The Journal of Experimental Biology 207, 1855-1863
Published by The Company of Biologists 2004
doi:10.1242/jeb.00992

Escape behavior and escape circuit activation in juvenile crayfish during prey–predator interactions

Jens Herberholz*, Marjorie M. Sen† and Donald H. Edwards

Department of Biology, Georgia State University, Atlanta, GA 30303, USA

*Author for correspondence (e-mail: biojhh@langate.gsu.edu)

†Present address: Emory University, PO Box 26177, Atlanta, GA 30322, USA

Accepted 22 March 2004

Summary

The neural systems that control escape behavior have been studied intensively in several animals, including mollusks, fish and crayfish. Surprisingly little is known, however, about the activation and the utilization of escape circuits during prey–predator interactions. To complement the physiological and anatomical studies with a necessary behavioral equivalent, we investigated encounters between juvenile crayfish and large dragonfly nymphs in freely behaving animals using a combination of high-speed video-recordings and measurements of electric field potentials. During attacks, dragonfly nymphs rapidly extended their labium, equipped with short, sharp palps, to capture small crayfish. Crayfish responded to the tactile stimulus by activating neural escape circuits to generate tail-flips directed away from the predator. Tail-flips were the sole defense mechanism in response to an attack and every single strike was answered by tail-flip escape behavior. Crayfish used all three known types of escape tail-flips during the interactions with the dragonfly nymphs. Tail-flips generated by activity in the giant neurons were predominantly observed to trigger the initial escape responses to an attack, but non-giant mediated tail-flips were often generated to attempt escape after capture. Attacks to the front of the crayfish triggered tail-flips mediated either by the medial giant neuron or by non-giant circuitry, whereas attacks to the rear always elicited tail-flips mediated by the lateral giant neuron. Overall, tail flipping was found to be a successful behavior in preventing predation, and only a small percentage of crayfish were killed and consumed.

Key words: crayfish, *Procambarus clarkii*, dragonfly nymph, *Anax junius*, predator, prey, escape.

Introduction

The neural circuits that underlie tail-flip escape behavior in crayfish have been intensively studied for more than 50 years by means of electrophysiological and anatomical methods (Wiersma, 1947; Furshpan and Potter, 1959; Zucker et al., 1971; Mittenthal and Wine, 1973; Reichert and Wine, 1982; Heitler et al., 1991; Yeh et al., 1996; Herberholz et al., 2002; Antonsen and Edwards, 2003; reviewed by Edwards et al., 1999). Three major circuits have been identified, two of them being controlled by giant interneurons that evoke a stereotyped, reflexive tail-flip response away from the stimulus and one being controlled by a non-giant system that produces tail-flips of more variable and less stereotyped forms (Wine and Krasne, 1972). The giant neuron mediated tail-flips are elicited by strong, phasic stimuli to either the rear (lateral giant mediated tail-flip, LG) or the front (medial giant mediated tail-flip, MG); the latter is also evoked by a fast approaching visual stimulus (Olson and Krasne, 1981). The non-giant mediated tail-flips (Non-G) are usually triggered by more gradual stimuli, have much longer response latencies and are also used during swimming (Kramer and Krasne, 1984). Excitation of the escape circuits is caused by stimulation of mechanosensory hairs that provide sensory input to primary afferents and interneurons. When the stimulus in the sensory field is strong enough, activation in the circuits will evoke the escape response. Tail-flips controlled by the giant neurons display very different forms. The MG-activated tail-flips produce a fast, straight and backwards ‘jump’, while the LG-activated tail-flips result in an upward, jack-knife motion (Wine and Krasne, 1972). Activation of the much less understood Non-G system, however, produces tail-flips that can take a variety of forms and are therefore behaviorally indistinguishable from the other two types (Wine and Krasne, 1982). To differentiate between the three neural escape circuits in intact animals it is necessary to use implanted electrodes (Glanzman and Krasne, 1983; Krasne et al., 1997; Herberholz et al., 2001) or bath electrodes to record the giant neuron activity and muscle potentials that are characteristic of each type of tail-flip (Herberholz et al., 2001).

Previous studies that examined predation on crayfish reveal little detail of the actual escape behavior and were mostly

concerned with ecological implications (Dye and Jones, 1975; Stein and Magnuson, 1976; Stein, 1977; DiDonato and Lodge, 1993; Garvey et al., 1994; Hill and Lodge, 1994; Söderbäck, 1994; Blake and Hart, 1995; Correia, 2001). These studies were either carried out in the field or in large test chambers (i.e. outdoor pools) where interactions between large predators (e.g. smallmouth bass) and crayfish were observed. In these conditions, it was possible to determine when crayfish escaped from an attack but it was not possible to determine which type of tail-flip the crayfish used to escape.

Dragonfly nymphs are opportunistic aquatic predators that hunt other invertebrate larvae including conspecifics (Merrill and Johnson, 1984; Wissinger, 1989; Johansson and Johansson, 1992; Wissinger and McGrady, 1993), tadpoles (McCollum and Leimberger, 1997; Barnett and Richardson, 2002) and small fish (Crumrine and Crowley, 2003). The predatory strike of dragonfly nymphs is based on hydraulic mechanisms. The animals close the anal valve and contract the abdominal dorso-ventral muscles to generate a rapid increase in hemolymph pressure that is followed by labial extension (Pritchard, 1965; Olesen, 1972). The fast movement of the labium is accomplished by storing muscular energy during the preparatory period of the strike (Tanaka and Hisada, 1980; Kanou and Shimozawa, 1983).

We found that dragonfly nymphs reliably attack juvenile crayfish and consume them after capture. Moreover, both animals are small enough to be tested in an aquarium that allows high-speed video recordings in combination with recordings of electric field potentials by use of bath electrodes. Therefore, they represent an ideal model for studying the details of prey–predator interactions in the laboratory. For 50 years, in the absence of any concrete behavioral evidence, it was assumed that the escape circuits in crayfish are activated in response to attacks from predators. Here, we test this hypothesis for the first time by measuring the activity of the escape circuits in response to attacks from natural predators.

Materials and methods

Adult female crayfish (*Procambarus clarkii* Girard) with eggs were obtained from a commercial supplier (Atchafalaya Biological Supply Co., Raceland, LA, USA) and then isolated in small water-filled plastic containers (8.5×15×8.5 cm). The free-swimming offspring were separated from the mother, kept in communal tanks until big enough for the experiments and then isolated in small containers for at least six days before being used. Dragonfly nymphs (*Anax junius* Drury) were obtained from a commercial supplier (Carolina Biological Supply Company, Burlington, NC, USA) and isolated in small plastic containers filled with water after arrival. Crayfish were fed with food pellets, and dragonfly nymphs with mealworms twice per week (not on the day of testing).

