Preparing for escape: anti-predator posture and fast-start performance in gobies

Håkan Turesson[1], Andrea Satta[1,2] and Paolo Domenici[1,2,*]

[1]IMC-International Marine Centre and [2]CNR-IAMC-c/o International Marine Centre, Località Sa Mardini, 09072 Torregrande, Oristano, Italy

*Author for correspondence (paolo.domenici@iamc.cnr.it)

SUMMARY

The adoption of postures as a response to threats is often interpreted in terms of predator detection or signalling (e.g. vigilance and defence display). The possibility that an alternative or additional function of anti-predator postures might be to enhance the subsequent escape has been largely unexplored. Here, we use black goby (*Gobius niger*) to test the hypothesis that a postural curvature caused by a bending response (i.e. a slow muscle contraction which bends the body with no forward displacement) induced by a weak stimulus (WS) may affect escape responses. Three experiments were carried out. (1) Control and WS-stimulated fish were startled using lateral mechanical stimuli, to test whether the orientation of the postural C-bend affected escape direction and performance. Postural curvature was defined as positive when escapes were towards the convex side of the postural C-shape, and negative when they were towards the concave side. Locomotor performance increased with postural curvature, although fish showed a preference for escaping away from the stimulus regardless of postural curvature. (2) Control and WS-stimulated fish were startled from above, hence minimising the directionality of the threat on the horizontal plane. WS-stimulated fish showed a bias towards escaping from a positive curvature, thereby enhancing their locomotor performance. (3) Field observations with stimuli coming from above showed that gobies escape most often towards the convex side of the postural C-shape. By escaping from positively curved postures, most of the initial tailsweep is directed backwards and may provide more thrust than when starting from straight or negatively curved postures. Hence, the anti-predator posture adopted by alerted benthic fishes may 'prepare' them for their subsequent escape response because it conveys an advantage when they are attacked from above (a likely occurrence), although when gobies are stimulated horizontally, escape direction may be favoured over high locomotor performance when the two trade off.

Key words: anti-predator posture, anti-predator response, escape response, gobies.

INTRODUCTION

Fish respond to approaching danger in a number of ways, which may depend on the perceived level of threat as well as on the conspicuousness of the prey (Godin, 1997). In addition to escape responses, other response types which do not imply much displacement of the body, such as freezing, changes in opercular frequency, erection of spines and fins, and the adoption of body postures, have been observed in threatened fish (Godin, 1997; Huntingford et al., 1994; Ylonen et al., 2007). The adoption of specific postures in prey animals as a response to potential threats has been interpreted in various ways, including vigilance (Lima and Dill, 1990; Wisenden et al., 1995), aggressive/defensive display (Huntingford et al., 1994; Sherbrooke, 2008; Stankowich, 2009), pursuit deterrence (Caro, 1995; Cooper, 2007; Smith, 1997), facilitation of crypsis (Bealor and Krekorian, 2002), size advertising (Honma et al., 2006; Sherbrooke, 2008) and death feigning (Gerald, 2008; Gyssels and Stoks, 2005; Honma et al., 2006). The possibility that an alternative (or additional) function of certain anti-predator postures might be that of enhancing performance in a subsequent escape has been largely unexplored. Nevertheless, postural changes prior to unsteady manoeuvres (e.g. predator strikes) have been observed in some species of fish, e.g. pike (Webb and Skadsen, 1980), leading to a higher locomotor performance. Similarly, some of the responses to threats, such as fin erection and postural changes (Godin, 1997), may also function in enhancing performance in the subsequent escape, as suggested for the fin erection commonly observed at the onset of escape responses (Eaton et al., 1977).

The most extreme response to a threatening stimulus is the escape response which, in fish, corresponds to a high energy burst of swimming associated with a short latency and high acceleration and speed (Domenici and Blake, 1997; Eaton and Hackett, 1984). Escape responses in fish are therefore important in avoiding predation (Walker et al., 2005) and their kinematics, performance and physiology have been well studied (Domenici and Blake, 1997; Wakeling, 2006). Escape responses consist of a fast contraction of the axial musculature, bending the body into a C-shape (stage 1), following by a contralateral contraction (stage 2) (Domenici and Blake, 1997). S-shaped escape responses (S-starts) have also been observed in certain species (Hale, 2002). While earlier work considered stage 1 as a 'preparatory stage' and stage 2 as a 'propulsive stage' (Weihs, 1973), recent work based on PIV (particle image velocimetry) has shown that stage 1 can also provide thrust (Tytell and Lauder, 2008). Escape responses are usually mediated by the Mauthner cells, two bilateral giant neurons, and other associated neurons in the brainstem escape network (Eaton et al., 2001). The brainstem escape network can be stimulated by sensory input from sound, mechanical vibration, electrical field or visual cues (Eaton et al., 2001).

Several external (e.g. temperature, oxygen) and intrinsic factors (e.g. body size and shape) influence time–distance performance in fast starts (Domenici and Blake, 1997; Domenici et al., 2007; Domenici et al., 2008; Langerhans et al., 2005; Wakeling, 2006). However, the ability to escape predators does not depend solely on locomotor performance but also on a number of variables that are

related mainly to the behavioural decisions of fish (Domenici et al., 2007; Fuiman et al., 2006; Walker et al., 2005). These components include responsiveness (the proportion of individuals responding to stimulation), the escape latency (time between the onset of the stimulus and the first visible movement), the reaction distance and the directionality (whether stage 1 is directed away or towards the threat) (Domenici, 2009; Domenici et al., 2007). All these factors need to be taken into account in order to fully evaluate escape performance in fish (Walker et al., 2005).

Using black gobies (*Gobius niger*), here we tested the hypothesis that both posture and the subtle changes in posture induced by relatively weak stimulation may affect the escape response, which is then triggered by a subsequent and stronger stimulation. The black goby (*Gobius niger*) is a benthic, bottom-dwelling species that can be found sitting on the bottom with a variety of postural curvatures, from relatively straight to C-shaped. The most extremely curved postures can occur as the result of a peculiar bending response when gobies are alerted by a weak stimulation. The bending response consists of a slow and subtle movement of the tail and usually occurs within seconds of stimulation. It is therefore hypothesised that this bending response may represent an escape 'preparation' as observed in other animals, although based on very different mechanisms (Bennet-Clark, 1975; Card and Dickinson, 2008). Specifically, here we tested the following main hypotheses: (1) whether escape direction and performance in fish startled using lateral mechanical stimuli were affected by the alerting weak stimulation and the resulting changes in postural curvature, (2) whether escape performance in fish startled using a stimulus from above was affected by the alerting weak stimulation and (3) whether fish observed in the field showed any preference in the direction of escape relative to the postural C-bend.

MATERIALS AND METHODS
Fish maintenance

Black gobies (*Gobius niger* L.) were wild-caught specimens from the brackish water lagoon of Marceddì, in Sardinia, Italy. Because of their territorial behaviour, gobies were kept individually in a space of 20 cm×30 cm (water depth 15 cm) in a flow-through recirculating system. The temperature in the holding tanks was 20–22°C. The gobies were fed pieces of oligochaetae daily, but they were starved for 24 h prior to the experiments.

