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Escape trajectories of the rockpool prawn (*Palaemon elegans*) in response to visual and mechanosensory stimuli.

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Abstract

Small crustaceans, such as the rockpool prawn *Palaemon elegans* Rathke, respond to the approach of predators by executing a version of the crustacean tail-flip response, known as the ‘jack-knife’. We used two types of stimulus to investigate the escape behaviour of *P. elegans*, specifically the escape trajectories taken by individual prawns. The first stimulus consisted of mechanosensory cues, while the second stimulus included visual and mechanosensory cues. Responses to the two stimulus types differed, with the combined cues from the second stimulus type resulting in escapes over longer distances, and with greater directionality, compared to those following purely mechanosensory stimulation. Altering the direction of approach of the ‘predator’ affected the proportion of escapes that were to the side opposite from the eliciting stimulus, and strongly influenced escape trajectories. Such unpredictability in the escape direction of *P. elegans* may be an example of so-called ‘Protean’ behaviour.

Keywords

Protean behaviour; escape responses; *Palaemon elegans*; predator evasion

Introduction

Two important categories of anti-predator defences are ‘predator avoidance’ and ‘predator evasion’. Predator avoidance strategies aim to reduce the number of predator-prey encounters; this can include morphological adaptations such as crypsis (Troscianko et al. 2013) and behavioural adaptations such as reduced activity in the presence of predators (Blake and Hart 1993). Predator evasion refers to behaviours shown by individual animals attempting to escape from an approaching predator, including rapid movements away from the predator (Dill 1974; Blake and Hart 1993; Neil and Ansell 1995; Martin and Lopez 1996; Domenici and Blake 1997; Arnott et al. 1998). Such high speed manoeuvres may be enough to enable an animal to escape predation.

However, predators can learn (or evolve) strategies to overcome anti-predator defences. This drives an evolutionary ‘arms race’ with prey evolving new defences and predators evolving corresponding adaptations to defeat them (Dawkins and Krebs 1979). One way of enhancing escape behaviour, which might limit the scope for predator learning and adaptation, is the inclusion of unpredictability within the escape response. For example, prey might randomly select from two or more possible responses to threats, or when under pursuit they may make apparently random movements that make it more difficult for predators to anticipate their trajectory and capture them. These types of ‘unpredictable’ anti-predator defences are sometimes referred to as ‘protean behaviour’ (Driver and Humphries 1988). Seemingly unpredictable behaviour can increase the survival probability of prey; moths which incorporate erratic and irregular manoeuvres in their evasive behaviour are more likely to escape predation than those that do not (52.7% survival vs. 2.2% survival; Acharya and Fenton 1999), and fruit flies making erratic turns are less likely to be captured by hunting dragonflies (Combes et al. 2012).

The main predator evasion response in decapod crustaceans is the widely-studied ‘tail flip’ (Neil and Ansell 1995). This involves rapid flexion of the abdomen, which typically propels the animal backwards and away from an approaching threat. This can be modified to propel the animal upwards and forwards in response to a threat from behind, and more precise steering of the tail-flip is possible for fine adjustment of trajectory (Neil and Ansell 1995). For smaller crustaceans, however, since the

abdomen and cephalothorax are more evenly weighted, the tail flip is often modified to produce the ‘jack-knife’ response, and the abdominal flexion begins only after a roll to one side, such that the animal is propelled laterally (Neil and Ansell 1995; Arnott et al. 1998). The lateral roll can be to the left or to the right, which can introduce a simple level of unpredictability into the response, and a wide range of potential trajectories are possible, introducing further unpredictability.

The relevant neuronal control mechanisms have been most extensively studied in the crayfish (*Procambarus clarkii*), an Astacidean decapod crustacean, in which the escape circuit reduces response latencies by using giant neurones (which increase conduction velocity) and multiple electrical synapses (which minimize synaptic delay) (see Edwards et al. 1999 for review). Rearward tail flips are triggered by medial giant interneurons (MGs), a bilateral pair of single neurons with the cell body located in the brain, which respond to anterior stimuli. A somersaulting tail flip is triggered by the lateral giant interneurons (LGs), a bilateral pair of chained neurons connected by electrical synapses, which respond to posterior stimuli. These giant neurones trigger only single tail flips, which are considered to be fixed in their pattern of action (Wine and Krasne 1982). Repetitive tail flips involve many non-giant neurons that are widely distributed throughout the nervous system (Reichert et al. 1981). Non-giant tail flips usually cause a crayfish to swim away from the source of a stimulus (Reichert and Wine 1983). They not only follow giant mediated tail flips, but can also be initiated voluntarily, or occur in response to predatory strikes when the giant interneurons do not fire (Herberholz et al. 2004). Importantly, non-giant tail flips have significantly longer latencies than giant tail flips (Reichert et al. 1981; Reichert and Wine 1983; Herberholz et al. 2004).