In a series of 41 single experiments, one crayfish and one dragonfly nymph were paired in a small test chamber (8×4.5×4 cm) filled with deionized water (height, 3 cm). None of the animals was used in more than one experiment. Animals were size matched for each experiment, with all crayfish (mean ± S.D., 2.1±0.2 cm; range, 1.7–2.6 cm; measured from rostrum to telson) being within 50–59% of the size of the dragonfly nymph (mean ± S.D., 3.9±0.4 cm; range, 3.1–4.7 cm; measured from tip of head to end of abdomen). Two lights were directed towards the test chamber to provide sufficient illumination for the high-speed video recordings. All animals were checked for physical intactness before the experiments and no crayfish molted within four days prior to or two days after the experiment. In a few experiments, the dragonfly nymphs did not attack the crayfish within 20 min after both animals were introduced to the test chamber. The animals were removed and no data were included in the analysis. Results from three experiments (7%) were excluded from the analysis because the video- and electrical recordings were not clear. Thus, a total of 38 experiments (i.e. attacks) were eventually used for analysis and statistical procedures. Non-parametric tests (Jandel SigmaStat® 2.0 and GraphPad Prism® 4.0) for independent data [Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks, Mann–Whitney rank sum test and Fisher's exact test] were applied for statistical comparison.

Field potentials from the aquarium bath were recorded with a pair of silver wire electrodes (1 mm outer diameter, insulated except at the tips) placed at either end of the test chamber. The signal was AC-coupled and amplified (1000×; A-M Systems, Sequim, WA, USA), displayed on an oscilloscope and simultaneously recorded on a personal computer with Axoscope software (Axon Instruments, Union City, CA, USA).

The behavior of the animals was taped with a high-speed video camera (5 ms frame^{-1}; JC Labs, San Mateo, CA, USA) from above and the side by means of a mirror angled at 45° from the base of the aquarium. Another mirror reflection of the oscilloscope trace in the bottom half of each video frame was used to correlate the recorded behavior and the electric field potentials recorded by the oscilloscope as well as the computer. The camera was connected to a video-recorder as well as a monitor, and data were stored in S-VHS format. The recordings were started shortly before the animals were introduced into the test arena and stopped some time after an attack by the dragonfly nymph had taken place. The behavior of the prey and predator were also recorded in a larger arena using a digital video camera (Canon XL1-S). All video recordings were digitized by use of Adobe Premier® and Dazzle* Digital Video Creator™, and single frames were used for analysis and illustrations.

Video- and field potential recordings were also used to monitor attacks from isolated dragonfly nymphs directed towards mock prey by use of a Puritan® cotton-tipped cleaning stick (length, 15 cm) or a small piece of black tape on a string moved randomly in front of them.

Under the same experimental conditions (but with a video-camera side view only) we also measured the response latencies of individual escape tail-flips generated by six previously isolated crayfish of a similar size (2.4±0.2 cm) as used during prey–predator interactions. The animals were stimulated to tail-flip with a handheld glass probe by tapping

them to the front or rear at different intensities. Sharp taps were administered to evoke giant mediated tail-flips and gentler taps to evoke non-giant tail-flips (cf. Herberholz et al., 2001). The latency of each response was measured by counting the video-frames between the contact of the probe on the crayfish's body and the activation of the respective escape behavior displayed as the initial movement of the crayfish. A minimum of five tail-flips of each type was collected from each animal.

Identification of a tail-flip depended on the correlation between the high-speed video recording of the tail-flip and the simultaneously recorded field potential. Electrical recordings from the bath electrodes warrant identification of LG- or MG-mediated tail-flips by their large, phasic motor giant (MoG) neuron potentials and the immediately preceding LG or MG neuron action potentials (Herberholz et al., 2001). The identification of the giant mediated tail-flips, however, was somewhat complicated in our study because the nymphs produced muscle potentials during attacks that were big enough to be recorded by the bath electrodes. These potentials started shortly before an attack and passed into the measured potentials from the crayfish tail-flip activity. Consequently, the giant neuron spikes were rarely identifiable in our recordings. The MoG potentials, however, were unaffected by the nymph's signal and were always of large amplitude. Thus, we felt confident in identifying the giant mediated tail-flips from this characteristic feature. MG-evoked tail-flips have a larger amplitude and a shorter duration of the MoG potential and can be used to discriminate between the two giant mediated tail-flips (cf. Herberholz et al., 2001). In addition, single frame analysis of the high-speed videography allowed us to distinguish between the two giant mediated tail-flips (upward and backward movements are indicative of LG and MG tail-flips, respectively). Non-G mediated tail-flips cannot be discriminated behaviorally from giant mediated tail-flips, but their electrical recordings lack the large MoG potentials and consist of much smaller and more erratic fast flexor (FF) muscle potentials only (cf. Herberholz et al., 2001).

Results

General behavior of prey and predator

After the animals were placed in the test chamber, the crayfish usually moved to explore the arena while the dragonfly nymphs remained stationary. When a crayfish entered the visual field of a dragonfly nymph, however, the nymph turned towards the crayfish in preparation for an attack (Fig. 1A). By contrast, the crayfish displayed no change in behavior when the predator was present. The crayfish continued its exploration and often moved towards the predator. The nymphs sometimes stalked the crayfish to reduce the distance between them and attacked by rapidly expanding the labium in a fast strike at the crayfish (Fig. 1B). The mean distance between the dragonfly nymph and crayfish during the attacks was 7.9±3.0 mm (mean ± S.D.; range, 2–13 mm, N=38; measured between the nymph's head and the target). The distances between the predator and prey did not differ significantly for the three different types of tail-flips that were elicited by the attacks (P>0.1; Kruskal–Wallis one-way ANOVA on ranks). The mean length of the prementum (the distal part) of the nymphs' labium was 9.1±1.5 mm, and the crayfish were often hit when the labium was only partially extended. The nymph never missed the crayfish and each attack was answered by a tail-flip escape response. Only in one case did the crayfish respond to an attack (i.e. tail-flip away) prior to being hit by the nymph's labium, whereas physical contact elicited the escape in all other cases. Thus, there was no indication that the escape behavior