Experiment 1: stimulation in the horizontal plane
Marking

The gobies were marked dorsally using white colour (titanium dioxide, powder) fixed with tissue adhesive (Vetbond[TM], 3M, St Paul, MN, USA), with one spot (2 mm in diameter) on each side of the centre of mass [CM, defined as the centre of mass of the fish when stretched straight (Webb, 1978)], located under the fifth ray of the anterior dorsal fin at 0.35 total body length from the snout as determined from five frozen specimens. This was done immediately before putting the goby in the experimental arena and the whole procedure took approximately 1 min. No anaesthetic was used. Marking allowed automatic tracking of the CM using the software Winanalyse (WINanalyse, Mikromak, Berlin, Germany).

The experimental arena and the stimuli

A circular arena with a light grey bottom and edges (1.03 m diameter, height 0.78 m) was used for the experiments. The lower perimeter of the arena was angled outwards at 45 deg. (Fig. 1) to minimise attraction to the tank edge. The arena was illuminated with four 150 W spotlights, placed 0.6 m above the water surface,
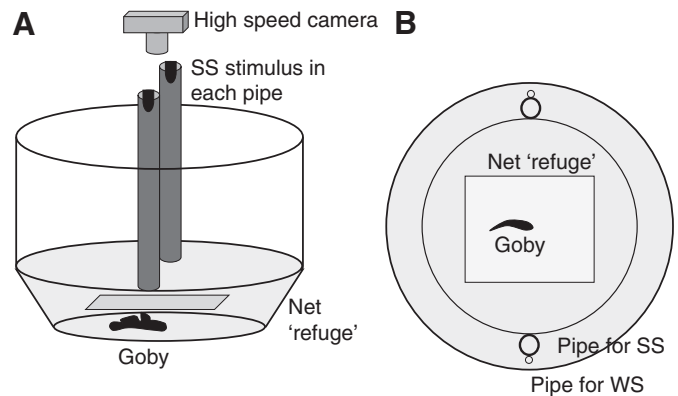

Fig. 1. The experimental arena viewed from the side (A) and from above (B). The goby is sitting on the bottom of the tank, under a 'net' refuge, and is filmed from above, through the net. The WS (weak stimulus) and SS (strong stimulus) were released from the sides, at random from either the left or right, in order to avoid a tank side effect. The startle stimulus fell within a pipe to prevent the goby from seeing it before it touched the water surface (see text for more details).

by the side of the tank. Water depth was 130 mm and the bottom diameter was 0.77 m (surface diameter 1.03 m). A plastic net (51 cm×30 cm) with a 5.5 mm mesh was provided, hung by transparent monofilament strings, half-way between the bottom and the surface in the centre of the arena (Fig. 1). The net provided minimal shading, sufficient to induce the gobies to position themselves under it, while allowing them to escape in any direction as well as allowing filming through the net. The gobies were startled when under the net and always >2 body lengths from the arena's edge to minimise edge effects on escape direction or performance (Eaton and Emberley, 1991). A weak stimulus (WS, i.e. 7 ml water, released from a 6 mm diameter pipe 22 cm above the water surface from either side of the tank, 0.49 m from the tank centre) was used to induce bending behaviour. Preliminary tests showed that this WS did not elicit a fast-start escape response, but was often successful in eliciting a bending response.

A strong stimulus (SS) consisting of a plastic cylinder (38.3 g, 6.4 cm diameter, 24 cm) was released from 1.23 m above the water surface by switching off an electromagnet, and fell into one of two grey plastic pipes (10.9 cm outer diameter, positioned on either side of the tank, 0.44 m from the tank centre), ending 10 mm above the water surface, to prevent the goby from seeing the stimulus before it made contact with the water surface (Lefrançois et al., 2005). An opening in the pipes allowed the camera to record when the SS hit the water so that escape latency could be measured. As for the WS, one of two randomly chosen SS was provided to prevent any tank side effects (Fig. 1). Water temperature in the experimental tank was 20–21.5°C. The experiment was filmed from above with a high speed camera at 500 frames s[-1] (MotionPro, Redlake MASD, San Diego, CA, USA).

The experimental protocol

Gobies were stimulated with lateral WS and SS (45–135 deg. from the line passing from CM to the snout). Lateral stimulation was chosen in order to maximise the directionality of the responses (Domenici and Blake, 1993b) and hence to test whether having a C-bend posture oriented away from or towards the stimulation had an effect on the direction of the response. The experiments were performed on three treatment groups. The first group was a control

group, for which the WS was not used (the no WS group). The other two groups were stimulated with the WS 30 s before the SS. The second group was first given a lateral WS from the same direction as the SS, i.e. ipsilateral WS group. The third group was given the WS from the opposite side to the SS direction and was defined as the contralateral WS group. In blocks of three, the first two fish were randomised into their treatment group immediately prior to the stimulation, while the third fish was given the remaining treatment in its block. This resulted in $N=20$ for the no WS group, $N=22$ for the ipsilateral WS group and $N=17$ for the contralateral WS group. The uneven number of replicates in the treatment groups depended on technical problems that forced us to discard some replicates. Fish were stimulated with the SS at random from the right or left side. The postural curvature of the fish before stimulation could be oriented with the concave or convex side to the stimulus. A single goby was left in the arena for a minimum of 90 min, and always under the centred net refuge. The spotlights were then turned on. The goby was left undisturbed for at least a further 30 min before stimulation (WS or SS, depending on treatment).

Experiment 2: stimulation from above

A second experiment was carried out using a stimulus dropped from directly above the fish. The aim of this experiment was to test the hypothesis that the escape performance of fish alerted by a WS differ from those of non-alerted fish when using a stimulus coming from above, thus avoiding potential bias in the direction of escape due to horizontally directional stimulation as used in experiment 1. In order to avoid the ripples of the stimulus from distorting the image, a glass square aquarium was used ($1\,m \times 1\,m \times 0.5\,m$), with a mirror placed at 45 deg. underneath it, which allowed filming from below. A circular net (45 cm in diameter) was used in order to prevent fish from using the corners as shelters. The arena was covered laterally by a black screen so that the experimenter was not visible to the fish. The arena was illuminated with two 150 W spotlights, placed by the side of the tank, 0.6 m above the water surface. Water depth was 20 cm.

Gobies were introduced into the centre of the arena, and kept within a circular net (20 cm in diameter) attached to two transparent strings, for a minimum of 60 min. After this period, the circular net was lifted using the transparent strings, so that the fish did not see the experimenter. The fish were startled using a SS after a minimum of 5 min. The startling SS consisted of a white plastic ball (4 cm in diameter, weight 39.6 g) dropped from 80 cm directly above the fish (<0.5 body lengths away from the CM of the fish). A small (4 mm) metal hook was inserted into the ball, allowing to release it using an electromagnet, and a transparent string was attached to the metal hook in order to prevent the ball from hitting the bottom of the tank.

Two treatments were used: (a) fish were startled 30 s after being stimulated with a WS (the same WS as used in experiment 1); (b) the fish were startled directly without any WS (control treatment).

Water temperature in the experimental tank was 20–21.5°C. The experiment was filmed using a high speed camera at 500 frames s^{-1} (MotionPro).