Caridean decapod shrimps are considered to have the most sophisticated escape circuitry of all crustaceans (Faulkes 2008), since in addition to two pairs of giant fibres there is also extensive myelination of motor giant fibres and other smaller fibres (Johnson 1924; Holmes 1942). Myelination of these fibres contributes to rapid conduction (Holmes et al. 1941; Hartline and Colman 2007), with their highly organised sheaths and nodes probably permitting them to propagate nerve impulses by saltatory conduction (Heuser and Doggenweiler 1966; Xu and Terakawa 1999).

Arnott et al. (1998, 1999) studied the escape trajectories of a caridean decapod, the brown shrimp *Crangon crangon*, which inhabits areas of open sediment in shallow coastal waters. This species uses predator avoidance (self-burial in sediment) and resorts to predator evasion using a ‘jack-knife’ response when detected. Escape responses were triggered using both artificial stimuli and natural predators (juvenile cod, *Gadus morhua*). Relative to their body orientation, escape trajectories tended to be in relatively constrained directions, but were modified in response to the direction of the triggering stimulus. Furthermore, the attack direction also influenced the proportion of escapes that were to the animal’s left or right, with a balanced ratio when the attacks were from the front, but more biased to one side or the other for attacks from the sides.

The rockpool prawn (*Palaemon elegans* Rathke) is a species of similar size, which uses a similar ‘jack-knife’ response, but which lives in a more topographically varied environment (subtidally on rocky shores and in coastal rockpools). We investigated the responses of *P. elegans* to an artificial stimulus similar to that used by Arnott et al. (1999) as well as to an alternative stimulus which was intended to provide no visual information regarding the attack direction. We hypothesised that properties of the escape response of this species (escape directions and distances travelled) would differ between these two stimuli.

Methods

Experimental animals

Rockpool prawns (*Palaemon elegans*) were collected from rockpools in Kames Bay, Great Cumbrae, Scotland, using handnets. Animals were transferred to constant temperature (10°C) holding tanks prior to use in experimental trials. Water from the holding tanks was used in all experiments and was changed regularly.

High-speed video system

Trials were recorded at 250 frames per second using a Photron Fastcam-PCI high-speed video camera with a Computar TV Zoom Lens (1:1.2/12.5-75). The camera was operated from a dedicated portable PC, using Photron Motion Tools (Version 1.05). Illumination was provided by two Class 1 Tungsten Halogen lamps.

Escape reaction experiments

An opaque container was used as the arena for the experimental study to prevent visual cues from outside the enclosure influencing escape behaviour; this was a large white polystyrene box (internal dimensions: length: 495 mm, width: 315 mm, depth: 310 mm). The enclosure was mounted on four stands to allow access to the underside. The camera was positioned directly above the centre of the arena, directed downwards, and the lamps were positioned at each end of the arena. The arena was filled with water to a depth of 25 mm. A small object of known size was placed within the camera field of view to provide size calibration for the video analysis. An individual prawn was placed in an opaque black plastic cylinder (50 mm internal diameter) at the centre of the trial arena and left to settle for 5 minutes before trials began. A trial began as soon as the cylinder was lifted up and out of the enclosure.

Two types of stimulus were used to trigger escape responses. Stimulus 1 (S1) was executed by striking one haphazardly selected side of the enclosure, or the underside of the enclosure. This stimulus was intended to communicate no visual information on threat direction to the test subject, although mechanosensory cues may still have provided some directional information. Stimulus 2 (S2) was intended to convey clear visual information to the test animal regarding the threat direction, in addition to any mechanosensory cues. A hole was made through the side of the enclosure at a height of 100 mm, through which a thin metal rod with a wide flat plastic head (40 mm width) was inserted, such that the plastic head was under the water surface, parallel to the bottom of the tank. This allowed the experimenter to move the rod without having to lean over the side of the tank and potentially provide additional visual stimuli to the animal. Once the cylinder containing the test animal was lifted clear of the enclosure, the rod was moved rapidly towards the animal. The high speed camera software stores a continuously updated 10 second 'buffer' of video footage, which is only permanently saved to the hard drive after the user indicates that an experimental trial has ended. Only trials in which escape reactions were successfully triggered were stored in this way. Between individual trials, animals were placed in the opaque black cylinder for sixty seconds before the next trial.

At a minimum, ten replicate trials were recorded for each individual for each stimulus type, with a break of at least one hour between the two sets of experiments. Some

individuals were subjected to larger numbers of trials for additional analyses. The order of experimentation was random; animals were not consistently subjected to one type of stimulus trial before the other.

Video analysis

Video sequences were analysed using Image Pro Plus for Windows, Version 4.5.0.29. Several measurements were taken from video sequences of each trial (Fig. 1). The initial orientation and starting position of the animal were marked in the frame immediately prior to the visible start of the tail-flip response. The orientation was recorded as a line drawn from the tip of the cephalothorax to the end of the telson, and the position of the animal was taken as a point directly between its eyes. The end of a tail-flip was taken as the first frame in which the animal had returned to a fully upright orientation. At this point, its new position was marked (again taken as the point directly between the animal's eyes), allowing the Escape Distance to be measured (the distance between the new and original positions). Escape Distances were expressed in 'body lengths' – absolute distance travelled divided by the absolute length of the animal.

