Marine and Freshwater Behaviour and Physiology
December 2006; 39(4): 245–258

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The impacts of flow on chemical communication strategies and fight dynamics of crayfish

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(Received 29 May 2006; in final form 18 August 2006)

Abstract
Signal transmission is influenced by the physics of an environment. Consequently, a physical effect on sensory signals can influence how animals send or sample sensory information. Habitat-specific physics may constrain or enhance signal transmission (e.g. sound transmission in a flowing river versus a still pond) and provide a mechanism for the evolution of sensory biases. This study investigated how the transmission of chemically mediated social signals in crayfish is influenced by two different aquatic environments. Agonistic bouts between crayfish were performed under lotic (flowing water) and lentic (nonflowing, still water) conditions. When crayfish (*Orconectes rusticus*) collected from a lotic system (river) interacted under lotic conditions, we noted that dominant *O. rusticus* spent more time upstream than subordinate *O. rusticus*. *Orconectes rusticus* positioned themselves randomly and spent equal amounts of time with respect to upstream and downstream in the nonflowing environment. We tested another species, *Orconectes virilis*, collected from a nonflowing environment (lake) and they showed no positional preference when tested in flow. Additionally, both *O. rusticus* and *O. virilis* took longer to reach high fight intensities under flow conditions. It was possible to visualize *O. rusticus* urine release, and they released urine more often when upstream of an opponent in a flow environment during these agonistic bouts. These results suggest that *O. rusticus* collected from lotic environments release urine to maximize the transmission of chemical cues to a fight opponent. It appears that crayfish may adapt their signalling processes based upon their long-term ambient environments.

Keywords: *Crustacean, crayfish,* Orconectes*, behaviour, aggression, water flow, flowing water, still water, environmental*

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ISSN 1023–6244 print/ISSN 1029–0362 online/06/040245–258
DOI: 10.1080/10236240600980608

Introduction

Environments structure how animals send and receive signals by constraining signal transmission and reception (Dusenbery 1992). Consequently, long-standing existence within one type of environment can drive evolutionary sensory biases (Endler and Basolo 1998). An animal's sensory solution to complex environmental physics can be reflected by a unique nervous system and consequential behaviour with its adaptive limitations, biases, and distortions (Wehner 1987). As an example of environments structuring communication, bats find prey when navigating through a forest by using echolocation strategies that are dependent upon the density of the vegetative mosaic within a forest (Pavey et al. 2001). Another example of complex environments affecting acoustic signals is seen in decapod crustaceans that are able to detect substrate vibrations at sensitivities sufficient to inform of the proximity of mates, competitors or predators in variety of environments (for a review, see Popper et al. 2001). These sensory and behavioural solutions are convergent strategies that resolve acoustic complexity. Other senses can be influenced by environmental conditions; certain fish species are able to detect electrical signals within an environment by suppressing noise at a number of levels that include behaviour, receptor anatomy, and physiology (Teeter et al. 1980; Montgomery and Bodznick 1999). In terms of adaptation to light, a number of terrestrial and aquatic animals use polarized light and each organism has a unique solution to the demands on the neural circuitries mediating this underlying behaviour (Dyer 1996; Wehner 2001; Dacke et al. 2002). The common theme for each of these examples is that animals communicate in complex environments by using sensory systems that are ''tuned'' to the relevant or dominant stimuli within an environment.

Communication occurs when information is exchanged between a sender and a receiver, resulting in an alteration of behavioural patterns for one or both of the participants (Enquist 1985; Dusenbery 1992; Bradbury and Vehrencamp 1998). In the chemical senses, organisms control the temporal and/or spatial components of a chemical signal in order to facilitate the transfer of information for recognition (Moore and Atema 1988). These temporal and spatial characteristics are important aspects of the signal with which an animal's sensory system can be ''tuned'' (Vickers et al. 1998). For example, lobsters and insects release pheromones during mating seasons that attract mates for copulation (Atema and Engstrom 1971; Jones and Hamilton 1998). Furthermore, the spatial and temporal release of signals is important for behavioural decisions that can influence whether decapod crustaceans enter territories, mate with certain individuals, or engage individuals in acts of aggression (Caldwell 1992; Bergman et al. 2005). Therefore, chemically mediated social signals can be important to the survival and reproductive success of signal senders and receivers.