Measurements for experiments 1 and 2

Temperature and fish length were measured directly after the fast start. No gobies were used more than once, to avoid habituation to the startle stimulus, and if one replicate failed for technical reasons after the fish was startled, the fish was not re-used. Videos were analysed frame by frame using the software Winanalyse and the
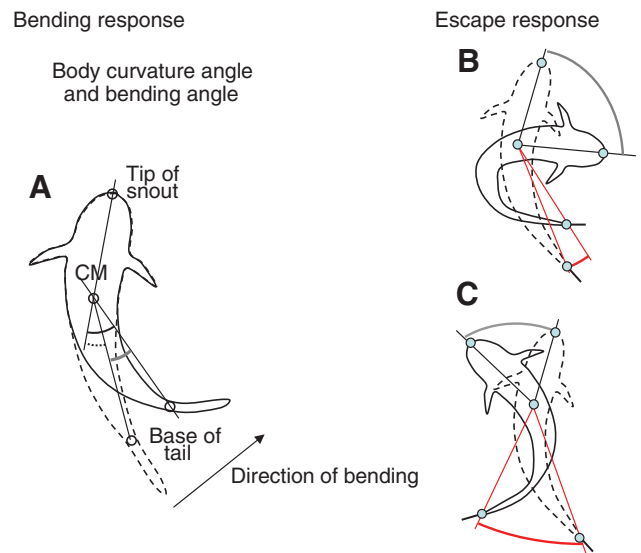

Fig. 2. (A) Fish outlines before (dashed outline) and after (continuous outline) a bending response. Body curvature angle was measured as 180 deg. minus the angle between two lines (originating from the tip of the snout and the base of the tail, respectively) intercepting at the centre of mass (CM). For a straight fish this angle is 0 deg. C_i (the initial postural curvature of the fish, i.e. prior to WS) and C_{ss} (the postural curvature of the fish prior to SS) are shown as the dotted and the continuous curve, respectively. Bending angle (grey curve) was defined as the angular increment from the initial postural curvature (dashed outline) to the curvature attained after the bending response (continuous outline). (B,C) Examples of gobies escaping towards the concave (B) and towards the convex side of the body (C). The initial position (dashed outline) and the position at the end of stage 1 (continuous outline) are shown. The CM, tip of the head and base of the tail are indicated by blue circles. TR_{max} is calculated as the maximum angular velocity of the segment linking CM with the head as it rotates in order to produce stage 1 angle (shown by the curved grey line). TF_{max} is calculated as the maximum angular velocity of the segment linking CM with the base of the tail as it rotates in order to produce the tail flip angle (indicated by the curved red line).

following three points were digitised: the CM, the tip of the snout and the base of the tail (Fig. 2).

Experiment 3: field observations

In a complementary field experiment, a startling stimulus coming from straight above the fish was used as in experiment 2, in order to minimise the directionality of the stimulation in the horizontal dimension. The aim of this experiment was to test whether gobies startled in the field have a preferential side of contraction in stage one of their escape responses, relative to the direction of their postural curvature. The stimulus used was a white plastic ball (4 cm in diameter, weight 39.6 g) released 3 m above the water surface. The site of the field experiment was located near a series of piers where water depth was in the range 0.4–1 m. Twenty-eight black gobies were stimulated while resting on the bottom with a C-shaped postural curvature. Escape direction in relation to body curvature (i.e. whether the escape response was directed towards the concave or convex side of the body) was recorded using a high speed field camera (Ranger, Fastec Imaging, San Diego, CA, USA) at 500 Hz.

Definition of variables

Responsiveness to stimulus (WS or SS): the proportion of fish that responded to stimulation with a bending response, an escape response or no response.

C_i: the initial postural curvature of the fish (i.e. prior to WS), calculated as the angle supplementary to the angle between the tip of the snout, the CM and the base of the tail (Fig. 2). A straight fish therefore has a curvature $C_i=0$.

C_{ss}: the postural curvature of the fish prior to SS. The absolute value of C_{ss} is calculated as for C_i but, for C_{ss}, curvature bears a positive sign when the escape response that follows SS is towards the convex side, and a negative sign when it is towards the concave side (Fig. 2).

Bending angle: the change in the curvature angle due to a bending response (Fig. 2).

Directionality: the proportion of away responses over the total (away+towards responses). Away and towards escape responses were defined as those in which the first movement of the head in stage 1 was away or towards the SS, respectively (Domenici and Blake, 1993b). Directionality was measured only for experiment 1, because in experiment 2 the startling stimulus hit the surface directly above the fish.

Escape latency: the time elapsed from the frame in which the SS hits the water surface until the first detectable movement by the goby. For experiment 2, fish occasionally escaped before the stimulus hit the water. In these cases, a negative value of latency was used corresponding to the time elapsed between the onset of the response and contact between the stimulus and the water surface.

$S1A$: stage 1 angle (in responses to SS), the angle between the straight lines passing from the tip of the snout to the CM before the fast start and at the end of stage 1 (Fig. 2). The end of stage 1 was defined as the reversal of the turning direction of the head (Domenici and Blake, 1997), after which stage 2 starts, ending at the further reversal of the head direction.

D_{30}: the distance between the CM of the fish at the frame before the first visible response and 30 ms later (30 ms was chosen as a fixed time occurring during stage 2 in all individuals) (Meager et al., 2006).

TR_{max}: maximum turning rate of the head, the maximum angular velocity of the line passing through the snout and the CM during stage 1.

TF_{max}: maximum turning rate of the tail flip, the maximum angular velocity of the line passing through the CM and the base of the tail (Fig. 2) during the first tail flip of the escape response.

D_{30}, TR_{max} and TF_{max} were measured only for the escape responses (all triggered by SS). To obtain D_{30}, TR_{max} and TF_{max}, the raw data were derived with respect to time using a five-point differentiation-based smoothing method (Lanczos, 1956).

RESULTS
Experiment 1
Responsiveness and directionality

A total of 59 black gobies were used in analysable replicates (fish total length 11.2±0.3 cm; mean ± s.e.). Each fish was used once and in only one of the three treatments (Table 1): no WS, ipsilateral WS and contralateral WS. The two WS treatments included 39 fish. None of the gobies escaped as a response to the WS, and 28 (71%) responded with a bending response (mean bending angle 10.6±1.9 deg., mean ± s.e.) while 11 showed no response (29%) (Fig. 3). The percentage of fish showing a bending response was 79% in fish with postural curvature (C_i) oriented towards the WS and 68% when oriented away from the WS (not significantly different; $\chi^2=0.48$, d.f.=1, $P>0.25$). The gobies responded with further bending in the direction of the original curvature, irrespective of whether they were stimulated from the concave or convex side of the body; thus none of them was reversing curvature direction

Table 1. The responses of gobies to SS following different WS treatments

	No WS	Ipsilateral WS	Contralateral WS	Total
No escape				
Remained still	0	2	0	2
Bending response	8	11	10	29
Escape				
Away responses	11	6	6	23
Towards responses	1	3	1	5
Total	20	22	17	59

SS, strong stimulus; WS, weak stimulus.

or straightening out. The 11 gobies that did not respond with bending stayed immobile.

Fish from the different WS treatments reacted to a SS with an escape response (away or towards) or with a bending response. Only in two cases did the fish show no response at all (Table 1; Fig. 3). There was no difference between the three treatments in the proportion of escaping/non-escaping gobies in response to SS ($\chi^2=1.91$, d.f.=2, $P>0.25$). Showing a bending response or no response to WS did not affect the proportion of escaping/non-escaping gobies ($\chi^2=1.15$, d.f.=1, $P>0.25$). Similarly, there was no difference in the proportion of away/towards escape responses among the three treatments ($\chi^2=2.27$, d.f.=2, $P>0.10$). Pooling all treatments, significantly more gobies escaped away from the SS than towards it (82% away vs 18% towards; $\chi^2=1.57$, d.f.=1, $P<0.001$).