[Figure 1 near here]

The 'Escape Bearing' was measured by assuming that the animal's initial orientation pointed towards 0° and taking a bearing from the animal's starting position to its position after the tail flip had been completed. The 'Escape Angle' was then taken to be the smallest angle between the initial orientation of the animal and a line drawn from the animal's starting position to its end position (representing its escape trajectory). For Escape Bearings $\leq 180^\circ$ the Escape Bearing and Escape Angle are identical; when the Escape Bearing is $> 180^\circ$, the Escape Angle is calculated by subtracting the Escape Bearing from 360° . All Escape Angles are therefore between 0 and 180° . Whether an escape was to the left (left-handed escape) or to the right (right-handed escape) of the initial axis of orientation was also recorded.

To check for possible directionality in the responses to S1 stimuli, the direction of escape was also recorded relative to the side of the enclosure that was struck to generate the stimulus. The Screen-relative Escape Bearing (SEB) was measured as the

angle between a line running from the animal's starting position to the side of the enclosure which was struck, and a line drawn from the animal's starting position to its end position (representing the escape trajectory). A SEB of 0° thus indicates that the escape trajectory was directly towards the side hit, while a SEB of 180° occurs when the animal moves directly away. For S1 trials where the stimulus was generated by striking the underside of the enclosure, SEBs were measured relative to the top of the camera field of view.

For S2 trials, the 'Attack Bearing' was measured as the bearing (relative to the animal's axis of orientation) from the starting position of the animal to the nearest point on the metal rod used to elicit an escape response. This bearing was taken in the frame immediately prior to the visible start of the tail-flip response. Attack Angle was calculated from Attack Bearing in the same way as Escape Angle was calculated from Escape Bearing (such that all Attack Angles were within the range 0-180°). It was also noted whether the escape response was left-handed or right-handed, and whether it was 'contralateral' or 'ipsilateral' relative to the stimulus. A contralateral escape is towards the opposite side of the animal to the eliciting stimulus, and an ipsilateral escape is one where the escape response is towards the same side as the stimulus. For example, if the metal rod approached from the left side of the animal, a contralateral escape would involve the animal escaping to its right, while an ipsilateral escape would involve the animal moving to its left. The final orientation of the animal after S2 trials was also recorded (in the same manner that orientation was recorded prior to the start of the reaction), and the rotation of the axis relative to its starting orientation was measured as the angle between these two lines. Escape trajectories were also calculated relative to the eliciting stimulus. The Escape Trajectory Bearing (ETB) was calculated using the Attack Bearing (AB) and Escape Bearing (EB), using either Equation 1 or Equation 2.

$$\text{If } EB > AB, \text{ then } ETB = EB - AB. \quad (\text{Equation 1})$$

$$\text{If } EB < AB, \text{ then } ETB = 360 - (AB - EB) \quad (\text{Equation 2})$$

Escape Trajectory Angle (ETA) was calculated from ETB in the same way that Escape Angle is calculated from Escape Bearing (If $ETB \leq 180^\circ$, $ETA = ETB$; when $ETB > 180^\circ$, $ETA = 360^\circ - ETB$).

Data Analysis

Data analyses were carried out using Minitab version 16.1.0 (Minitab Inc.), Sigmaplot version 12.5 (Systat Software, Inc.), R version 3.0.2 (R Development Core Team 2008) with packages *pgirmess* and *psych*, and Oriana v2.02c (Kovach Computing Services).

Results

A total of 268 responses to Stimulus 1 and 611 responses to Stimulus 2 were recorded, from trials conducted on a total of 43 animals. Data from only 21 animals were used to compare responses to the two stimulus types; these 21 animals were selected on the basis that they were tested at least 10 times with each stimulus. Animals responded to both stimuli by executing a ‘jack-knife’ response (Neil and Ansell 1995; Arnott et al. 1998), and tail flips were elicited in virtually all cases; failures to respond were not analysed. Multiple flips were observed for both types of stimulus (9.7 % of escapes from S1 and 11.0 % of escapes from S2), but the typical response to either stimulus was a single tail flip. Therefore the following analyses of escape properties only consider the first tail flip. In rare cases (1 out of 268 responses to S1 and 9 out of 611 responses to S2) no lateral roll occurred and the escape trajectory was approximately directly backwards.

Stability of tail-flip parameters over multiple trials

Considering only S1 trials (conducted on 21 animals), there was no significant change in Escape Distance between the first measured tail-flip and the tenth (Paired t-test, $n = 21$, $t = -0.10$, one tailed $p = 0.541$). There was also no significant effect of repeated stimulation (over the course of 10 replicate S1 trials) on Escape Angle (two-way ANOVA; $p > 0.05$; Table 1), although there was some variation among individual animals.