It has been theorized that aggressive display signals provide important information on the social status of potential combatants (Bell and Gorton 1978). This information is utilized by organisms to reduce the number and intensity of agonistic encounters (Francis 1988; Caldwell 1992; Adamo and Hanlon 1996). It has become evident that agonistic ability or social status is chemically communicated by crayfish (Zulandt Schneider and Moore 2000; Zulandt Schneider et al. 2001; Bergman et al. 2003; Bergman and Moore 2005). They also have the ability to modify their chemical signals by controlling urine release and immediate fluid flow (Breithaupt and Eger 2002; Bergman et al. 2005). If we apply Wehner's matched filter hypothesis (1987), we would expect that crayfish have communication strategies that are shaped by the flow environment of their native habitats. In other words, crayfish from lotic (flow) habitats may be able to use background flow to facilitate chemical communication by placing themselves upstream from the receiver. Conversely, crayfish

from lentic (nonflowing) habitats should not adopt this strategy because lentic habitats do not have directional flow; these crayfish may facilitate communication by self-generated currents (Bergman et al. 2005).

Crayfish are commonly found in both lotic and lentic environments (Englund and Krupa 2000; Bergman and Moore 2003), and the use of chemical signals in these environments is influenced by the physics of the local water flow. Crayfish urine is projected using self-generated currents to facilitate communication within lentic (still water) environments (Breithaupt and Eger 2002; Bergman et al. 2005); however, this projection may be limited within lotic (flowing water) systems. The results of this study will clarify whether different chemical communication and behavioural strategies are used when an organism's sensory system is exposed to different environmental conditions.

Materials and methods

Lotic crayfish used in experiments 1 and 3

Crayfish, *Orconectes rusticus*, were collected from a lotic environment, the Portage River near Bowling Green State University (BGSU), Ohio. Intermolt male *O. rusticus* were physically and socially isolated in a flow-through holding tank in the laboratory. *Orconectes rusticus* were housed at a regulated temperature (23°C) and light : dark cycle (14 : 10) for a minimum of 1 week prior to the experiments. They were fed one commercial rabbit pellet three times per week and were used once during the course of either experiment 1 or 3. *Orconectes rusticus* were size-matched within 95% for carapace and chelae size, and no less that 90% for weight of participants for all trials. In each trial, one crayfish was marked with white correction fluid on the dorsal side of the carapace to later distinguish between the two fight participants. Upon completion of the study, all markings were removed and *O. rusticus* were returned to the Portage River.

BGSU flume setup for experiments 1 and 3

Agonistic fight trials were conducted in a 3650 L recirculating flume (complete dimensions: $568 \times 57\,cm^2$; working section of flume: $244 \times 57 \times 61\,cm^3$). The working section of the flume was further reduced, using an egg-crating cage ($60 \times 60 \times 50\,cm^3$), to ensure that crayfish would interact. The cage was fitted with a 3.0 cm plastic lip that prevented crayfish from climbing out. The flume was filled with aged-tap/dechlorinated water (average water temperature, 19.4 ± 0.8°C) to a depth of 20 ± 0.8 cm. The frame of the flume (working section and reservoir tanks) was constructed of stainless steel with glass as the sides and bottom of the working section. The bottom of the flume was lined with a plastic sheet that had a sand substrate fixed in place using a 5 min fixing epoxy. A sheet of polycarbonate core honeycomb (2.54 cm diameter holes) and a sheet of fluorescent light grating (169 mm diameter holes) wrapped with fibreglass screen (1 mm diameter holes) were positioned upstream as collimators. Flow was regulated with a centrifugal pump (WEG, Model # 005180P3E184JM) powered by an adjustable speed drive (Baldor, Model # ID15H205-E). Flow velocity was measured at three points along the centre and each wall of the flume (nine points) with a Marsh–McBirney Model 2000 flow meter. The background accumulation of odours was prevented by using a diatomaceous earth hot-tub filtre system between trials. The filtre utilized a diatomaceous earth filter that removed organic particles as small as 3–4 μm. The flume was completely drained and refilled approximately every 3 weeks.

Experiment 1: Lotic/lentic agonistic bouts with lotic crayfish

Two size-matched crayfish from a lotic habitat (*O. rusticus*) were selected per fight trial and allowed to fight under one of two conditions. Fights were performed under either lentic ($0\,cm\,s^{-1}$) or lotic ($10 \pm 0.3\,cm\,s^{-1}$) conditions within the recirculating flume. *Orconectes rusticus* that fought under lentic conditions had a mean (±SEM) carapace size of 34.8 ± 0.8 mm, chelae size of 34.4 ± 1.1 mm, and a weight of 16.1 ± 0.3 g. *Orconectes rusticus* that fought under lotic conditions had a mean (±SEM) carapace size of 36.0 ± 0.7 mm, chelae size of 35.8 ± 1.0 mm, and a weight of 17.4 ± 0.3 g. They were isolated within 3 cm diameter PVC pipe shelters that were cut in half and had a plastic grating fixed to the front to confine the crayfish within the shelter for a 15 min acclimation period. Shelters were placed directly across from each other in the middle of the walls of the reduced working section, perpendicular to the flow. Fight trials were performed under diurnal conditions. After acclimation, *O. rusticus* were released from their shelters and allowed to interact for 20 min. Twenty trials were run for each flow regime.