Bending angle

In fish reacting to WS with a bending response, the bending angle was not affected by whether the WS was delivered towards the
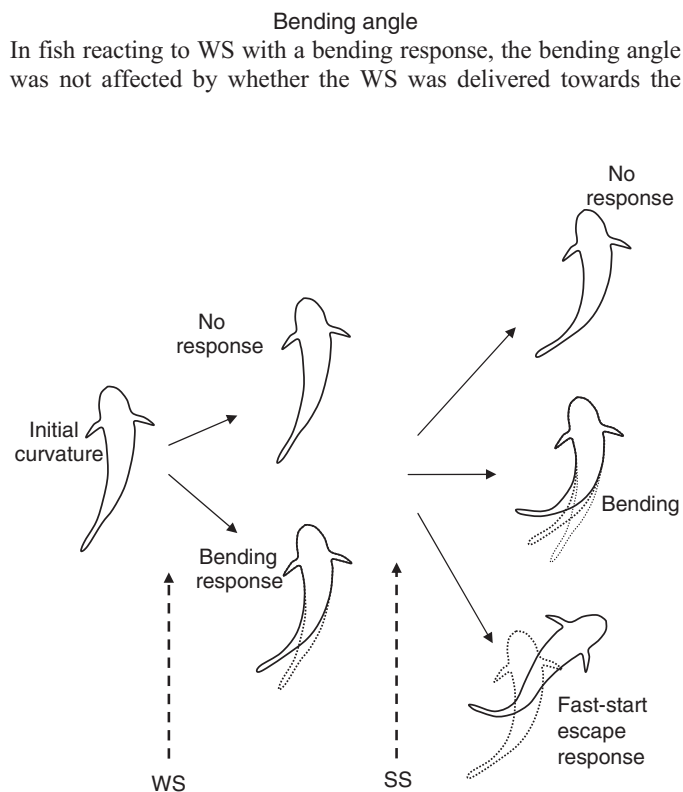

Fig. 3. Possible responses observed as a reaction to WS and SS. The initial position implied a certain degree of curvature (initial curvature). The WS caused 71% bending responses and 29% no responses. The SS caused 47% escape responses, 49% bending responses and 3% no responses in the three treatments.

convex (bending angle 9.8+2.9 deg., N=11) or the concave side of the C-bend (bending angle 11.2+2.6 deg., N=17; Mann–Whitney U=84, P>0.5).

The effect of stimulus type was tested by comparing those fish that showed a bending response to WS vs fish that showed a bending response to SS in the no WS treatment, i.e. in order to ensure a fair comparison between fish that were stimulated directly with a SS or a WS. Stimulus type had a significant effect on bending angle (responses to WS, 10.6±1.9 deg., N=28; responses to SS, 22.6±4.9 deg., N=8; Mann–Whitney U=52, P<0.05)

Fast-start escape latency
Escape latency was not affected by WS treatment (non-parametric ANOVA, Kruskal–Wallis test, P>0.25), or by C_{SS} (linear regression, R^2=0.001, P>0.5). The 28 gobies that performed an escape response had a mean latency of 67.0±10.4 ms (mean ± s.e., range 10–252 ms), and the latency distribution showed a bimodal pattern with one peak at about 10–20 ms and a second peak at about 70–100 ms (Fig. 4). Gobies starting towards the SS had a shorter latency than those starting away from the SS (26.8±7.3 ms vs 75.7±11.9 ms, Mann–Whitney U=21.5, P<0.05; Fig. 4).

The effect of postural curvature (C_{ss}) on performance and kinematics
WS treatment had no effect on performance (Kruskal–Wallis test; P>0.5, P>0.1, P>0.5, P>0.1 on D_{30}, $S1A$, TF_{max} and TR_{max}, respectively). Fish exhibited an escape response to SS starting from various postural curvatures (Fig. 5). The postural curvature had a significant effect on locomotor performance and tail kinematics, as both D_{30} and TF_{max} increased significantly with C_{ss} (Fig. 6). On the other hand, both S1 angle and TR_{max} were independent of C_{ss} (linear regressions for $S1A$ R^2=0.055, P>0.1, N=28; for TR_{max} R^2=0.017, P>0.25, N=28) and they were, on average, $S1A$=67.95±4.23 deg. and TR_{max}=3978±112 deg. s^{-1}.

Experiment 2
Bending angle
A total of 39 black gobies were used in analysable replicates (fish total length 10.04±0.19 cm, mean ± s.e.), with N=19 and N=20 for the alerted (i.e. stimulated with a WS) and non-alerted treatment, respectively. All fish from both treatments responded to the startling stimulus. All alerted fish performed a bending response upon release of the WS, and the resulting bending angle was on average 10.3±1.1 deg. As in experiment 1, gobies responded with further
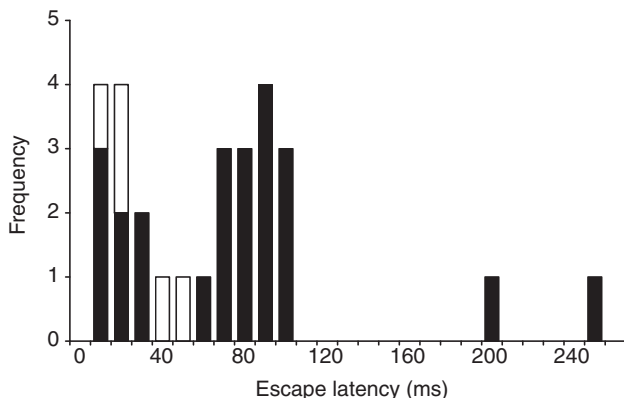

Fig. 4. Frequency distribution of the escape latencies. Open bars and filled bars indicate towards and away responses, respectively.

bending in the direction of the original curvature. As a result of the bending response, alerted fish showed a larger C_{ss} than the control (alerted fish 16.6±1.5 deg., non-alerted fish 7.9±1.4 deg.; Mann–Whitney U=62, P<0.001). Postural curvature prior to WS did not differ between treatments (alerted fish 6.2±0.9 deg., non-alerted fish 7.9±1.4 deg.; Mann–Whitney U=178.5; P>0.75).

Direction of escape relative to the postural curvature
In alerted fish, a significantly higher number of individuals (84% of the total) escaped towards the convex side of the postural C-shape (i.e. Fig. 2C) than towards the concave side (i.e. Fig. 2B; binomial test, P=0.01). No difference in these proportions was found in non-alerted individuals (45% of all the escape responses were towards the convex side of the C-shape; binomial test, P>0.5). The proportion of individuals escaping towards the convex side of the C-bend was higher in alerted than in non-alerted fish (χ^2=6.5, d.f.=1, P<0.05).

Fast-start escape latency
No differences were found in escape latencies when alerted and non-alerted fish were compared (6.1±1.2 ms vs 5.3±2.1 ms, for alerted and non-alerted individuals, respectively, Mann–Whitney U=187, P>0.5).

The effect of WS on locomotor performance
Alerted fish showed higher D_{30} and TF_{max} than control (non-alerted) fish (t-test, Table 2). No statistically significant differences between alerted and non-alerted fish were found when comparing TR_{max} and stage 1 angles (t-test, Table 2).