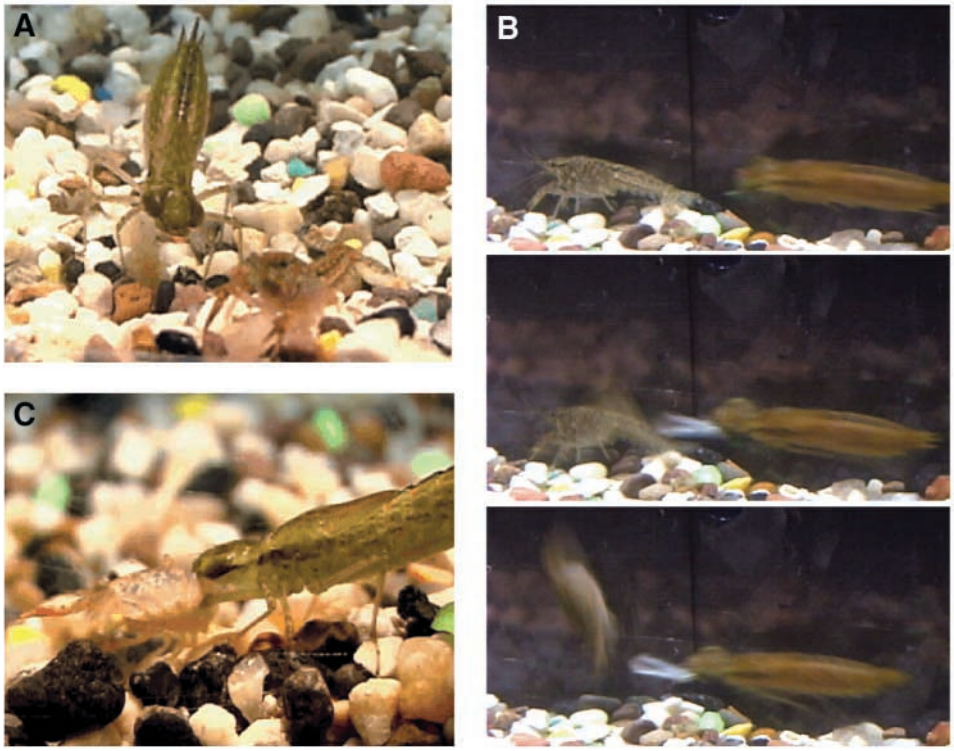


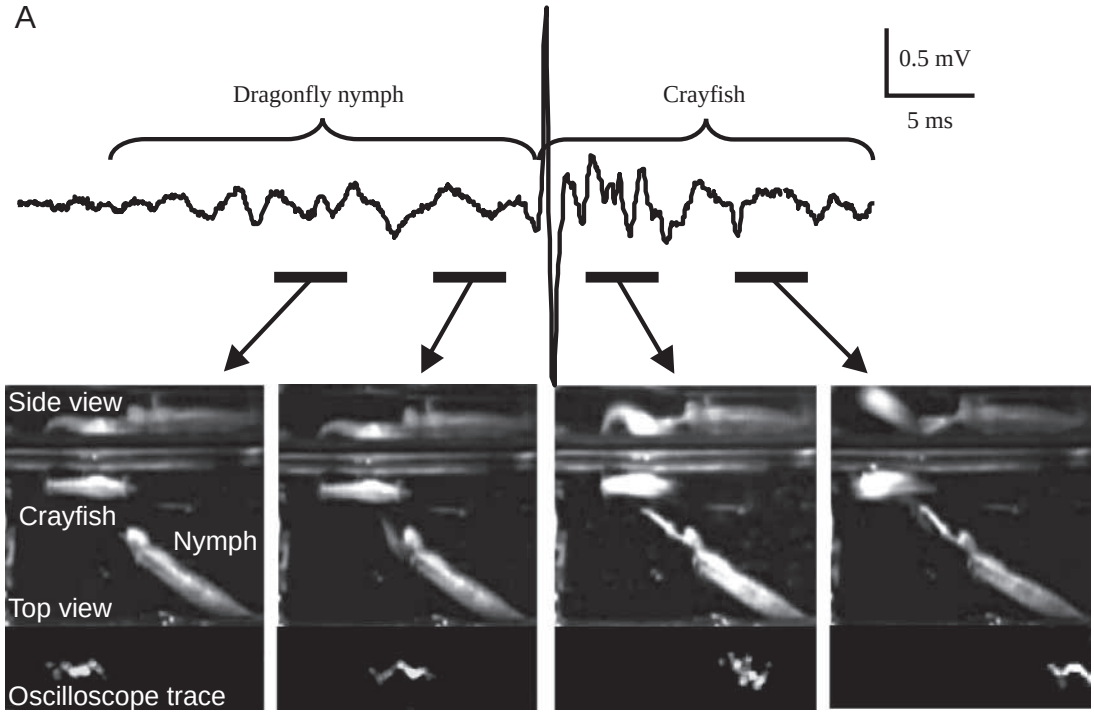
Fig. 1. Prey–predator interactions between juvenile crayfish and dragonfly nymphs recorded with a digital video camera. (A) Single video frame showing a dragonfly nymph (*Anax junius*; left) in preparation for attacking a juvenile crayfish (*Procambarus clarkii*; right). (B) Three video frames, from top to bottom, illustrating a predatory strike by the dragonfly nymph (right; note the extension of the white-colored labium in the middle frame) that evokes an escape tail-flip by the crayfish (left, bottom). (C) Single video frame showing a dragonfly nymph feeding on a captured crayfish.

was evoked by a possible hydrodynamic signal preceding the approaching labium. The nymphs only attacked moving crayfish whereas crayfish that remained motionless never evoked an attack. The nymphs would often continue to observe the quiescent crayfish for a while but would lose interest if the crayfish did not resume motion within a few minutes. The mean latency between the start of the experiments and the nymphs' attacks was 176±202 s (range, 17–911 s, *N*=38). After capture, the nymph would retract the labium with the attached prey and begin to lacerate the crayfish with its powerful mouthparts (Fig. 1C). Following capture, the restrained crayfish often used additional tail-flips in an attempt to escape.

Field potential recordings

All known and previously described types of escape tail-flips were evoked by attacks from the dragonfly nymphs. The muscle potentials generated during these escape responses were recorded with a pair of bath electrodes in the test chamber. Fig. 2A illustrates the combination of an electrical recording and the simultaneously recorded video sequence during an MG-mediated escape tail-flip produced by the crayfish in response to a frontal attack from the dragonfly nymph. The recording from the bath electrodes is shown on top. Examples of the corresponding video frames showing the behavior of the animals (top view and side view *via* mirror image) as well as the corresponding oscilloscope trace for each frame are shown on the bottom. For better illustration, the reflected oscilloscope traces were flipped horizontally to match with the electrical recordings sampled on the computer. The first two frames show parts of the nymph's attack with the opening of the labial palps and the extension of the labium, respectively. The third frame shows the moment when the escape behavior is initiated shortly after the labium hit the body of the crayfish. The last frame shows a later part of the escape behavior with the crayfish being propelled back and away from the predator.