Lentic crayfish used in experiment 2

Orconectes virilis crayfish were used in experiment 2 because we have located populations that are located in different hydrodynamic habitats when compared to *O. rusticus*. *Orconectes virilis* were collected from Maple Bay in Burt Lake, Michigan. Intermolt male *O. virilis* were housed in flow-through outdoor metal holding tanks. *Orconectes virilis* were isolated for at least one week and fed on detrital material that accumulated in the tanks. They were size-matched within 90% for carapace and chelae size. In each trial, one *O. virilis* was marked with white correction fluid on the dorsal carapace to later distinguish between fight participants. All marking were later removed and *O. virilis* were released back into Burt Lake after testing was completed.

UMBS artificial stream setup for experiment 2

An artificial stream ($16 \times 1 \times 0.2\,m^3$) was constructed with concrete cinder blocks and 4 mm plastic sheeting. Stream water was used directly from the Maple River and an underground well system into a 1 m mixing area ahead of the 11.7 m stream section, resulting in a background concentration of natural odours. In order to avoid excessive particulate matter build-up, the flow-through pipes were equipped with nylon stockings secured to the ends of the flow pipe to filtre out the course particulate organic matter and macroinvertebrate fauna. Free stream velocity was measured at the beginning of each trial by a Marsh–McBirney 2000 flow meter. Water depth was maintained at 20 ± 0.3 cm. Collimators consisting of three sheets of plastic egg crating (1.7 cm diameter holes) covered with fibreglass sheeting (1 mm diameter holes) were placed in the upper part of the stream to create a laminar incoming flow. The stream contained an 11.7 m flow conditioning section in front of the 2.7 m working section and 1.6 m outflow section. The working section was partitioned off from the rest of the stream with two sheets of plastic egg crating. The end of the stream was covered with a $1.5 \times 0.35\,m^2$ board that contained 2.54 cm diameter holes evenly spaced throughout to maintain a constant depth and flow rate. The water exited the artificial stream and reentered the Maple River approximately 200 m downstream of the intake for the stream lab. To facilitate visual identification of *O. virilis* and behaviours, the bottom of the flume was painted white.

Experiment 2: Lotic/lentic agonistic bouts with lentic crayfish

Two size-matched crayfish from a lentic habitat (*O. virilis*) were selected per fight trial and allowed to fight under one of two conditions. Fights were performed under either lentic ($0\,cm\,s^{-1}$) or lotic ($10 \pm 0.5\,cm\,s^{-1}$) conditions within the artificial stream. *Orconectes virilis* that fought under lentic conditions had a mean (±SEM) carapace size of 33.2 ± 1.0 mm and chelae size of 31.4 ± 0.8 mm. *Orconectes virilis* that fought under lotic conditions had a mean (±SEM) carapace size of 34.3 ± 0.9 mm and chelae size of 30.8 ± 0.8 mm. Fight trials were performed under diurnal conditions. After acclimation, *O. virilis* were released from their shelters and allowed to interact for 20 min. Twenty trials were run for each flow regime.

Experiment 3: Urine visualization

Fight trials in this experiment were conducted only using *O. rusticus* given the results that *O. rusticus* modified its behaviour in experiment 1. This is in contrast to *O. virilis*, which did not observably modify its behaviour in response to flow conditions in experiment 2. The lentic and lotic conditions in experiment 1 were repeated in a similar manner, with the exception that crayfish were injected with a fluorescein dye. A 3 ppt sodium fluorescein (Sigma F-6377) solution dissolved in dechlorinated water was injected at a dose of $0.01\,mL\,g^{-1}$ body mass into the pericardial region of the crayfish (Breithaupt and Eger 2002; Bergman et al. 2005). The carapace was dried and a small strip of labelling tape was placed over the future injection site (dorsal carapace). A 1 mL syringe with a 26.5 ga needle was used to inject the fluorescein dye. The syringe and needle were spun slowly to pierce through the tape and carapace. Once the dye was injected, the needle was removed and an additional piece of tape was placed over the needle hole. Superglue (Duro) was quickly applied to seal the injection site to prevent leaking of the dye from the needle puncture. Superglue on the carapace has been demonstrated not to adversely affect crayfish behaviour, particularly the fight initiator or eventual winner (Bergman et al. 2003). After two size-matched *O. rusticus* were injected, they were allowed a minimum of 1 h prior to a trial to reacclimate to an approximate pre-injection state. During this time, fluorescein pools in the urinary bladder and consequently allows for the observance of urine that is released during an agonistic bout (Breithaupt and Eger 2002; Bergman et al. 2005). This method has been demonstrated to be an effective technique for visualizing urine release in crustaceans (Breithaupt and Eger 2002; Bergman et al. 2005). This previous work has demonstrated that 100% of the crayfish take the fluorescein into their urine, although the behavioural decision to release urine during agonistic interactions is variable (Breithaupt and Eger 2002; Bergman et al. 2005). Given this previous work, we are confident that those trials that included crayfish that did not release urine were due to some other factor rather than the failure to sequester the fluorescein in the urine. For this reason, we include all injected crayfish in part of the analysis. After this recovery phase, the size-matched *O. rusticus* were separated visually and mechanically within closed PVC shelters within the flume for 15 min. Lentic conditions remained at $0\,cm\,s^{-1}$ as in previous experiments; however, lotic conditions were performed at a flow velocity of $5.5 \pm 0.2\,cm\,s^{-1}$. The reduced flow velocity was used to increase the probability of observing fluorescein released with the urine before it dissipated when carried downstream. The BGSU flume was fitted with four black lights (15 W, GE #F15T8) and fights were conducted under dark conditions to maximize the illumination of the fluorescein dye. We have previously demonstrated using these methods that *O. rusticus* releases urine when fighting both under dark and lighted conditions (Bergman et al. 2005); however, the finer aspects of chemical communication could conceivably be altered by the availability of visual information.