[Table 1 approximately here]

Directionality of responses to Stimulus 1

When S1 stimuli were generated by striking the underside of the tank, the distribution of Screen-relative Escape Bearings (SEBs) was not significantly different from a uniform circular distribution (Rayleigh's $Z = 0.765$, $n = 135$, $p > 0.05$). However, when S1 stimuli were generated by striking the side of the tank, SEBs were not uniformly distributed (Rayleigh's $Z = 3.916$, $n = 132$, $p < 0.05$), and 60.6% of escapes were away from the side of the tank that was struck ('away responses' with Escape Trajectory Bearings between 90° and 270°). This was significantly different from the 50% away responses that would be expected if there was no directional response to the stimulus (Exact binomial test, $p < 0.01$, Alternative Hypothesis – proportion of ETBs between 90° and 270° is $> 50\%$). However, in several respects escapes did not differ when the side of the enclosure was struck instead of the underside: mean Escape Distance of initial tail-flip ($t = 1.5$, d.f. = 252, $p > 0.05$); proportions of left-handed and right-handed escapes (not significantly different from 50/50 in either case; Exact binomial test, $p > 0.05$); mean Escape Angle ($t = -0.63$, d.f. = 258, $p > 0.05$); and variance in Escape Angle (Bartlett's $K^2 = 2.65$, d.f. = 1, $p > 0.05$). Therefore, when comparing these aspects of escape behaviour between S1 and S2 trials (see below), all S1 data were pooled.

Comparison of responses in S1 and S2 trials

For comparison of the behaviour of *P. elegans* in S1 and S2 trials, data were only considered for 21 individuals where 10 or more replicate trials were recorded for both stimulus types (where an individual animal was subjected to more than 10 trials of either type, only the first ten were used in the comparative analyses). This left data from 420 trials (10 trials of each stimulus type for each of individual).

The mean distance covered by an individual animal in a single tail flip (averaged over 10 trials) was greater in S2 trials than in S1 trials (Table 2; Paired t-test, $t = 6.952$, d.f. = 20, $p < 0.01$). For both stimuli (not taking into account the precise direction from which the stimuli originated), the proportions of left- and right-handed escapes were not significantly different from 50/50 (Exact binomial test, $n = 210$, $p > 0.05$ for both S1 and S2, Alternative Hypothesis - Percentage of left-handed escapes $\neq 50\%$).

[Table 2 approximately here]

Escape Bearings were not uniformly distributed (Fig. 2) for either stimulus type (S1 - Rayleigh's $Z = 71.874$, $n = 210$, $p < 0.01$; S2 - Rayleigh's $Z = 54.255$, $n = 210$, $p < 0.01$). In both S1 and S2 trials, left-handed and right-handed escapes had similar Escape Angle distributions, both in terms of the mean Escape Angle (S1 - $t = -0.7344$, d.f. = 208, $p > 0.05$; S2 - $t = 0.6911$, d.f. = 208, $p > 0.05$) and the variance in Escape Angle (S1 - Bartlett's $K^2 = 0.5647$, d.f. = 1, $p > 0.05$; S2 - Bartlett's $K^2 = 0.5396$, d.f. = 1, $p > 0.05$). Therefore Escape Angle data for left- and right-handed escapes were pooled for each stimulus type.

[Figure 2 approximately here]

Distributions of Escape Angles differed between S1 and S2 trials (Fig. 2; Table 2). Mean Escape Angle was significantly smaller in S2 trials than S1 trials ($p < 0.05$; Table 3), and there was significant variation among individual animals ($p < 0.01$; Table 3). There was also significantly greater variance in Escape Angle in S2 trials compared to S1 trials (Bartlett's $K^2 = 59.5856$, d.f. = 1, $p < 0.01$).

[Table 3 approximately here]

Influence of attack direction on escape trajectory in S2 trials

For analysis of the effect of attack direction on escape trajectory, data from 538 S2 trials, conducted on all 43 animals, were considered. Trials in which Attack Bearing/Angle or Escape Bearing/Angle could not be measured from the footage were excluded.

In 208 of the 538 S2 trials examined, the stimulus rod came into contact with the test animal. The Escape Distance of tail flips following contact with the stimulus rod was somewhat larger (mean ED = 2.38) than where there was no contact (mean ED = 2.12) but there was no significant variation among individual prawns (Table 4). Whether or not the rod made contact with the test animal had no significant effect on the Escape Angle, and there was no significant variation among individual animals in

Escape Angle; in contrast, Escape Angle was significantly affected by Attack Angle (Table 4).