The muscle potential produced by the predator was recorded by the bath electrodes and always preceded the signal



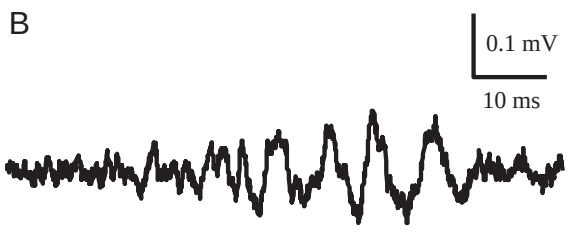


Fig. 2. Correlation between field potentials and simultaneously recorded behavior from prey and predator during an attack. (A) A medial giant neuron mediated escape tail-flip produced by the crayfish in response to a frontal attack from the dragonfly nymph. The electrical recording from the bath electrodes is at the top and shows the potential generated by the predator attack followed by that of the prey escape. Below that trace, four video frames show the behavior of the animals (top view and side view *via* mirror image). The bars and arrows between them indicate the timing of each frame relative to the bath potential. The bottom of each frame displays the oscilloscope trace of that portion of the bath potential. The first two frames illustrate the initial period of the strike with the opening of the labial palps and the extension of the labium, respectively. The last two frames illustrate the successful escape response of the crayfish. For further explanation, see text. (B) Field potential measurement of the signal generated by the dragonfly nymph while attacking a mock prey. The initial part of the recording consists of small deflections that become larger towards the end of the potential.

generated by the crayfish (Figs 2A, 3). This signal usually started with small deflections when the nymphs opened the palps on either side of the labium that became larger when the labium was extended during the strike (Fig. 2A). The mean duration of the predators' muscle potentials was 33.6±7.9 ms (11–49 ms, *N*=38; measured from the beginning of the signal to the start of the potential produced by the crayfish's escape circuits). The durations did not differ significantly for the three different types of tail-flips that were elicited ($P>0.05$; Kruskal–Wallis one-way ANOVA on ranks). The mean duration of the extensions (measured by counting the video frames between the onset of the movement of the labium and the moment when the target was hit) was 14.8±3.7 ms (*N*=38) and was much shorter than the actually recorded muscle potentials. Since each video frame represents a period of 5 ms, the calculated response latencies from the measured number of frames are only a demonstration of the time window in which the behavior occurred. Moreover, the measured extensions did not include the initial part, i.e. the preparation of the strike when the nymphs opened the labial palps.

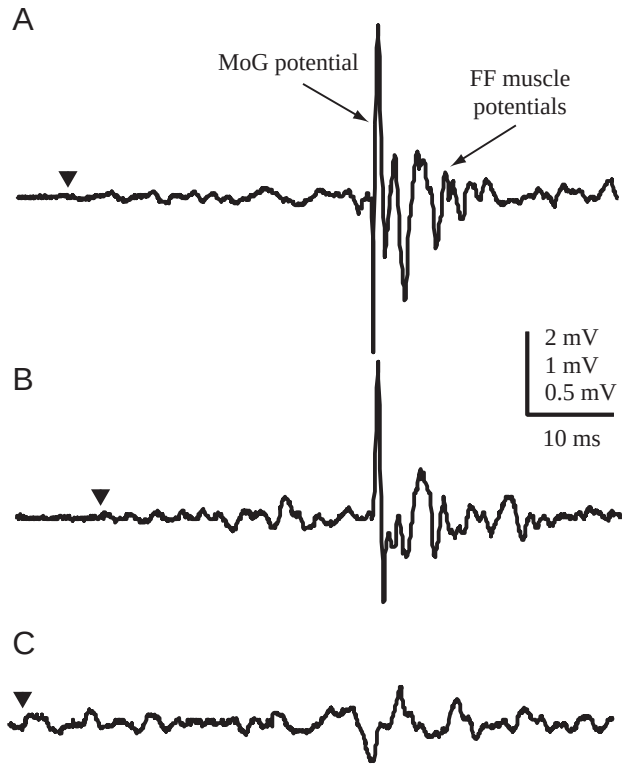


Fig. 3. Field potentials recorded with bath electrodes during predatory strikes. The onset of the predator's muscle potential is indicated with an arrowhead in all traces. (A) An electrical recording of potentials produced during a medial giant neuron mediated escape tail-flip. The large and phasic motor giant (MoG) neuron potential is followed by fast flexor (FF) muscle potentials. (B) Muscle potential recorded during a lateral giant mediated escape tail-flip. A smaller phasic MoG neuron potential is visible, followed by FF muscle potentials. (C) Muscle potential recorded during a non-giant mediated tail-flip. The signal consists of small FF muscle potentials only, and no large and phasic potential can be seen.

The signal of the predator was also measured in eight isolated nymphs that were stimulated to launch an attack against mock prey. We found that the signal duration in these experiments averaged 46.5±5.9 ms (*N*=17), more than 10 ms longer than during prey–predator interactions (Fig. 2B). Thus, in the electrical recordings taken while the nymphs attacked crayfish, the later part of the signal emitted by the dragonfly nymphs was masked by the larger signal generated by the crayfish.

Fig. 3 shows examples of three types of recorded muscle potentials that represent the three different types of tail-flips. The recordings from MG- and LG-mediated tail-flips both show the large and phasic MoG potentials as well as the following FF muscle activity (Fig. 3A,B). The Non-G-mediated tail-flip produced a very different crayfish response consisting of a much smaller and less phasic potential that can be attributed to FF muscle activity only (Fig. 3C). Extensor muscle potentials that usually follow the FF muscle potentials with some delay were small and rarely identifiable in our recordings. The arrowheads shown above the traces indicate the beginnings of the predators' muscle potentials.

Initial escape responses to an attack

Each single attack from the dragonfly nymph was answered with a tail-flip escape response by the crayfish (100%, *N*=38). The attacks were directed to most parts of the crayfish's body with the majority aimed at the head and thorax and fewer directed towards the abdomen (Fig. 4A). Rarely was the attack directly aimed at the appendages (i.e. claws, walking legs, antennae, antennules). In some cases, however, the nymph hit appendages first before the strike was passed on to the body. Most attacks evoked activity in the MG neuron (63%) and less evoked activity in the LG neuron (24%; Fig. 4B). Only five attacks (13%), all directed to the front of the animal, were answered by Non-G-mediated tail-flips that were identified by their characteristic muscle potential recordings (Fig. 4B).

