however both sets of fish achieved similar turning angles during the C-bend. An increased egg mass may have affected swimming performance by increasing the girth and consequently changing the drag profile of the fish.

Therefore, it is possible that changes in swimming performance were due to either direct effects of the increased egg mass and girth on drag and/or the muscle power to body mass ratio of the fish, or indirect effects due to a decrease in physiological condition due to the gravid state.

Similar decreases in locomotory performance have been found in gravid snakes (Seigel *et al.*, 1987; Brodie, 1989) and lizards (Bauwens & Thoen, 1981; Sinervo *et al.*, 1991). The effect of increased drag due to the egg mass is thought to be the major reason why aquatic gravid snakes carry a smaller relative mass of eggs than terrestrial gravid snakes (Shine, 1988). In studies on gravid snakes (Seigel *et al.*, 1987) and lizards (Sinervo *et al.*, 1991) some of the reductions in locomotory performance were maintained for several weeks after oviposition indicating that reproduction impairs locomotory performance at least partially by affecting the physiological condition of the gravid female.

Gravid snakes (Brodie, 1989) and lizards (Bauwens & Thoen, 1981) adapt their behaviour to make greater use of crypsis and shorter escape routes to shelter to avoid predation. Gravid birds also show significant decreases in locomotory activity (Houston *et al.*, 1995). Such behavioural changes could represent an energetic trade-off with decreased locomotory activity and increased gonad development (Shine & Schwarzkopf, 1992). There appears to be a decrease in both swimming and feeding activity of gravid short-horn sculpin in the laboratory (James & Johnston, unpubl. obs.). Indeed wild fish caught during early egg maturation have large quantities of food in their stomach and a comparatively small mass of eggs; however, as spawning approaches they have a much larger mass of eggs and no food in their stomachs (James & Johnston, unpubl. obs.). Gravid short-horn sculpin may also rely more heavily on crypsis and the presence of spines around the head to deter would-be predators than postspawning fish, or alternatively gravid fish may be more vulnerable to predation.

CONTRACTILE PROPERTIES OF MUSCLE

The change in reproductive state from postspawning to gravid affected significantly the contractile properties of fast myotomal muscle in short-horn sculpin (Table III). Muscle from gravid fish had faster twitch and tetanus kinetics and more rapid maximum unloaded shortening velocity (V_0). Maximum isometric muscle stress and power output decreased significantly even though myofibrillar protein and water contents were similar (120 mg g^{-1} wet mass and 86%, respectively; Table I). The lack of change in water and protein contents indicates that the period of starvation prior to spawning may be relatively short in the gravid short-horn sculpin. Muscle water content in cod *Gadus morhua* L., does not change until after 5 weeks of starvation, indicating that during early

phases of starvation energetic substrates are derived by mobilization of lipids from the liver (Love, 1980). The decrease in power output in gravid fish was likely to be due primarily to the decrease in ability to produce force, with very similar decreases in maximum isometric force and maximum power output, of 35 and 36%, respectively. Such a decrease in muscle power output could account at least partially for the decreased kinematic performance of the gravid fish as Wakeling & Johnston (1998) have demonstrated that fast-start performance is limited by muscle power output in a wide range of fish.

POSSIBLE MECHANISMS AFFECTING THE PHYSIOLOGICAL STATE OF GRAVID FISH

In this study decreases in swimming activity in gravid fish may have caused a shift in the contractile properties of muscle. In previous studies on rats subjected to hind limb suspension for 1 week (Alley & Thompson, 1997) and in rabbits with their ankle angle immobilized at 90° for 2 weeks (Cotter *et al.*, 1991) there was a decrease in soleus muscle mass and maximum force production, but an increase in contraction velocity/decrease in twitch times. These changes in contractile times due to muscle disuse mirror the changes with gravid state found in the present study.

In the present study we have shown that a change in reproductive state in the short-horn sculpin is accompanied by significant increases in mass of ovaries (39% of total fish mass in gravid fish compared with 1.0% in postspawning fish) and consequently girth (Table I). These increases in girth are liable to result in the anterior abdominal muscle being physically stretched. Stretch of muscle in mice has been shown to cause an increase in the number of sarcomeres in series to maintain an optimal sarcomere length for force generation (Williams & Goldspink, 1978). In the present study the muscle fibre length (l_0) for maximal twitch force, when expressed as a proportion of total fish length, was found to be significantly longer ($P < 0.05$) in gravid than postspawning fish. However, at l_0 there was no significant difference ($P > 0.29$) in sarcomere length between gravid and postspawning fish, respectively. Therefore, it is likely that the number of sarcomeres in series increases in abdominal muscle fibres during egg maturation. After spawning short-horn sculpin lack tone in the anterior abdominal muscle, further evidence that the muscle has been stretched (James & Johnston, unpubl. obs.). Stretch of rabbit latissimus dorsi muscle for 6 weeks causes an increase in serial sarcomere number and decreases in maximum isometric stress, power output and twitch times similar to those found in the present study (James *et al.*, 1997).

CONCLUSION

In the present study a change in reproductive state initiated significant changes in swimming performance and the contractile properties of muscle independently of seasonal variations in temperature and photoperiod. The changes in swimming performance were probably due to the added mass of eggs, the associated drag and the changes in muscle properties. The changes in the contractile properties of anterior abdominal muscle were probably non-adaptive being secondary consequences of the reproductive state due to the effects of muscle disuse and physical stretch. In order to differentiate between the possible causes

(muscle disuse or stretch) of the changes in contractile properties, future work could include comparing the properties of muscle fibres that were (anterior abdominal myotomes) or were not (rostral myotomes) subjected to physical stretch in gravid fish. The anterior abdominal muscle comprises only 17% of the total muscle mass of the short-horn sculpin (James & Johnston, unpubl.), therefore, the majority of fast muscle fibres would not be subjected to stretch. However, all locomotory muscle in gravid short-horn sculpin may be affected by muscle disuse atrophy. Therefore, changes in contractile properties of muscle combined with the increased egg mass were probably the major factors contributing to the observed decreases in escape performance.

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