[Table 4 approximately here]

Stimulus direction affected the proportions of contralateral and ipsilateral escapes (Fig. 3). When the stimulus was to the front or rear of the animal, the proportions of ipsilateral and contralateral escapes were not significantly different. Lateral stimuli resulted in greater proportions of escapes being directed to the contralateral side, especially when the Attack Angle was between 90° and 120° (Fig. 3). However, only when the eliciting stimulus had an Attack Angle between 60° and 150° were the proportions of ipsilateral and contralateral escapes found to be significantly different from an equal distribution (Exact binomial test, $p < 0.01$, Alternative Hypothesis - Percentage of contralateral escapes $> 50\%$).

[Figure 3 approximately here]

Attack Angle also affected Escape Angle (Table 4). S2 trials were split into six groups based on the sector from which the ‘attack’ stimulus originated (Table 5). Median Escape Angle differed significantly among escapes in each of the six groups (Kruskal-Wallis $H = 122.97$, d.f. = 5, $p < 0.01$; Table 5). Attacks towards the front of the animal resulted in larger Escape Angles (distributions shifted further towards the rear relative to escapes in S1 trials), while attacks towards the rear sectors resulted in relatively forward shifted escape distributions (Fig. 4). Following tail flips, the orientation of the animal had also been rotated (either clockwise or anti-clockwise) with respect to the orientation immediately prior to reaction. Typically this rotation was clockwise following left-handed escapes (305 out of 313 left-handed escapes) and anti-clockwise if the escape was right-handed (217 out of 225 right-handed escapes). The amount of rotation was strongly and significantly negatively correlated with the Escape Angle (Spearman’s $\rho = -0.748$, $p < 0.01$, $n = 507$; trials where rotation of axis could not be accurately measured were excluded). Large Escape Angles were associated with very small rotation angles, while relatively small escape angles were associated with significantly larger rotation angles.

[Table 5 approximately here]

[Figure 4 approximately here]

Escape trajectories relative to Stimulus 2

Escape Trajectory Bearings were not uniformly distributed (Fig. 5a; Rayleigh's $Z = 144.474$, $n = 537$, $p < 0.01$), nor did they correspond to a von Mises distribution (Watson's $U^2 = 0.156$, $p < 0.01$). Escapes were mostly oriented away from the stimulus (82.5% of Escape Trajectories were between 90° and 270°). Escape Trajectory Angles had a distribution that was skewed towards 180° (Fig. 5b), but with an apparent peak between 150° and 160° (the modal class).

[Figure 5 approximately here]

Discussion

*Escape behaviour of *Palaemon elegans**

Two types of stimulus were presented to individual *P. elegans*, and both triggered 'tail flip' escape responses, which were usually single flips. The first stimulus type, Stimulus 1, most likely consisted of purely mechanosensory cues received by the individual via the water and the floor of the enclosure. This stimulus was intended to minimise or eliminate precise directional information. However, the weak (but significant) tendency for animals to move away from the side of the enclosure that was struck indicates that individuals were able to determine the direction of threat, at least in some cases. This tendency was comparatively weak; only 60.6% of escapes in S1 trials were 'away' responses, compared to 82.5% in S2 trials. This may be because the mechanosensory cues gave only approximate information on the direction of threat. This could prevent accurate directional responses, but still be enough to subtly shift the distribution of escapes such that more were away from the threat. A second possibility is that uncontrolled variability in the intensity of strikes to the side of the enclosure (the force used to create the stimulus was not standardised) may have meant that directional cues were strong in some trials even if weak or non-existent in others, creating a small 'away' bias in the overall distribution of escapes. When tail flip responses were elicited by striking the underside of the tank, the circular distribution of escapes was uniform, and no directional trend was detected.

The second stimulus type seemed to provide more precise directional information, and was multisensory; both visual and mechanosensory cues were generated by the approaching rod. Animals moved further in S2 trials than in S1 trials. This difference in response may have resulted from the different modes of detection involved (Stimulus 2 included visual information as well as potentially differing mechanosensory cues), or it could be an additive effect of more than one cue being present in S2 trials. In S2 trials where the rod approached close enough to make contact with some part of the animal, escape distances were even greater, which potentially supports an additive effect, since these trials included an additional cue – physical contact with the ‘predator’. Other species are known to modify their escape reactions based on the properties of an approaching threat; zebra danios (*Brachydanio rerio*) react earlier to faster, larger predators (Dill 1974). It is also possible that with more complete and reliable information about the approach direction of a threat, an animal can confidently ‘invest’ in a longer escape flight from a predator. When there is no clear indication (or only a weaker, less reliable indication) of the location of a threat, it may be more profitable to move a shorter distance, since any movement may actually bring the animal closer to a potential predator. Smaller displacements under uncertainty could thus reduce the risk associated with a poor choice of escape trajectory. When the stimulus is a ‘false alarm’ normal foraging may resume more quickly after a shorter escape reaction, reducing opportunity costs. A similar effect has been demonstrated for crayfish, where individuals which responded to a threat by executing a full tail flip took longer to reach a food source than those which froze in response (Liden et al. 2010). Larger escape distances can also be associated with an increased risk of detection by other predators (Bateman and Fleming 2014).