For the purpose of this study, small alterations in urine release seem largely negligible because we do not attempt to compare these fights to fights under lighted conditions. Shelters were removed after the acclimation period, and the crayfish were allowed to interact for 15 min. In the trials when *O. rusticus* did not release any observable urine, it was not possible to determine whether crayfish either withheld urine or if the injection procedure failed. Size-matched *O. rusticus* did not release any discernible urine in twelve out of a total of 32 trials.

Behavioural and statistical analyses

A digital video camera (Canon XL-1) was positioned 1 m above the respective flume bottoms to record trials. All fight trials were analyzed using a blind design where the observer that analyzed the trials was not the same experimenter that conducted the trials. The identities of initiating and winning animals were recorded for each encounter. The crayfish that first engaged an opponent in physical contact was regarded as the fight initiator. The winner (dominant) was determined as the crayfish that remained in place after its opponent (i.e. the loser or subordinate) retreated or tail-flipped away. Once status was determined, it could then be applied to categorize crayfish for subsequent analyses. The intensity of fights was analyzed using a modified ethogram (see Bergman and Moore 2003). All interactions were analyzed by examining the behaviour of both participants. Trials were additionally analyzed for the proportion of trial time that crayfish spent upstream and number of bouts per trial for each condition.

Throughout this article, we refer to upstream and downstream for both lentic and lotic conditions. The upstream half of the fight arena is always relative to the flow direction of the flume whether flow was present or not, and taken in relation to a fight opponent. A crayfish position could be classified in one of three categories – upstream, downstream, or perpendicular to the flow when engaged with an opponent. The proportion of trials spent in the upstream position was analyzed using a multiple comparisons for proportions contingency table ($q_{0.05,\infty,2} = 2.772$) that allows for testing analogous to the Tukey or Student–Newman–Keuls tests (Zar 1999). Significant results are represented by giving $q_{0.05,\infty,2} > 2.772$ from the multiple comparisons test, which is equivalent to $p < 0.05$. A one-way MANOVA with a Tukey *post hoc* comparison test was performed to examine for significant differences in fight intensities and average number of bouts per trial. The number of urine releases and the time spent releasing urine was analyzed in experiment 3. A factorial ANOVA with a Tukey *post hoc* analysis was used on the time spent releasing urine during agonistic interactions. Fluorescein released with the urine was additionally recorded in terms of status, number of urine release events per *O. rusticus* and position within the flume relative to their opponent during release. The proportion of urine releases by *O. rusticus* was analyzed using a multiple comparisons for proportions contingency table ($q_{0.05,\infty,6} = 4.030$). Significant results are represented by giving $q_{0.05,\infty,6} > 4.030$ from the multiple comparisons test, which is equivalent to $p < 0.05$.

Results

Experiment 1

Dominant status *O. rusticus* collected from lotic habitats showed a significant preference to be upstream in relation to subordinate crayfish during agonistic