The relationship between postural curvature (C_{ss}) and performance and kinematics
Similar to experiment 1, C_{ss} had a significant effect on locomotor performance and tail kinematics. Both D_{30} and TF_{max} increased significantly with C_{ss}, and these relationships were not different between treatments (ANCOVA, P>0.1 and P>0.25 for D_{30} and TF_{max}, respectively, Fig. 7). $S1A$, TR_{max} and latencies were independent of C_{ss} in both treatments (linear regressions for alerted fish, $S1A$ R^2=0.073, P>0.25, TR_{max} R^2=0.017, P>0.5, latencies R^2=0.002, P>0.5; for non-alerted fish, $S1A$ R^2=0.007, P>0.5, TR_{max} R^2=0.034, P>0.25, latencies R^2=0.042, P>0.25).

Experiment 3: field experiment
In the field experiment with the stimulus coming from above the fish, 28 gobies were stimulated while resting in a C-shaped posture. Twenty-one of these gobies escaped towards the convex side of the postural C-shape, while seven initiated the escape towards the concave side. A binomial test showed that significantly more gobies escaped towards the convex side of the postural C-shape (P=0.02).

DISCUSSION
Responses to stimulation in the horizontal plane
Most gobies responded to the WS with a bending response, while some stayed motionless, and none showed an escape response. This is in line with our observations from natural habitats, where black gobies respond to weak disturbances (such as a slow approach; P.D., personal observations) with a bending response. The gobies did not appear to make use of the directional information of the WS for their bending response as they always bent their body in the direction in which they were already curved prior to WS stimulation. The direction of the WS did not have any effect on the bending angle either, which was approximately 10 deg. on average in all cases.
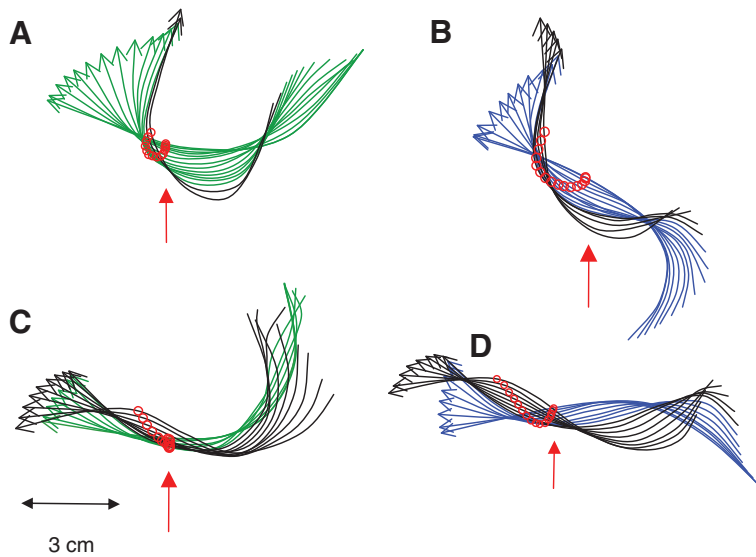
Fig. 5. Tracings of four examples of escape responses in the black goby. Midline and CM (red circles) of the fish at 2 ms intervals, i.e. from the onset of the response to 30 ms later. Arrows indicate the head. Stage 1 tracings of fish initiating escape towards the concave side are green (A,C), and stage 1 tracings of fish starting towards the convex side are blue (B,D). Stage 2 tracings are black in all examples. All examples are responses away from the stimulus, whose direction is indicated by the red arrow. A and B show escape responses with a large turning angle, C and D those with a small turning angle.

Bending angle was, however, affected by the type of stimulus (WS or SS). Bending angles in response to SS were significantly larger than those in response to WS (22.6 deg. *vs* 10.4 deg., respectively). Therefore, the bending response is not a fixed, stereotypical response, because it is affected by the strength of the stimulation. The bending behaviour we observed occurs in other species (P.D., personal observations), especially in benthic fish (e.g. Blennidae, Scorpionidae) in which the maintenance of a postural curvature may be facilitated by contact with the ground. Bending behaviour is relatively slow, of the order of 0.5–2 s. This implies that it is probably not controlled by the Mauthner cell complex, unlike the escape response. The slow motion of the bending response may serve the purpose of avoiding perception by predators, while creating a curved posture which may improve locomotor performance in a subsequent escape response (see below).

Black gobies reacted with a stronger response to the SS than to the WS. Roughly half of the fish (47%) stimulated with a SS showed an escape response, about 49% of the fish showed a bending response similar to WS-stimulated fish but with larger bending angles and only 3% did not show any response. The responsiveness to SS was not affected by the WS treatment, i.e. gobies showed a similar proportion of escape responses, whether they had received an ipsilateral, a contralateral or no WS. In addition, the proportion of escape responses observed was not affected by whether there was a prior bending response or no response to a WS. Similarly, the directionality was not affected by the WS treatment, with most responses being away from the SS (82%), similar to observations on other teleost fish (Domenici and Batty, 1994; Domenici and Batty, 1997; Domenici and Blake, 1993b). Hence, receiving a weak ipsilateral stimulation (WS) prior to the startling stimulus did not increase the chances of responding away from the stimulus. In other fish species (goldfish *Carassius auratus* and cichlids *Haplochromis burtoni*) a directional visual stimulus displayed 10–100 ms prior to an acoustic non-directional startling stimulus was shown to affect escape direction, presumably by driving one of the Mauthner cells closer to firing threshold (Canfield, 2003; Canfield and Rose, 1996). In our experiment, the interval between WS and SS was relatively long (30 s), which may allow the Mauthner cells enough time to return to their normal firing threshold.

Similar to the results on responsiveness and directionality, WS had no effect on escape latency in black gobies. Hence ipsilateral or contralateral stimulation with WS does not make gobies more 'ready' to escape than having received no stimulation at all prior to SS. The latency distribution showed a bimodal pattern (Fig. 4) with peaks at approximately 20 and 80 ms. A similar pattern was also found in other fish species and may reflect different neural pathways for short and long latencies (Domenici and Batty, 1997; Eaton et al., 2001). In the present study, 'towards' responses had shorter latencies than 'away' responses. Longer latencies thus seem
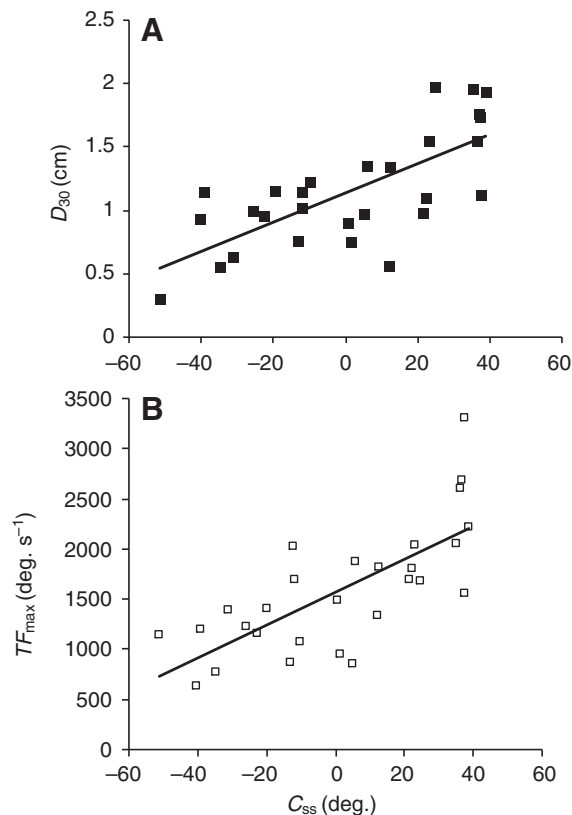

Fig. 6. (A) The relationship between D_{30} and C_{ss}. $D_{30}=0.0115C_{ss}+1.14$; $R^2=0.52$, $P<0.001$, $N=28$. (B) The relationship between TF_{max} and C_{ss}. $TF_{max}=16.32C_{ss}+1570$; $R^2=0.53$, $P<0.001$, $N=28$.