It is also interesting to note that statistically significant variation in Escape Angle was apparent between individuals in S1 trials (see Table 1), while individual animals did not appear to vary significantly in how they responded to more clearly directional stimuli (individual was not a significant factor influencing Escape Angle, Table 4b). This suggests that small individual variation in ‘preferred’ escape trajectory could be suppressed when responding to more clearly directional stimuli.

In any case, our results imply that *P. elegans* can modify not only the direction but also the strength of their tail flips. The neuronal pathways mediating the escapes in the present study were not investigated, and neither were the response latencies to either stimulus measured. Consequently it is not possible to draw definitive conclusions from our data about the neural control mechanisms underlying the modulation of the escapes. Studies on crayfish have concluded that plasticity in their tail flips is most often associated with non-giant circuitry (Wine and Krasne 1982; Edwards et al. 1999; McCarthy et al. 2004; Liden and Herberholz 2008), although evidence is accumulating that giant fibre tail flips can also be modulated by multimodal (visual and hydrodynamic) sensory systems (Newland et al. 1992; Neil and Ansell 1995; Liu and Herberholz 2010). However, since Caridean decapod shrimps are known to possess non-giant interneurons and motor giant fibres that are myelinated (Johnson 1924; Holmes et al. 1941; Hartline and Colman 2007) and are thus capable of rapid and perhaps salutatory conduction (Heuser and Doggenweiler 1966), it may be difficult to use response latency alone as a criterion for distinguishing between giant- and non-giant-mediated tail flips. This is unlike the case for macruran decapods, in which the non-giant interneurons lack myelination. Indeed, many aspects of escape behaviour of shrimps (Arnott et al. 1998, 1999) and prawns (present study) are not easily explained by what is known from crayfish escape responses (Faulkes 2008), and remain to be investigated.

Factors affecting escape trajectories

Following S1 stimuli, Escape Angles (relative to the original orientation of the animal) were centred on a mean angle of approximately 122°, within a relatively constrained range of less than 72° (Fig. 2; Table 2). Relatively fixed escape angles with respect to body orientation (referred to as ‘turn angle’ in some studies) have been identified in other species (Domenici et al. 2011b). For some species it has been suggested that biomechanical constraints may determine the ‘default’ escape trajectory (Arnott et al. 1999). However we recorded substantial variability around the mean Escape Angle, particularly when escape reactions were elicited using Stimulus 2. While escapes in many directions are biomechanically possible, it may be that tail flips with Escape Angles of around 120° require the least steering effort and can

therefore be executed more easily or quickly; use of a preferred Escape Angle may be a way of reducing reaction times.

Actual escape trajectories may be a compromise between favoured Escape Angles and the need to select an optimum trajectory with respect to an attacking predator. Stimulus direction is an important element influencing the escape trajectory in a range of taxa, including teleost fish (Eaton and Emberley 1991), amphibians (Dill 1977), insects (Camhi and Tom 1978; Domenici et al. 2008), and crustaceans (Arnott et al. 1999). *Palaemon elegans* also modify their escape trajectories based on the direction of the stimulus. When attacks originated from in front of the animal (Attack Angles up to 90°), Escape Angles were similar to those after non-directional stimuli (Fig. 4; Table 5). When attacks originated from behind the animals, however, the distributions of Escape Angles were shifted forwards (Fig. 4d, e, f).

Considering the direction of escapes relative to the threatening stimulus (ETB and ETA, Fig. 5) there was a peak in ETA between 150° and 160° (Fig. 5b). For any predator-prey system, there may be an optimum escape trajectory, balancing the need to move away from the predator against the need to move out of its attack path (Weihs and Webb 1984; Domenici et al. 2011a). Escapes with a trajectory of 180° (directly away from the predator, but also directly along the predator's axis of movement) are thought to be sub-optimal, since it is often the case that in a straight-line chase many predators can out-pace their prey. While some escapes in this study did follow a 180° trajectory relative to the 'predator' (Fig. 5a) there is a dip in the frequency of escapes with ETA greater than 160° (Fig. 5b). For a number of species, there seem to be peaks in the distribution of escape trajectories relative to the direction of the threat: one at 180° (directly away from the threat), and one at around 90-130° (Domenici et al. 2011a). This is true of *C. crangon*, which had peaks at approximately 180° and 130° (Arnott et al. 1999). Our data do not conform to this pattern, having only one apparent peak at around 160° (Fig. 5b), which is notable since in other ways (such as the preferred escape direction relative to initial body orientation) the escape behaviour of *P. elegans* is similar to that of *C. crangon*. The 160° peak is, however, consistent with theoretical estimates that the optimal evasion angle should be less than 21° from the line heading directly from the prey to the predator (Weihs and Webb 1984).