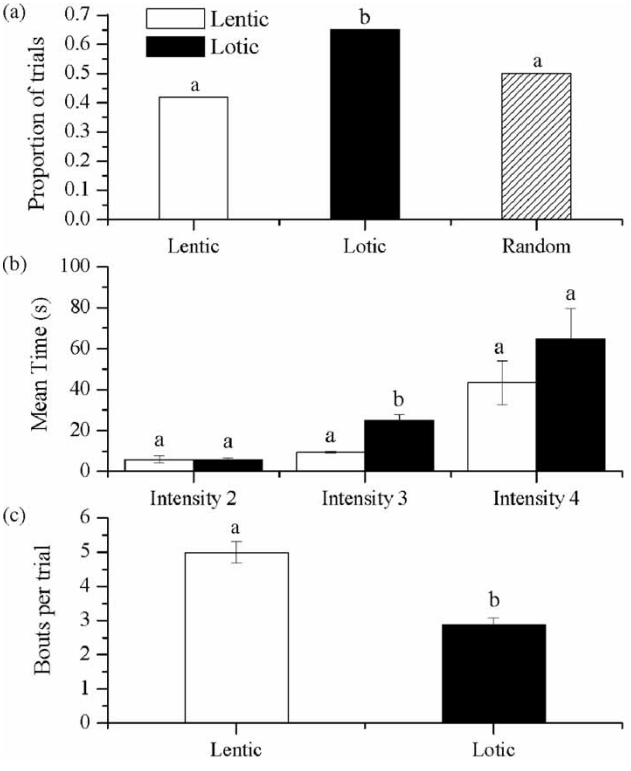

Figure 1. (a) Proportion of trials where dominant *O. rusticus* from lotic habitats spent more time upstream an opponent during lentic and lotic laboratory conditions. *Orconectes rusticus* spent a greater proportion of time upstream of an opponent in lotic conditions (black) compared to lentic (white) and random = 50% occurrence (crosshatched). Random bar was placed for comparison only; this bar contains no collected data. Different letters indicate a significant difference ($p < 0.05$). (b) The average time that fights took to reach each of the three intensity levels between *O. rusticus*. *Orconectes rusticus* fights progressed to intensity level 3 more rapidly in the lentic (white) condition compared to the lotic (black; $p < 0.05$). All other intensity levels were not significantly different. (c) Number of bouts per trial between *O. rusticus* under lentic and lotic conditions. *Orconectes rusticus* had significantly more bouts under lentic (white) compared to lotic (black) conditions ($p < 0.05$).

interactions in lotic conditions when compared to lentic conditions and random ($N = 20$; multiple comparisons for proportions; $p < 0.05$; Figure 1a). In the absence of flow, dominant *O. rusticus* did not show any preference for positions within the arena (Figure 1a). The time it took to reach intensity level 2, pushing with the chelae, was significantly longer under lotic (flow) conditions (25.2 ± 2.5 s) compared to lentic conditions for *O. rusticus* (9.4 ± 0.5 s; $p < 0.01$; Figure 1b). The time to reach intensity levels 2 (threat display with meral spread) and 4 (active chelae use by grasping) were not significantly different between lentic and lotic conditions for *O. rusticus* (intensity 2: 5.9 ± 1.6 s, 5.7 ± 1.1 s; intensity 4: 43.4 ± 10.6 s, 64.8 ± 14.7 s, respectively; $p > 0.05$; Figure 1b). Crayfish (*O. rusticus*) had significantly more agonistic bouts under lentic (no flow) conditions (5.0 ± 0.3) compared to lotic (flow) conditions (2.9 ± 0.2; $p < 0.01$; Figure 1c).

Experiment 2

Dominant *O. virilis* collected from lentic habitats showed no upstream preference in relation to subordinate crayfish during agonistic conditions in either lentic or lotic conditions, or compared to random ($N = 20$; multiple comparisons for proportions; $p > 0.05$; Figure 2a). The time to reach intensity level 4, grasping with the chelae, was significantly longer under lotic (flow) conditions (88 ± 16.9 s) compared to lentic conditions for *O. virilis* (40.3 ± 12.9 s; $p < 0.01$; Figure 2b). The time to reach intensity level 2 (threat display with meral spread) and 3 (pushing with chelae) were not significantly different between lentic and lotic conditions for *O. virilis* (intensity 2: 18.5 ± 5 s, 10.3 ± 2.1 s; intensity 3: 28.6 ± 10 s, 28.2 ± 6.7 s, respectively; $p > 0.05$; Figure 2b). *Orconectes virilis* had a similar