Table 2. Locomotor performance in alerted *vs* non-altered gobies

Treatment	D_{30} (cm)	TF_{max} (deg. s^{-1})	TR_{max} (deg. s^{-1})	Stage 1 angle (deg.)	N
Alerted	1.19±0.11	1559±89	5249±284	103.1±11.0	19
Non-alerted	0.83±0.10	1282±100	5227±292	111.3±11.1	20
t	2.5	2.1	0.05	0.52	
P	<0.05	<0.05	>0.5	>0.5	

D_{30}, distance covered in 30 ms; TR_{max}, maximum turning rate of the head; TF_{max}, maximum turning rate of the tail flip.

to be associated with an increased proportion of escapes directed away from the stimulus, as found in herring, *Clupea harengus* (Domenici and Batty, 1994; Domenici and Batty, 1997). It is likely that additional information processing that leads to a longer latency also allows for a more correct directional decision. Thus there appears to be a trade off between short latency and directional accuracy.

WS treatment had no effect on escape kinematics. Locomotor performance (D_{30}) increased with body curvature, i.e. it was highest in responses starting from tightly bent, positively curved postures.
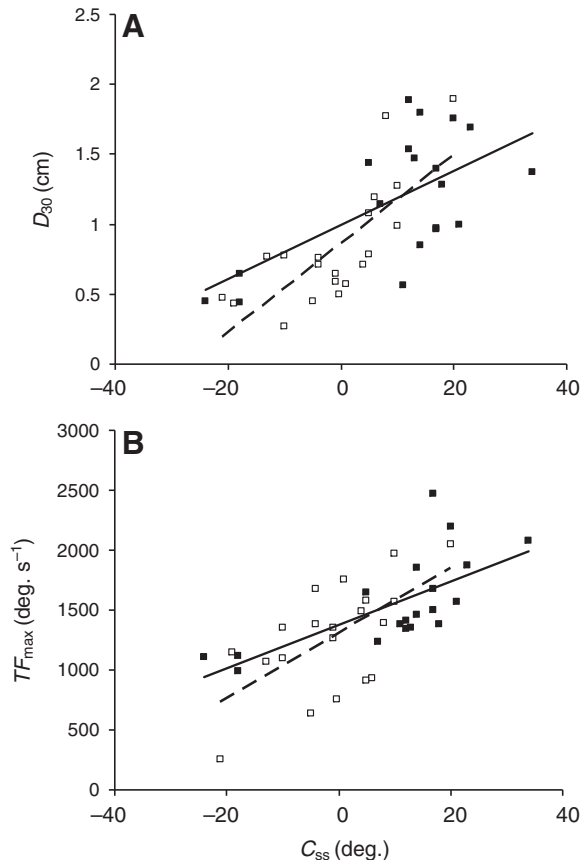

Fig. 7. (A) The relationship between D_{30} and C_{ss} (postural curvature prior to SS) in alerted (filled squares) and non-alerted (open squares) gobies. Alerted fish regression (continuous line): $D_{30}=0.019C_{ss}+0.99$; $R^2=0.39$, $P<0.01$, $N=19$; non-alerted fish regression (dashed line): $D_{30}=0.031C_{ss}+0.86$; $R^2=0.56$, $P<0.001$, $N=20$. The two regressions do not differ from each other (ANCOVA, $P>0.1$). (B) The relationship between TF_{max} and C_{ss} in alerted (filled squares) and non-alerted (open squares) gobies. Alerted fish regression (continuous line): $TF_{max}=18.1C_{ss}+1373$; $R^2=0.48$, $P<0.01$, $N=19$; non-alerted fish regression (dashed line): $TF_{max}=27.4C_{ss}+1308$; $R^2=0.39$, $P<0.01$, $N=20$. The two regressions do not differ from each other (ANCOVA, $P>0.25$).

No effect of body curvature was found on $S1A$ and TR_{max}. This suggests that the postural curvature had no effect either on the turning angle of the fish or on its head turning rate. The turning rate of the head is an important variable for assessing escape performance because it gives an indication of the agility of the fish (Domenici, 2001; Walker et al., 2005). At the mechanistic level, both TR_{max} and TF_{max} should give an indication of the bending rates. If a fish escapes from a postural C-bend, it may be expected that it will not reach values of bending rates as high as if it starts from a straight posture. However, our results suggest that postural curvature affects only the bending rate of the tail and not that of the head. This suggests that the postural curvature involves mainly pre-bending of the tail.

A high angular velocity of the tail may in part explain the higher locomotor performance (in terms of D_{30}) as a high angular velocity of the tail should provide a higher thrust. In addition, by escaping from positively curved postures, gobies may be able to orient the jets created by the caudal fin (Tytell and Lauder, 2008) in a more favourable direction than when escaping from a negatively curved position or even a straight position. Tytell and Lauder (Tytell and Lauder, 2008) show that the momentum of the jet produced by the caudal fin in stage 1 in a classic C-start escape starting from a straight posture actually opposes the escape trajectory of the fish, and acts to reduce the efficiency of the escape response. Effectively, stage 1 of escapes starting from positively curved postures resembles stage 2 of a typical escape response starting from a straight posture, as in this case gobies start from a posture that resembles the end of stage 1 (C-bend) of the escape responses observed in other species (Fig. 5). Therefore, in these responses, much of the tail sweep during stage 1 is directed backwards (as in stage 2 of more 'typical' escape responses previously observed) and therefore should provide more thrust than in the case of escapes starting from straight or negatively curved postures.

In experiment 1, we triggered startle responses with a lateral stimulus and most escape responses were in the direction away from the stimulus irrespective of the initial postural body curvature. In this case, therefore, the results show that the direction of escape (i.e. away from the stimulus) overrides the potential locomotor advantages of escaping towards the concave side of the body. These results are in line with the idea that escape direction may be favoured over high locomotor performance in fish when the two trade off (Domenici and Blake, 1993a).

Responses to stimulation from above

All fish responded to WS and SS from above. The reason for such a high responsiveness compared with experiment 1 may be related to the lack of shading on the fish (unlike in experiment 1), as well as stimulation from a shorter distance (i.e. from above). As a result of WS stimulation and the subsequent bending response (with a bending angle of approximately 10 deg.), the C_{ss} of alerted fish was more pronounced than that of non-alerted ones. Stimulation with WS also increased the probability of escaping from a positively

curved posture (Fig. 2C) compared with non-alerted fish whose escape responses were non-biased with respect to the orientation of the body curvature. This difference may be due to a combination of (a) escaping from a more pronounced C_{ss} in alerted fish, which may induce a preference for the side of contraction relative to the body curvature compared with fish that are almost straight (as in non-alerted individuals), and (b) a direct effect of WS stimulation on neural control. Given that WS stimulation had no effect on the temporal readiness (latency), the possibility that more pronounced curvature results in a biased escape direction relative to the postural bend is more likely than the direct effect of WS stimulation on neural control. Escape latencies were relatively short compared with those of experiment 1 and compared with values from other studies (Turesson and Domenici, 2007). A likely explanation for the short latencies observed is that the response of the fish may have been visual and therefore triggered prior to contact between the stimulus and the water surface, while the latency was calculated from the instant the stimulus hit the water.