Arnott et al. (1999) identified a 126° arc (63° to either side of the eliciting stimulus) into which *C. crangon* did not escape, which they described as an ‘exclusion envelope’. Our data show a similar property of the distribution of escape trajectories in *P. elegans*; only approximately 5% of escapes following S2 stimuli had Escape Trajectory Angles less than 60° (28 out of 537 trials; see Fig. 5b). The resulting 120° wide arc can be considered analogous to the ‘exclusion envelope’ of Arnott et al (1999)

‘Protean’ behaviour and predator evasion tactics in P. elegans

There are two main elements of the escape response of *P. elegans* where unpredictability can be introduced: the ‘choice’ of left-handed or right-handed escape, and the actual escape angle. Even if the typical Escape Angle is relatively fixed, a predator approaching from the front will still have only a 50% chance of successfully predicting whether an escaping prawn will move left or right. A predator approaching with an Attack Angle between 60° and 165° will trigger escapes that are somewhat more predictably contralateral, especially when the Attack Angle is between 90° and 105°, when almost no ipsilateral escapes occur (Fig. 3). This pattern (equal proportions of ipsilateral and contralateral escapes for frontal stimuli, and a contralateral bias for lateral stimuli) is found in other species, including crustaceans such as *C. crangon* (Arnott et al. 1999) and vertebrate larvae (Boothby and Roberts 1995). From the perspective of *P. elegans*, maximum unpredictability occurs when predators approach from the front (or the rear). Rotation of the body orientation of individual prawns during a tail-flip generally resulted in animals facing back towards their original position. This would tend to orient the animal so that it is facing towards the predator. This potentially aids the animal in preparing for another strike from the same predator, by increasing the unpredictability of any subsequent escape reactions.

From the predator’s perspective, the escape response is most predictable when individual *P. elegans* are approached from the side; very high proportions of escapes are contralateral for lateral approaches. Prey animals may also present a larger target when viewed from the side (Domenici and Blake 1997). Even in these circumstances, when it is easy to predict whether an individual will go left or right, there is still substantial variation around the modal Escape Angle, making accurate prediction of trajectory difficult. Furthermore, if we assume that predators in the marine

environment encounter prey at random, then the orientation of the prey with respect to the predator may be highly variable; under these circumstances, the trajectory of escaping prey can be considered to be effectively random, even if the Escape Angle of the prey is fixed with regard to its own body orientation (Domenici et al. 2011a).

Escape trajectories of fleeing prey are often predominantly ‘away’ responses (Domenici et al. 2011b); these are trajectories which correspond to an ETB between 90° and 270° . Most escape responses in many species, including angelfish (Domenici and Blake 1993b), lizards (Martin and Lopez 1996) and *Xenopus laevis* embryos (Boothby and Roberts 1995) are ‘away’ responses. Similarly, most escape trajectories of *P. elegans* were ‘away’ responses with an ETB between 90° and 270° (Fig. 5a). However, not all escapes were in this range; 17.5% of escape trajectories were ‘towards’ responses (escapes with ETB of less than 90° or more than 270°). These ‘towards’ responses may seem disadvantageous as they potentially move the animal closer to an approaching predator. It has been hypothesised that maintaining a proportion of counter-intuitive escapes back towards an attack may introduce an additional element of unpredictability and therefore help to confound predator learning; alternatively, ‘towards’ responses may be advantageous under some specific circumstances, or may represent tactical ‘mistakes’ (Domenici et al. 2011a).

Comparison with natural scenarios

Our experimental trials used an unnatural environment and unnatural stimuli. In the natural environment, escapes from predators occur in complex and heterogeneous habitats with obstacles and refuges, and in the presence of conspecifics and sometimes multiple predators. Escaping prey may use their environment to help with escapes, for example by always fleeing towards available refuges (Cooper 1997) or by allowing predators to get closer before fleeing if there are nearby refuges (Cuadrado et al. 2001). Animals may alter escape trajectories to avoid obstacles (Eaton and Emberley 1991), and escape trajectories can be modified by prior ‘sub-threshold’ stimuli (these may provoke some reaction but are not sufficient to trigger a full escape response). For example, *C. crangon* tend to escape away from the side which received a lateral sub-threshold stimulus prior to a stronger stimulus from the front (Arnott et al. 1999). Individual animals may respond differently to predatory attacks (or artificial stimuli) when solitary, compared to when they are aggregated in shoals (Domenici

and Batty 1997), and escape behaviours of some species may be altered when multiple predators are present (Cooper et al. 2007). Furthermore, animals may react differently to real predators compared to artificial stimuli (Dill 1974), may respond differently to different predator species (Staudinger et al. 2013), or may have a number of alternative escape strategies which could be used under a range of circumstances (Domenici and Blake 1991; Domenici and Blake 1993a; Bateman and Fleming 2014). Experimental trials using real predators (such as those conducted by Arnott et al. 1999) in more ‘realistic’ trial arenas would be the only way to verify that the results of this study apply during real predator-prey encounters, and to evaluate whether different escape trajectories (or alternative strategies) are more or less successful at evading predators.