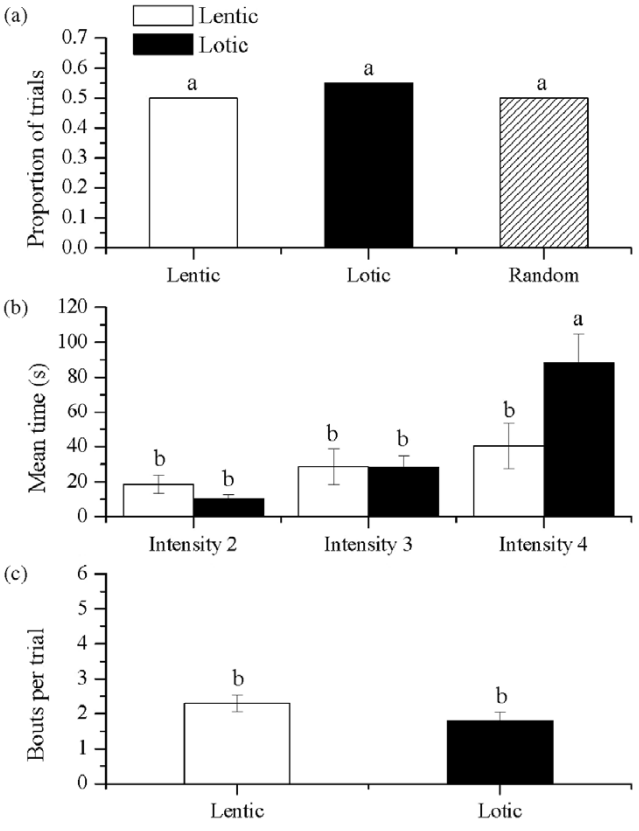

Figure 2. (a) Proportion of trials where dominant *O. virilis* from lentic habitats spent more time upstream of an opponent during lentic and lotic conditions. *Orconectes virilis* did not spend a greater proportion of time in any region of the arena relative to an opponent: lotic (black), lentic (white), and random = 50% occurrence (crosshatched). Random bar was placed for comparison only; this bar contains no collected data. Different letters indicate a significant difference. (b) The average time that fights took to reach each of the three intensity levels between *O. virilis*. *Orconectes virilis* fights progressed to intensity level 4 more rapidly in the lentic (white) compared to the lotic (black) condition ($p < 0.05$). All other intensity levels were not significantly different. (c) Number of bouts per trial between *O. virilis* under lentic and lotic conditions. There was no significant difference in the number of bouts between lentic (white) and lotic (black) conditions ($p > 0.05$).

number of bouts under lentic (no flow) conditions (2.3 ± 0.24) compared to lotic (flow) conditions (1.8 ± 0.25; $p > 0.05$; Figure 2c).

Experiment 3

There was no significant difference between number of release times of *O. rusticus* under lentic ($N = 32$) and lotic conditions ($N = 32$; $p > 0.05$). However, when examining the spatial position of *O. rusticus* when releasing urine, we observed statistical differences. When under lotic (flow) conditions, *O. rusticus* altered its pattern of urine release. Dominant *O. rusticus* released urine more often when perpendicular to the flow and in a fight ($N = 19$; 0.59) than when upstream ($N = 10$; proportion $= 0.31$; $q_{0.05,\infty,6} = 6.55$; $p < 0.05$) or downstream from an opponent ($N = 3$; proportion $= 0.09$; ($q_{0.05,\infty,6} = 12.83$; $p < 0.05$; Figure 3a). Furthermore, there was a greater probability of the dominant crayfish being upstream from an opponent than downstream when a flow was present ($q_{0.05,\infty,6} = 6.30$; $p < 0.05$; Figure 3a). When under lentic (no flow) conditions, dominant *O. rusticus* released urine similarly in regard to position when fighting within the enclosed arena (downstream: $N = 12$; proportion $= 0.35$; upstream: $N = 9$; proportion $= 0.26$; $q_{0.05,\infty,6} = 2.17$; perpendicular: $N = 13$; proportion $= 0.38$; $q_{0.05,\infty,6} = 0.70$; $p > 0.05$; Figure 3a).

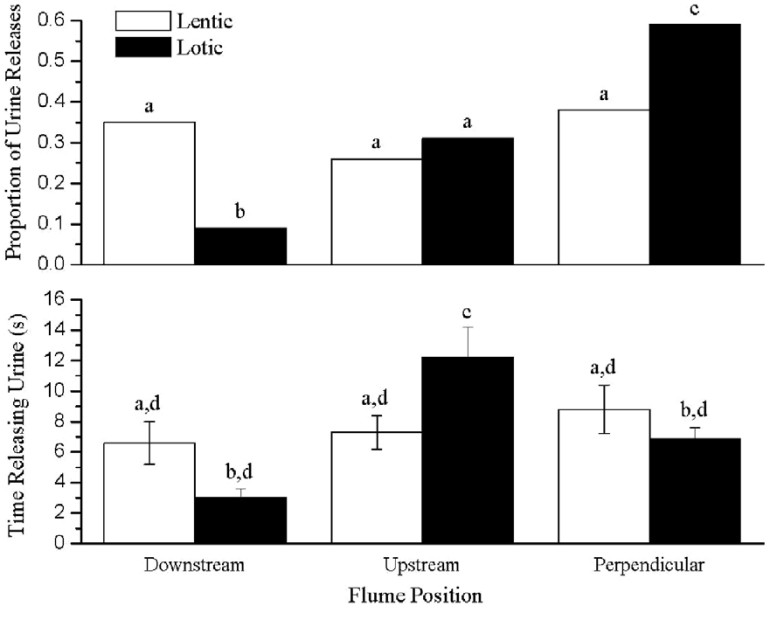

Figure 3. (a) Under lentic conditions (white), *O. rusticus* showed no difference in the likelihood to release urine containing fluorescein when fighting, regardless of position relative to opponent ($p > 0.05$). Under lotic conditions (black), *O. rusticus* released urine most often when across from an opponent while being perpendicular to the flow ($p < 0.05$). *Orconectes rusticus* released urine significantly more when upstream of an opponent than when downstream, but also significantly less compared to the perpendicular release ($p < 0.05$). (b) *Orconectes rusticus* released urine for a significantly longer periods of time when upstream from an opponent than when downstream or perpendicular under lotic conditions (black; $p < 0.05$). *Orconectes rusticus* did not alter the time spent releasing urine when under lentic conditions (white; $p > 0.05$). Different letters indicate a significant difference.