Alerted fish showed higher performance in terms of D_{30} and TF_{max} compared with non-alerted fish. This is probably due to the larger C_{ss} from which alerted fish were startled. In fact, as for fish stimulated from the side (experiment 1), these two variables increased with C_{ss} (Figs 6 and 7). The regressions between these variables and C_{ss} do not differ between the two treatments, therefore a plausible explanation for the higher performance in alerted fish is that they show a higher proportion of fish escaping with a high value of C_{ss}, as a result of the bending response. While a bending response may also cause a large negative value of C_{ss}, our results show that alerted gobies startled from above tend to escape mainly from positively curved postures, and therefore they show, on average, a higher performance than non-alerted fish.

Field observations

The bias towards escaping from a positively curved posture observed in experiment 2 was confirmed by field observations where fish were startled from above while resting in a curved position. Most fish initiated escape towards the convex side of their body (i.e. maximising locomotor performance). Therefore, in natural situations where threats may often come from above, or in other cases with little or no directional information on the threat in the horizontal plane, it is likely that the gobies will escape towards the convex side and benefit from a postural curvature such as that induced from an alerting WS. Little is known about postural changes in response to threatening stimulations. A similar phenomenon to the bending response observed in the gobies is the postural curvature (S-shape) observed in a number of predator species (Esocidae, Fistularidae, Aulostomidae, larval Engraulidae and Clupeidae) before they strike, which results in superior locomotor performance compared with attacks from a straight posture (Webb and Skadsen, 1980). Postural C-shaped curvatures can be adopted by other benthic species (e.g. Blenniidae, P.D., personal observation) and therefore it would be interesting to test whether they can be used by other prey species to increase escape performance.

General discussion

Our results have implications both for the mechanisms controlling the response to threatening stimuli and for the potential functional significance of anti-predator postures. Our results suggest that the bending response may be an anti-predator response, based on the fact that (a) fish stimulated with a strong stimulus which in many cases provokes an anti-predator (escape) response may also show a bending response as alternative, (b) field observations show that approaching gobies slowly causes them to perform a bending response, and (c) a bending response may enhance locomotor performance when fish are stimulated from above. Nevertheless, further field work is necessary to enable us to conclude that the bending responses can indeed be considered an anti-predator response. At the mechanistic level, the suggestion that the bending response is an anti-predator response would have implications for neural control, as anti-predator responses may fall into different (partly stimulus-dependent) categories showing increasing strength of the response, from no response, to the bending response, to an escape response. Nothing is known about the mechanisms controlling the bending response. It would be interesting to investigate the relationship between bending and escape responses, because while bending was always observed to be in the direction of the original postural curvature, escape was often in the opposite direction. Hence it is possible that the neural commands for escaping may inhibit the bending response, although both the latencies and the time required for escaping are so much shorter than those of the bending response that such an inhibitory mechanism may not be necessary.

In terms of anti-predator postures, ours is the first demonstration that a fish species can prepare for escape; that is, adopt a posture that can give them subsequent locomotor advantages once the escape is initiated in certain circumstances, i.e. when attacked from above, which is a likely occurrence in benthic fishes. While the adoption of specific postures as a response to predators has been described in many species of animal, their function has mainly been interpreted as being related to predator detection and signalling, e.g. in terms of vigilance, facilitating the assessment of any upcoming danger, and aggressive/defensive display, which may deter predators from attacking (Godin, 1997; Lima and Dill, 1990). On the other hand, postures that prepare for escape have been observed in certain legged animals: locusts, for example, prepare for escape by positioning their legs so that elastic energy is stored for a subsequent jump. Hindlegs are flexed into a 'cocked' position, which may or may not be followed by an escape jump (Bennet-Clark, 1975; Heitler and Burrows, 1977; Santer et al., 2005). Escape 'preparation' has also been observed in *Drosophila* flies which, when stimulated, execute postural adjustments so that leg extension will push them away from the stimulus, whether escape will eventually be performed or not (Card and Dickinson, 2008). In these cases, escape preparation is, however, related to either mechanical constraints (locust) or directional advantage (*Drosophila*), rather than to enhancing locomotor performance as observed here. Further work could test the possibility of locomotion-enhancing anti-predator postures in other animals. For example, various species of fish are known to erect their fins when threatened (Altbacker and Csanyi, 1990; Godin, 1997; Huntingford et al., 1994; Ylonen et al., 2007). While fin erection also occurs in conjunction with the escape response, it is possible that performing this behaviour prior to startle may enhance locomotion performance by increasing the surface area that produces thrust. Finally, the locomotor advantages of the postural curvature observed here do not exclude other potential functions. These include communicating to a predator that it is detected, facilitating predator assessment, or providing a warning signal to conspecifics (Smith and Smith, 1989). Two other gobid species, *Asterropteryx semipunctatus* and *Gnatholepis anjerensis*, perform predator recognition behaviour, called bobbing behaviour, where the fish bob up and down upon predator detection (Smith and Smith, 1989). Although communicative functions might possibly also explain the bending response of benthic fishes, the slow and subtle nature of the tail bend make communicative functions less likely than the enhancement of locomotor performance.

REFERENCES

Altbacker, V. and Csanyi, V. (1990). The role of eyespots in predator recognition and antipredatory behavior of the paradise fish, *Macropodus opercularis* L. *Ethology* **85**, 51-57.

Bealor, M. T. and Krekorian, C. O. (2002). Chemosensory identification of lizard-eating snakes in the desert iguana, *Dipsosaurus dorsalis* (Squamata: Iguanidae). *J. Herpetol.* **36**, 9-15.

Bennet-Clark, H. C. (1975). Energetics of jump of locust *Schistocerca gregaria. J. Exp. Biol.* **63**, 53-83.

Canfield, J. G. (2003). Temporal constraints on visually directed C-start responses: behavioral and physiological correlates. *Brain Behav. Evol.* **61**, 148-158.

Canfield, J. G. and Rose, G. J. (1996). Hierarchical sensory guidance of Mauthner-mediated escape responses in goldfish (*Carassius auratus*) and cichlids (*Haplochromis burtoni*). *Brain Behav. Evol.* **48**, 137-156.

Card, G. and Dickinson, M. H. (2008). Visually mediated motor planning in the escape response of *Drosophila. Curr. Biol.* **18**, 1300-1307.

Caro, T. M. (1995). Pursuit-deterrence revisited. *Trends Ecol. Evol.* **10**, 500-503.

Cooper, W. E. (2007). Escape and its relationship to pursuit-deterrent signalling in the cuban curly-tailed lizard *Leiocephalus carinatus. Herpetologica* **63**, 144-150.

Domenici, P. (2001). The scaling of locomotor performance in predator-prey encounters: from fish to killer whales. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **131**, 169-182.