Fig. 5 shows the distribution of hits to the crayfishs' bodies that excited the three different escape responses. The round circles indicate the position of the center of the labium on the crayfish's body. A schematic of the frontal part of the labium is shown to illustrate the size relation. Arrows around the crayfish demonstrate the direction of the attack (i.e. the position of the dragonfly nymph). A strong correlation between the evoked tail-flip escape response and the target of the strike can be seen: MG- and Non-G-mediated tail-flip responses were only elicited by attacks to the front part of the crayfish (head and thorax) while attacks to the abdomen evoked LG-mediated escapes only. The position of the nymph, however, varied with regard to the evoked responses.

The response latency of the different escape tail-flips was measured by counting the video frames between the contact of the labium on the crayfish's body and the activation of the respective escape behavior displayed as the initial movement of the crayfish. These latencies (Fig. 6A) were significantly different for the three different types of tail-flips ($P\leq0.01$; Kruskal–Wallis one-way ANOVA on ranks). The responses

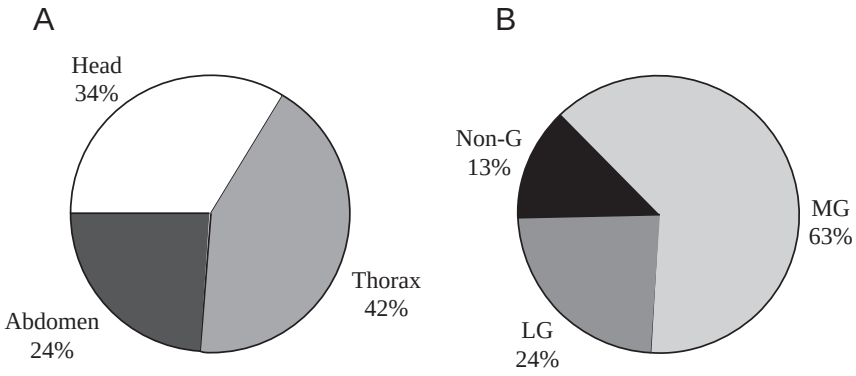


Fig. 4. Targets and percentage of escape responses. (A) More attacks are directed towards the anterior parts of the crayfish (head and thorax) than towards the posterior parts (abdomen). (B) Most escape responses are generated by activity in the medial giant neuron (MG) while lateral giant mediated escapes (LG) are less frequent and non-giant mediated escape tail-flips (Non-G) are rare.

triggered by activity in the Non-G circuit were significantly slower than responses generated by activity in the MG or LG neurons ($P \leq 0.01$ and $P \leq 0.05$, respectively; Mann–Whitney rank sum test). When compared with escape latencies measured in isolated crayfish (N=6, n=144) of the same size that were stimulated with a handheld probe, the latencies for MG-mediated tail-flips (predator, 10.0±1.5 ms; probe, 9.7±1.1 ms) and LG-mediated tail-flips (predator, 11.7±2.5 ms; probe, 12.6±1.3 ms) were found to be almost identical under both conditions. However, latencies for Non-G tail-flips were significantly shorter ($P \leq 0.01$; Mann–Whitney rank sum test) during predator-evoked escapes (predator, 16.0±2.2 ms; probe, 57.2±5.7 ms; Fig. 6A).

Overall, the initial tail-flip response proved to be a successful escape mechanism for juvenile crayfish to prevent predation from the dragonfly nymphs. In 45% of all cases (17 of 38), the initial tail-flip response catapulted the crayfish out of reach before the predator was able to restrain the prey. In all other cases (55%), the nymph caught the crayfish with the sharp labial palps, pulled it towards the body and started using its mouthparts to consume it. Since the neural escape circuits are always excited in these cases and the escape behavior is initiated before the crayfish is restrained, we were able to identify the type of initial tail-flip response in every case by combining the measurements from the bath electrodes with our video recordings.

The two giant mediated types of tail-flip escapes were found to be equally successful in preventing capture (Fig. 6B). The LG-mediated tail-flips provided an escape rate of 44% (four of nine), while MG-mediated tail-flips had an initial escape rate of 50% (12 of 24). Only one of five (20%) of the Non-G-mediated tail-flips, however, was successful in preventing capture (Fig. 6B).

Additional escape behavior after capture

Once caught, the crayfish often produced a series of Non-G-mediated tail-flips to escape from the nymph's grip (mean ± S.D., 4.5±3.8). Only in two cases did the crayfish not generate additional tail-flips after capture and in both cases the animals were killed and consumed.

Fig. 7A shows a trace recorded by the bath electrodes that displays the muscle potentials produced by predator and prey during and after the attack. A giant mediated tail-flip was generated first as an immediate response to the attack but was followed by three non-giant mediated tail-flips after the animal was captured. Although the subsequent tail-flips did not free the crayfish from the dragonfly nymph in this case, additional tail flipping after capture was generally very effective and produced many additional escapes with or without inflicted injuries (14 of 19; 74%). None of the crayfish that escaped injured (six of 21; 29%; typically, loss of a claw or a walking leg) died as a result of the injury.

The success of a captured animal's escape attempts depended on where the animal was attacked and held. Animals

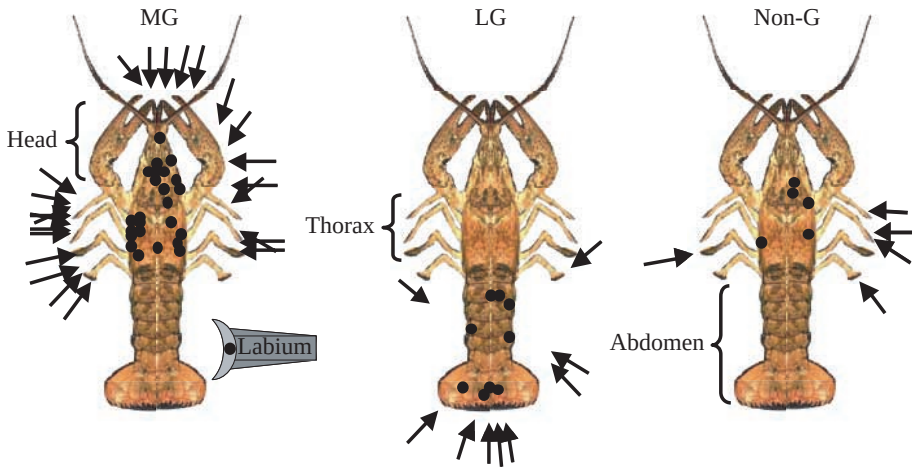


Fig. 5. Distribution of all strikes and relative positions of dragonfly nymphs during attacks that evoked the three different types of tail-flips. Black circles indicate the position of the center of the labium on the crayfish's body for each strike and type of escape response. A schematic of the frontal part of the labium is shown to illustrate the size relationship (bottom left). Arrows around the crayfish demonstrate the relative position of the dragonfly nymph when the attacks were delivered.