Orconectes rusticus released urine for significantly longer times when upstream from an opponent under lotic conditions (12.2 ± 2.0 s) than when downstream (3.0 ± 0.6 s; $p < 0.05$) or perpendicular to the flow (6.9 ± 0.7 s; $p < 0.05$; Figure 3b). When upstream to either a hypothetical or actual flow, dominant *O. rusticus* release urine for longer durations under lotic conditions (12.2 ± 2.0 s) than lentic conditions (7.3 ± 1.1 s; $p < 0.05$; Figure 3b). *Orconectes rusticus* did not alter the time spent releasing urine when under lentic conditions, whether they were upstream, downstream, or perpendicular to a hypothetical flow (7.3 ± 1.1 s; 6.6 ± 1.4 s; 8.8 ± 1.6 s respectively; $p > 0.05$; Figure 3b). There were no significant differences between dominant and subordinate crayfish for time spent releasing urine or number of releases ($p > 0.05$).

Discussion

Orconectes rusticus collected from lotic environments spend a greater proportion of fight trials in the upstream position under lotic conditions compared to fights in lentic conditions. *Orconectes virilis* collected from a lentic habitat did not show this same tendency to move upstream in either condition. This result indicates that *O. rusticus* that inhabit lotic environments have a tendency to move upstream when a perceptible flow is present. In fact, crayfish have been used in a number of orientation experiments because of their abilities to locate upstream odour sources (Bovbjerg 1970; Moore and Grills 1999; Keller et al. 2001). Perhaps it is not surprising then that crayfish collected from a lotic system would show an affinity to move upstream by orienting to mechanosensory stimuli present in the movement of water, which was also demonstrated by Momot (1966) and Hazlett et al. (1979) in real stream environments. In fact, the optimal strategy for an animal walking or crawling on a substrate while searching for the source of a chemical carried by shifting wind or current may be to move against the current (Dusenbery 1989, 1990). It is curious, however, that *O. virilis*, a species that lives in rivers and streams (Hazlett et al. 1974) and has exhibited upstream orientation behaviour (Adams et al. 2003), would not use the behavioural strategy to move upstream of a potential fight opponent in our experiments. *Orconectes virilis* collected from a lake do not appear to modulate their short-term behaviour in response to a flowing environment.

The presence of water flow had an additional effect on *O. rusticus* aggression. *Orconectes rusticus* pairs exhibited a greater number of agonistic bouts when experiencing lentic (no flow) conditions compared to the number of bouts under lotic conditions, and when compared to *O. virilis*. Not only did *O. rusticus* fight more often under lentic conditions, agonistic encounters progressed to high fight intensities (intensity 3 – pushing with chelae) at a faster rate compared to fights in lotic conditions. Although bout number did not differ between experimental conditions for *O. virilis*, agonistic encounters for this species progressed in a similar manner to *O. rusticus* in that they reached high fight intensities (intensity 4 – grasping with chelae) faster under lentic rather than lotic conditions. The complexity of flow in the lotic environment may be responsible for the longer times to increase to the higher intensities of fights and reduced number of bouts. The additional difficulty associated with chemical communication in a flowing environment may play an important role in mediating fight dynamics (Rose 1982). Moreover, Itagaki and Thorp (1981) demonstrated that *Procambarus clarkii* do not communicate chemically, or do so at least inefficiently, over distances greater than the effective range for visual communication. Vision plays an important role in decapod crustacean social interactions, particularly when the environment is relatively transparent (Vannini and Gherardi 1981; Bruski and Dunham 1987).

However, when water clarity is reduced by turbidity or darkness, crayfish may rely heavily on olfactory signals for communication.

Orconectes rusticus are thought to be endemic to the Ohio River and its tributaries (Taylor and Redmer 1996), where visual communication is limited due to the high turbidity of the rivers. Conditions in these rivers would likely have selected for chemical communication systems because of the decreased light transmission. *Orconectes virilis* is a species that is found in the more pristine rivers and lakes of the northern United States, but also now has a considerable overlap in habitat with *O. rusticus* due to the rapidly expanding range of *O. rusticus* (Hazlett et al. 1974; Bergman and Moore 2003). It is possible that the differences we observed could be a result of species-specific communication strategies. However, Bergman and Moore (2003) examined agonistic fight strategies of both *O. rusticus* and *O. virilis* in a lentic system and observed no differences in a number of agonistic characteristics used to evaluate fight performance. Given the lack of quantified field observations of fight dynamics in rivers, it is unknown how fight dynamics in lotic systems compare between these species. It seems likely that these two species consequently use chemical signals in a similar manner within a lentic system and chemical communication might be able to be utilized by the two species to chemically communicate status information in lotic systems.