Domenici, P. (2009). Escape responses in fish: kinematics, performance and behavior. In *Fish Locomotion: An Etho-Ecological Perspective* (ed. P. Domenici and B. G. Kapoor). Enfield, NH: Science Publishers.

Domenici, P. and Batty, R. S. (1994). Escape maneuvers of schooling *Clupea harengus. J. Fish Biol.* **45**, 97-110.

Domenici, P. and Batty, R. S. (1997). Escape behaviour of solitary herring (*Clupea harengus*) and comparisons with schooling individuals. *Mar. Biol.* **128**, 29-38.

Domenici, P. and Blake, R. (1997). The kinematics and performance of fish fast-start swimming. *J. Exp. Biol.* **200**, 1165-1178.

Domenici, P. and Blake, R. W. (1993a). The effect of size on the kinematics and performance of angelfish (*Pterophyllum eimekei*) escape responses. *Can. J. Zool.* **71**, 2319-2326.

Domenici, P. and Blake, R. W. (1993b). Escape trajectories in angelfish (*Pterophyllum eimekei*). *J. Exp. Biol.* **177**, 253-272.

Domenici, P., Lefrançois, C. and Shingles, A. (2007). Hypoxia and the anti-predator behaviour of fishes. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **362**, 2105-2121.

Domenici, P., Turesson, H., Brodersen, J. and Brönmark, C. (2008). Predator-induced morphology enhances escape locomotion in crucian carp. *Proc. Biol. Sci.* **275**, 195-201.

Eaton, R. C. and Emberley, D. S. (1991). How stimulus direction determines the trajectory of the mauthner-initiated escape response in a teleost fish. *J. Exp. Biol.* **161**, 469-487.

Eaton, R. C. and Hackett, J. T. (1984). The role of Mauthner cells in faststarts involving escape in teleost fish. In *Neural Mechanisms of Startle Behavior* (ed. R. C. Eaton). New York: Plenum Press.

Eaton, R. C., Bombardieri, R. A. and Meyer, D. L. (1977). Mauthner-initiated startle response in teleost fish. *J. Exp. Biol.* **66**, 65-81.

Eaton, R. C., Lee, R. K. K. and Foreman, M. B. (2001). The Mauthner cell and other identified neurons of the brainstem escape network of fish. *Prog. Neurobiol.* **63**, 467-485.

Fuiman, L. A., Rose, K. A., Cowan, J. H. and Smith, E. P. (2006). Survival skills required for predator evasion by fish larvae and their relation to laboratory measures of performance. *Anim. Behav.* **71**, 1389-1399.

Gerald, G. W. (2008). Feign *versus* flight: influences of temperature, body size and locomotor abilities on death feigning in neonate snakes. *Anim. Behav.* **75**, 647-654.

Godin, J. G. J. (1997). Evading predators. In *Behavioural Ecology of Teleost Fishes* (ed. J. G. J. Godin), pp. 191-236. Oxford: Oxford University Press.

Gyssels, F. G. M. and Stoks, R. (2005). Threat-sensitive responses to predator attacks in a damselfly. *Ethology* **111**, 411-423.

Hale, M. E. (2002). S- and C-start escape responses of the muskellunge (*Esox masquinongy*) require alternative neuromotor mechanisms. *J. Exp. Biol.* **205**, 2005-2016.

Heitler, W. J. and Burrows, M. (1977). Locust jump. 1. The motor programme. *J. Exp. Biol.* **66**, 203-219.

Honma, A., Oku, S. and Nishida, T. (2006). Adaptive significance of death feigning posture as a specialized inducible defence against gape-limited predators. *Proc. Biol. Sci.* **273**, 1631-1636.

Huntingford, F. A., Wright, F. P. I. and Tierney, J. F. (1994). Adaptive variation in anti-predator behaviour. In *The Evolutionary Biology of Sticklebacks* (ed. M. A. Bell and S. E. Foster), pp. 277-296. Oxford: Oxford University Press.

Lanczos, C. (1956). *Applied Analysis.* Englewood Cliffs, NJ: Prentice Hall.

Langerhans, R. B., Layman, C. A. and DeWitt, T. J. (2005). Male genital size reflects a tradeoff between attracting mates and avoiding predators in two live-bearing fish species. *Proc. Natl. Acad. Sci. USA* **102**, 7618-7623.

Lefrançois, C., Shingles, A. and Domenici, P. (2005). The effect of hypoxia on locomotor performance and behaviour during escape in *Liza aurata. J. Fish Biol.* **67**, 1712-1730.

Lima, S. L. and Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* **68**, 619-640.

Meager, J. J., Domenici, P., Shingles, A. and Utne-Palm, A. C. (2006). Escape responses in juvenile Atlantic cod Gadus morhua L.: the effects of turbidity and predator speed. *J. Exp. Biol.* **209**, 4174-4184.

Santer, R. D., Yamawaki, Y., Rind, F. C. and Simmons, P. J. (2005). Motor activity and trajectory control during escape jumping in the locust *Locusta migratoria. J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **191**, 965-975.

Sherbrooke, W. C. (2008). Antipredator responses by Texas homed lizards to two snake taxa with different foraging and subjugation strategies. *J. Herpetol.* **42**, 145-152.

Smith, R. J. F. (1997). Avoiding and deterring predators. In *Behavioural Ecology of Teleost Fishes* (ed. J. G. J. Godin), pp. 163-190. Oxford: Oxford University Press.

Smith, R. J. F. and Smith, M. J. (1989). Predator-recognition behavior in 2 species of gobiid fishes, *Asterropteryx semipunctatus* and *Gnatholepis anjerensis. Ethology* **83**, 19-30.

Stankowich, T. (2009). When predators become prey: flight decisions in jumping spiders. *Behav. Ecol.* **20**, 318-327.

Turesson, H. and Domenici, P. (2007). Escape latency is size independent in grey mullet. *J. Fish Biol.* **71**, 253-259.

Tytell, E. D. and Lauder, G. V. (2008). Hydrodynamics of the escape response in the bluegill sunfish, *Lepomis macrochirus. J. Exp. Biol.* **211**, 3359-3369.

Wakeling, J. A. (2006). Fast-start mechanics. In *Fish Biomechanics* (ed. R. E. Shadwick and G. V. Lauder), pp. 333-368. San Diego, CA: Academic Press.

Walker, J. A., Ghalambor, C. K., Griset, O. L., McKenney, D. and Reznick, D. N. (2005). Do faster starts increase the probability of evading predators? *Funct. Ecol.* **19**, 808-815.

Webb, P. W. (1978). Fast-start performance and body form in 7 species of teleost fish. *J. Exp. Biol.* **74**, 211-226.

Webb, P. W. and Skadsen, J. M. (1980). Strike tactics of *Esox. Can. J. Zool.* **58**, 1462-1469.

Weihs, D. (1973). The mechanism of rapid starting of slender fish. *Biorheology* **10**, 343-350.

Wisenden, B. D., Chivers, D. P. and Smith, R. J. F. (1995). Early warning in the predation sequence: a disturbance pheromone in Iowa darters (*Etheostoma exile*). *J. Chem. Ecol.* **21**, 1469-1480.

Ylonen, H., Kortet, R., Myntti, J. and Vainikka, A. (2007). Predator odor recognition and antipredatory response in fish: does the prey know the predator diel rhythm? *Acta Oecologica* **31**, 1-7.