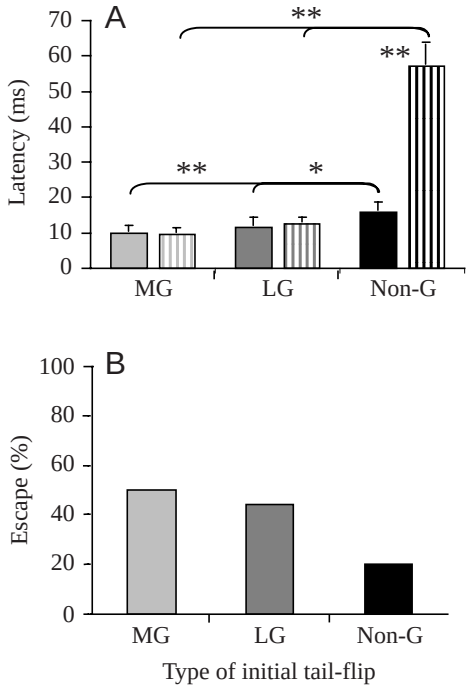


Fig. 6. Response latencies and success rates for escape for the three different types of tail-flips. (A) Latencies of crayfish escape after being hit by the dragonfly nymph (solid bars) or after being stimulated with a handheld probe (striped bars). During predator attacks, non-giant mediated tail-flips (Non-G) are executed with significantly longer latencies than medial giant (MG)- or lateral giant (LG)-mediated tail-flips. After stimulation with a probe, Non-G tail-flips also have significantly longer latencies than MG- or LG-mediated tail-flips. Non-G tail-flips evoked by the dragonfly nymph have significantly shorter latencies than probe-evoked ones. **P*≤0.05, ***P*≤0.01. (B) MG- and LG-mediated tail-flips have a higher escape rate than Non-G-mediated tail-flips.

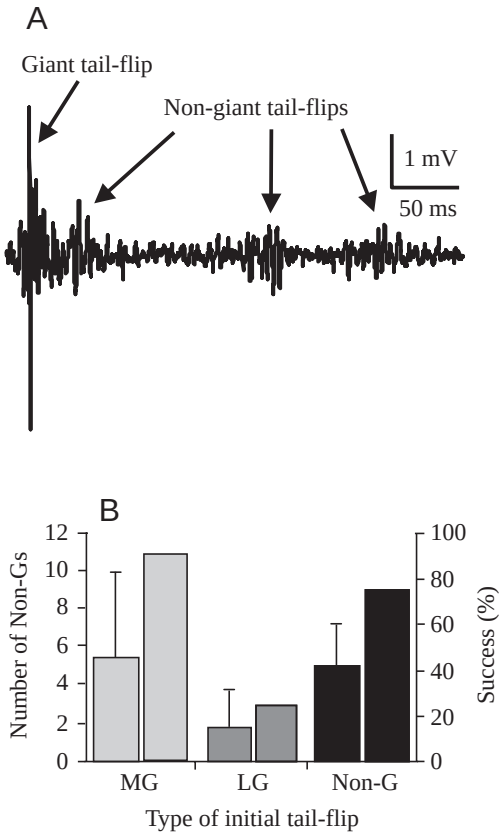


Fig. 7. Non-giant (Non-G)-mediated escape tail-flips after capture. (A) An initial giant mediated escape tail-flip was followed by three Non-G-mediated tail-flips after the dragonfly nymph captured the crayfish. Note the difference in amplitude between the giant and the Non-G escape responses. (B) After unsuccessful initial medial giant (MG)- and Non-G-mediated escapes, crayfish frequently used Non-G-mediated tail-flips that resulted in high percentages of additional escapes after capture. After unsuccessful lateral giant (LG)-mediated escapes, crayfish used few Non-G-mediated tail-flips that resulted in few additional escapes.

captured by frontal attacks were significantly more likely to escape than animals captured by abdominal attacks (81% and 20%, respectively; $P<0.05$; Fisher's exact test). That increased frequency of escape was associated with a greater number of Non-G tail-flips (Fig. 7B). Successful frontal attacks triggered unsuccessful MG or unsuccessful Non-G tail-flips that were followed by a high number of subsequent Non-G tail-flips (mean ± S.D., 5.4±4.5 and 5.0±2.2, respectively), and these additional tail-flips led to escape in 91% (10 of 11) and 75% (three of four), respectively, of all cases in which they were executed (Fig. 7B). Successful abdominal attacks that triggered unsuccessful LG tail-flips were followed by only 1.8±1.9 Non-G tail-flips, and these achieved an escape rate of only 25% (one of four; Fig. 7B). In these cases, the dragonfly nymphs caught the crayfish by closing the sharp labial palps around the tail or abdomen, which prevented the crayfish from executing non-giant mediated tail-flips or generating full force during non-giant mediated tail-flips. Consequently, only one crayfish that initially produced a LG-mediated tail-flip in response to the attack escaped after being caught. The differences in numbers of additional non-giant mediated escapes after MG-, LG- and Non-G-initiated tail-flips did not reach statistical significance ($P>0.1$; Kruskal–Wallis one-way ANOVA on ranks). They do, however, explain the high mortality rate of captured crayfish that initially tried to escape by LG-mediated tail-flips (see below).

Unsuccessful escapes and mortality rates

Only 18% (seven of 38) of all attacks from the dragonfly nymphs were fatal with the crayfish being killed and consumed. Crayfish that initiated an escape with MG-mediated tail-flips and were captured (50%) still suffered a low overall mortality rate of 8% (two of 24) because of the high number of successful Non-G-mediated tail-flips they generated after capture. Likewise, the unsuccessful Non-G-mediated tail-flips (80%) produced by the crayfish to avoid capture were compensated for by the high number of successful Non-G-mediated tail-flips that followed capture and resulted in a 20% (one of five) mortality rate. Crayfish that attempted LG-

mediated escapes and were captured (56%), however, experienced a high mortality rate of 44% (four of nine), mostly because subsequent Non-G-mediated tail flipping was disabled.