In addition, it is possible that the differences seen with our results could be attributed to subtle differences in experimental conditions. Although the laboratory flume and artificial stream were closely matched in flow dynamics and fight arena size, the *O. rusticus* trials were performed using dechlorinated tap water, whereas the trials with *O. virilis* were performed using natural stream water. The stream water was filtered to remove macroscopic organisms and debris, but undoubtedly the background chemical composition between the two experimental conditions differed. We are cautious about directly comparing laboratory experiments with more naturalistic experiments. Behaviours of crayfish are sometimes altered under laboratory conditions. This is thought to be due to heightened and prolonged aggression arising from spatial confinement of combatants (Bergman and Moore 2003). Although this caveat is important to keep in mind when interpreting these results, we have found no evidence to date that demonstrates that natural background chemicals alter social interactions. Since we matched the spatial constraints and flow dynamics between the two setups, we feel that any other differences resulting from laboratory versus naturalistic conditions do not negate our findings.

Olfaction is equally, if not more important, than visual cues for determining aggressive behaviours for crayfish (Zulandt Schneider et al. 2001; Bergman et al. 2003; Bergman and Moore 2005). One likely source of information for this olfactory-mediated behaviour may be the urine. Urine signals, excreted through nephropores located near the base of the antennae, are a likely source of chemical cues in decapods (McLeese 1973; Atema 1986; Zulandt Schneider et al. 2001). The presence of urine increases olfactory sampling mediated through antennule flicking during fights (Ameyaw-Akumfi and Hazlett 1975; Rutherford et al. 1996). In addition, the presence of urine alters fight dynamics by decreasing duration and intensity of interactions (Zulandt Schneider et al. 2001). Urine is also important in the establishment of dominance. When crayfish are prevented from detecting urine, any previous social status information is eliminated (Bergman et al. 2003). Thus, crayfish appear to rely on broadcasting and detecting chemical signals during social interactions.

Orconectes rusticus collected from lotic habitats were able to release urine at the appropriate times during a fight, i.e. when upstream of an opponent when under lotic (flow) conditions. Crayfish have the ability to manipulate the surrounding habitat by projecting

urine and its component substances several body lengths away from the broadcaster (Breithaupt 2001; Breithaupt and Eger 2002; Bergman et al. 2005), yet this manipulation is likely very limited when it comes to overcoming the flows experienced in a river or stream environment. Thus, being upstream from an opponent in a lotic environment would increase the probability to chemically communicate the status of the upstream crayfish to their opponent. *Orconectes rusticus* collected from a turbid river appear to be capable of communication over short distances when experiencing flow conditions by using appropriate behavioural strategies when fighting. *Orconectes rusticus* shift communication strategies by releasing urine more often when upstream or across from an opponent when perpendicular to the flow. It appears that *O. rusticus* from lotic habitats have modified a communication strategy that is well suited to their environment. Whether this modification occurs through natural selection or as a learned behaviour over very short periods of time with experience is yet to be determined.

In summary, we can provide three possible interpretations of our results. First, our results may be due to species differences in the use of chemical signals and the ability to respond to different flow habitats. Second, differences in background chemicals in the two experimental conditions could alter the way in which crayfish use chemical signals during social interactions. Finally, it is possible that the differences in the hydrodynamics of home habitats have placed different constraints on chemical communication and crayfish have adopted signalling strategies that match their environments (Wehner 1987). If this last interpretation is correct, crayfish may have the neural complexity and flexibility essential for learning processes (Basil and Sandeman 2000; Sandeman and Sandeman 2000; Panksepp and Huber 2004). This neural architecture would give crayfish the ability to adapt using different behavioural communication strategies learned over a relatively short time. It is likely that crayfish are flexible enough in their communication strategies that lentic *O. virilis* could adopt a more proficient communication strategy within lotic environments with experience. More investigation will be required to ultimately determine whether adapting chemical signalling processes is a unique phenomena to *O. rusticus* or if this behavioural strategy is something that is more general and applicable across a variety of crayfish species and even other decapod crustaceans.

Acknowledgments

The authors would like to thank the Laboratory for Sensory Ecology, NSF (IBN #0131320) to P.A.M., and Sigma Xi Grant-in-Aid of Research to D.A.B. (#12030027) for their contributions to this research. All experiments comply with the current laws and protocols concerning invertebrate care in the United States.

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