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Table 1. Two way ANOVA (GLM) for effect of repeated stimulation (Trial number; fixed factor) and identity of individual animals (Animal; random factor) on Escape Angle for S1 trials

Source	DF	SS	MS	<i>F</i>	<i>p</i>
Animal	20	8366.140	418.307	3.744	< 0.001
Trial number	9	499.708	55.523	0.497	0.875
Residual	180	20109.922	111.722		
Total	209	28975.770	138.640		

Table 2. Summary data for escapes during experimental trials

	Mean Escape Distance (body lengths)	Mean Escape Angle ($\pm$ s.d.)	Median Escape Angle	Range of escape angles
Stimulus 1	1.73	126.60 ($\pm$ 11.77)	125.71	71.92
Stimulus 2	2.24	122.62 ($\pm$ 20.36)	121.91	119.78

Table 3. Two-way ANOVA (GLM) for effect of stimulus type (Stimulus, fixed factor) and individual identity (Animal; random factor) on Escape Angle.

Source	DF	SS	MS	<i>F</i>	<i>p</i>
Stimulus	1	1666.3	1666.3	5.31	< 0.05
Animal	20	23499.6	1175.0	3.75	< 0.01
Stimulus*Animal	20	6271.8	313.6	1.38	0.127
Error	378	85801.2	227.0		
Total	419	117239.0			

Table 4. Effects of contact with the stimulus rod during S2 trials, on (a) Escape Distance (ANOVA) and (b) Escape Angle (ANCOVA). Fixed factor: ContactY/N. Random factor: Animal. Attack Bearing is the covariate in (b).

a. Effect of contact on Escape Distance					
Source	DF	SS	MS	<i>F</i>	<i>p</i>
Animal	42	13.92	0.33	0.64	0.924
ContactY/N	1	3.72	3.72	7.68	< 0.01
Animal*ContactY/N	42	21.78	0.52	1.35	> 0.05
Error	425	163.35	0.38		
Total	510	202.96			

b. Effect of contact on Escape Angle					
Source	DF	SS	MS	<i>F</i>	<i>p</i>
Attack Angle	1	35995.1	35995.1	114.10	< 0.001
Animal	42	17100.1	407.1	1.39	0.147
ContactY/N	1	98.9	98.9	0.33	0.567
Animal*ContactY/N	42	12334.7	293.7	0.93	0.597
Error	451	142271.4	315.5		
Total	537				

Table 5. Comparisons between median Escape Angles for escapes resulting from Stimulus 2 ‘attacks’ within each of six sectors. There was significant overall variation in the median Escape Angle ($p < 0.01$). Pairwise tests were conducted using routine ‘kruskalmc’ in R (R Development Core Team 2008) package *pgirmess*. ‘Attack Sectors’ are 30° sectors from which stimuli originated (**a – f** in this table correspond to **a – f** in Fig. 4)

Attack Sector	n	Median Escape Angle (°)	Significant differences at $p < 0.05$
a (0-30°)	120	131.54	d, e, f
b (30-60°)	106	129.75	d, e, f
c (60-90°)	110	124.30	e, f
d (90-120°)	93	114.41	a, b, f
e (120-150°)	63	110.16	a, b, c, f
f (150-180°)	46	102.60	a, b, c, d, e

Figure 1. Measurements extracted from video footage of escape responses. Solid grey line indicates axis of orientation of animal before and after responses. **a.** Escape Distance, **b.** Escape Bearing, **c.** Escape Angle (identical to Escape Bearing for escapes oriented between 0° and 180°), **d.** Attack Bearing (applicable to S2 trials only), **e.** Attack Angle (applicable to S2 trials only, and identical to Attack Bearing for attacks originating between 0° and 180°), **f.** rotation of orientation axis.

Figure 2. Distribution of Escape Bearings in S1 (left) and S2 (right) trials. The 0-180° axis corresponds to the orientation of the animal in the video frame immediately prior to the visible start of the tail-flip response, with the animal facing towards 0°.

Figure 3. Effect of Attack Angle in S2 trials on proportion of contralateral escapes (escapes to the opposite side of the animal to the attack stimulus). Bars marked * show significant departure from a 50/50 distribution at $p < 0.01$ (Exact binomial test, Alternative Hypothesis - Percentage of contralateral escapes $> 50\%$).

Figure 4. Distribution of Escape Angles for escapes in S2 trials with Attack Angles within each of six 30° sectors: **a** 0-30°, **b** 30-60°, **c** 60-90°, **d** 90-120, **e** 120-150°, **f** 150-180° (see inset). Dashed line shows the median Escape Angle in each case, solid line shows median Escape Angle for all escapes following S1 (for reference).

Figure 5. **a.** Distribution of Escape Trajectory Bearings (ETB) relative to stimulus direction for S2 trials. Bars represent a class width of 6° and each concentric circle marks a frequency of 5 (max 25), $n = 537$ trials. **b.** Distribution of Escape Trajectory Angles (ETA) for the same trials.