Discussion

We found that tail flipping is the exclusive defense mechanism used by juvenile crayfish in response to attacks from dragonfly nymphs. All three known and previously described neural circuits that control tail flipping are used with some success. We were able to identify the different tail-flips by combining the electrical recordings of the bath field potentials generated by active neural circuits and muscles with the behavior displayed by the crayfish. This technique was first applied to identify LG-mediated tail-flips in adult crayfish when they were at rest, walking backward or displaying a defense response (Beall et al., 1990). More recently, it was used to identify which tail-flip circuits mediated escape responses by juvenile crayfish during intraspecific agonistic interactions (Herberholz et al., 2001), and proved equally viable for this study. Giant and non-giant mediated tail-flips were easily distinguished by means of the recorded field potentials, and single frame analysis of the high-speed video recordings allowed us to discriminate between the two types of giant mediated escape responses (Figs 2, 3).

The bath electrodes were sensitive enough to record field potentials generated by the predator before and during its attack. The muscle potentials produced by the dragonfly nymphs in our experiments showed an increase in amplitude over time, reflecting previously reported recordings made with implanted electrodes (Tanaka and Hisada, 1980). The potentials recorded with bath electrodes were much shorter, however, than described from electromyograms, and the largest deflections were observed during the extension of the labium, i.e. after the onset of the strike. Only the extensor and the adductor muscles of the labium are active at this time (Tanaka and Hisada, 1980), and the later part of the recorded field potentials may therefore be attributed to activity in these muscles. On the other hand, it seems unlikely that these small muscles in the labium solely account for the large potentials recorded with the bath electrodes during labial extension. Possibly, the dorso-ventral muscles that create the increase in hydrostatic pressure are in part responsible for these large potentials and are active longer than previously reported (Olesen, 1972; Tanaka and Hisada, 1980).

Most dragonfly attacks were directed at the head and thorax of the crayfish (Figs 4A, 5), perhaps because the crayfish's exploratory tour of the aquarium led it to approach the stationary predator. Each strike was then answered by an attempted tail-flip escape. As a consequence of the strong and phasic nature of the predatory strike, most of the evoked tail-flips were mediated by activity in the giant neurons (Figs 4B, 5). The type of giant mediated escape response depended on the target of the attack but not on the position of the attacker. LG-mediated escapes were only evoked by strikes to the abdomen, whereas MG-mediated escape tail-flips were only evoked by strikes to the head and thorax (Fig. 5). This is consistent with earlier descriptions of the receptive fields of LG and MG as being non-overlapping and restricted to the abdomen and cephalothorax, respectively (Wine and Krasne, 1972). A few attacks to the anterior part of the crayfish's body elicited Non-G-mediated tail-flips (Figs 4B, 5). We found no apparent differences in target, position of the predator or physical parameters of the strike that would distinguish these attacks from the ones that evoked MG-mediated escapes. Nonetheless, the Non-G-mediated tail-flips had significantly longer response latencies than the giant mediated escapes (Fig. 6A). However, the Non-G latencies were shorter than those measured in earlier studies that evoked tail-flips by tactile stimulation with a tapping rod (Wine and Krasne, 1972; Reichert and Wine, 1983; Kramer and Krasne, 1984). They were also shorter than Non-G latencies measured in this study after stimulation with a glass rod under the same conditions in animals of similar size, whereas latencies for MG- and LG-mediated tail-flips did not differ whether evoked by nymph attacks or by a tapping rod (Fig. 6A). The longer latencies for Non-G tail-flips have been attributed to the 'voluntary' nature of the escapes in which the animals make decisions about the direction and angle of the response before the tail-flip is executed (Wine and Krasne, 1972; Reichert and Wine, 1983). The processing time required to set up the Non-G system was therefore considered to account for the long response latencies after stimulation (Reichert and Wine, 1983). The surprisingly short response latencies for Non-G-mediated escapes reported here during predator attacks reveal an interesting possibility; the Non-G system may be primed by the perception of the approaching predator (e.g. the labial extension), thereby reducing the latency of the Non-G response that follows the actual physical contact. However, because of the low number of observed Non-G tail-flips in response to predator attacks and the relatively low temporal resolution of our high-speed system, additional experiments are required to test whether crayfish actually use sensory information provided by the predator to prepare the Non-G escape response prior to the tactile stimulation.

The crayfish used in our study were successful in employing tail-flip escapes to prevent capture and so experienced low mortality rates. Crayfish that are smaller or larger in size relative to the predator, however, may experience different mortality rates during interactions with dragonfly nymphs. Non-G tail-flips were less successful in preventing capture than giant mediated tail-flips (Fig. 6B), probably because of the longer response latencies measured for Non-G-mediated escapes as compared with giant mediated escapes (Fig. 6A), and because Non-G tail-flips generate less thrust than giant mediated tail-flips. Although not efficient for initial escape, activation of the Non-G system was common after capture (Fig. 7A) and generated many additional escapes (Fig. 7B). These additional tail-flips were predominantly used after unsuccessful MG- and Non-G-mediated escapes (Fig. 7B) but rarely produced after initial LG tail flipping. Attacks that

elicited LG-mediated escapes were always directed to the rear of the crayfish, and closure of the labial palps around the tail or abdomen prevented the crayfish from executing Non-G tail-flips or generating full force during attempted Non-G tail-flips.

Tail flipping without giant fiber activity is considered the early condition of escape in crustaceans (Heitler et al., 2000). We found that Non-G-mediated tail-flips in juvenile crayfish are ineffective for escaping the fast and precise strikes generated by predators such as dragonfly nymphs. The co-existence with predators delivering phasic stimuli during attacks may have led to the evolution of the giant fibers that enable powerful, short-latency escape responses. Activation of either the MG or LG neuron generates rapid and powerful tail-flip responses that allow crayfish to escape from sudden attacks. However, the enduring importance of the ancestral form of escape is still apparent: although not very successful during initial escapes, the Non-G-mediated tail-flips are of great importance for the overall survival, accounting for many additional escapes after capture.

We would like to thank Laurel Johnstone, Issac Greenbride and Aaron Miller for their help. This study was supported by research grants (NIH) RO1-NS26457 and (NSF) IBN 0135162 to D.H.E.

References

Antonsen, B. L. and Edwards, D. H. (2003). Differential dye coupling reveals lateral giant escape circuit in crayfish. *J. Comp. Neurol.* **466**, 1-13.

Barnett, H. K. and Richardson, J. S. (2002). Predation risk and competition effects on the life-history characteristics of larval Oregon spotted frog and larval red-legged frog. *Oecologia* **132**, 436-444.

Beall, S. P., Langley, D. J. and Edwards, D. H. (1990). Inhibition of escape tailflip in crayfish during backward walking and the defense posture. *J. Exp. Biol.* **152**, 577-582.

Blake, M. A. and Hart, P. J. B. (1995). The vulnerability of juvenile signal crayfish to perch and eel predation. *Freshwater Biol.* **33**, 233-244.

Correia, A. M. (2001). Seasonal and interspecific evaluation of predation by mammals and birds on the introduced red swamp crayfish *Procambarus clarkii* (Crustacea, Cambaridae) in a freshwater marsh (Portugal). *J. Zool.* **255**, 533-541.

Crumrine, P. W. and Crowley, P. H. (2003). Partitioning components of risk reduction in a dragonfly–fish intraguild predation system. *Ecology* **84**, 1588-1597.

DiDonato, G. T. and Lodge, D. M. (1993). Species replacements among *Orconectes* species in Wisconsin Lakes: the role of predation by fish. *Can. J. Fish. Aquat. Sci.* **50**, 1484-1488.

Dye, L. and Jones, P. (1975). The influence of density and invertebrate predation on the survival of young-of-the-year *Orconectes virilis*. *Freshwater Crayfish* **2**, 529-538.

Edwards, D. H., Heitler, W. J. and Krasne, F. B. (1999). Fifty years of a command neuron: the neurobiology of escape behavior in the crayfish. *Trends Neurosci.* **22**, 153-161.

Furshpan, E. J. and Potter, D. D. (1959). Transmission at the giant motor synapses of the crayfish. *J. Physiol.* **145**, 289-325.

Garvey, J. E., Stein R. A. and Thomas, H. M. (1994). Assessing how fish predation and interspecific prey competition influence a crayfish assemblage. *J. Ecol.* **75**, 532-547.

Glanzman, D. L. and Krasne, F. B. (1983). Serotonin and octopamine have opposite modulatory effects on the crayfish's lateral giant escape reaction. *J. Neurosci.* **3**, 2263-2269.

Heitler, W. J., Fraser, K. and Edwards, D. H. (1991). Different types of rectification at electrical synapses made by a single crayfish neurone investigated experimentally and by computer simulation. *J. Comp. Physiol. A* **169**, 707-718.

Heitler, W. J., Fraser, K. and Ferrero, E. A. (2000). Escape behaviour in the stomatopod crustacean *Squilla Mantis*, and the evolution of the caridoid escape reaction. *J. Exp. Biol.* **203**, 183-192.

Herberholz, J., Antonsen, B. L. and Edwards, D. H. (2002). A lateral excitatory network in the escape circuit of crayfish. *J. Neurosci.* **22**, 9078-9085.

Herberholz, J., Issa, F. A. and Edwards, D. H. (2001). Patterns of neural circuit activation and behavior during dominance hierarchy formation in freely behaving crayfish. *J. Neurosci.* **21**, 2759-2767.

Hill, A. M. and Lodge, D. M. (1994). Diel changes in resource demand: competition and predation in species replacement among crayfishes. *Ecology* **75**, 2118-2126.

Johansson, A. and Johansson, F. (1992). Effects of 2 different caddisfly case structures on predation by a dragonfly larva. *Aquatic Insects* **14**, 73-84.

Kanou, M. and Shimozawa, T. (1983). The elicitation of predatory labial strike of dragon fly larvae in response to a purely mechanical stimulus. *J. Exp. Biol.* **107**, 391-404.

Kramer, A. P. and Krasne, F. B. (1984). Crayfish escape behavior: production of tailflips without giant fibre activity. *J. Neurophysiol.* **52**, 189-211.

Krasne, F. B., Shamsian, A. and Kulkarni, R. (1997). Altered excitability of the crayfish lateral giant escape reflex during agonistic encounters. *J. Neurosci.* **17**, 709-716.

McCollum, S. A. and Leimberger, J. C. (1997). Predator-induced morphological changes in an amphibian: predation by dragonflies affects tadpole shape and color. *Oecologia* **109**, 615-621.

Merrill, R. J. and Johnson, D. M. (1984). Dietary niche overlap and mutual predation among coexisting larval Anisoptera. *Odonatologica* **3**, 387-406.

Mittenthal, J. E. and Wine, J. J. (1973). Connectivity patterns of crayfish giant interneurons: visualization of synaptic regions with cobalt dye. *Science* **179**, 182-184.

Olesen, J. (1972). The hydraulic mechanism of labial extension and jet propulsion in dragonfly nymphs. *J. Comp. Physiol.* **81**, 53-55.

Olson, G. C. and Krasne, F. B. (1981). The crayfish lateral giants as command neurones for escape behaviour. *Brain Res.* **214**, 89-100.

Pritchard, G. (1965). Prey detection by dragonfly larvae (Odonata; Anisoptera). *Can. J. Zool.* **43**, 271-289.

Reichert, H. and Wine, J. J. (1982). Neural mechanisms for serial order in a stereotyped behaviour sequence. *Nature* **296**, 86-87.

Reichert, H. and Wine, J. J. (1983). Coordination of lateral giant and non-giant systems in crayfish escape behavior. *J. Comp. Physiol.* **153**, 3-15.

Söderbäck, B. (1994). Interactions among juveniles of two freshwater crayfish species and a predatory fish. *Oecologia* **100**, 229-235.

Stein, R. A. (1977). Selective predation, optimal foraging and the predator-prey interaction between fish and crayfish. *J. Ecol.* **58**, 1237-1253.

Stein, R. A. and Magnuson, J. J. (1976). Behavioral response of crayfish to a fish predator. *Ecology* **57**, 751-761.

Tanaka, Y. and Hisada, M. (1980). The hydraulic mechanism of the predatory strike in dragonfly larvae. *J. Exp. Biol.* **88**, 1-19.

Wiersma, C. A. G. (1947). Giant nerve fibre system of the crayfish. A contribution to comparative physiology of synapses. *J. Neurophysiol.* **10**, 23-38.

Wine, J. J. and Krasne, F. B. (1972). The organization of escape behavior in the crayfish. *J. Exp. Biol.* **56**, 1-18.

Wine, J. J. and Krasne, F. B. (1982). The cellular organization of crayfish escape behavior. In *The Biology of Crustacea*, vol. 4 (ed. D. C. Sandeman and H. L. Atwood), pp. 241-292. New York: Academic Press.

Wissinger, S. A. (1989). Seasonal variation in the intensity of competition and predation among dragonfly larvae. *Ecology* **70**, 1017-1027.

Wissinger, S. and McGrady, J. (1993). Intraguild predation and competition between larval dragonflies: direct and indirect effects on shared prey. *Ecology* **74**, 207-218.

Yeh, S. R., Fricke, R. A. and Edwards, D. H. (1996). The effect of social experience on serotonergic modulation of the escape circuit of crayfish. *Science* **271**, 366-369.

Zucker, R. S., Kennedy, D. and Selverston, A. I. (1971). Neural circuits mediating escape responses in crayfish. *Science* **173**, 645-650.