The impact of increased turbidity, as a result of global climate change, on the stress and alarm signaling in the crayfish, Orconectes virilis

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Abstract

The objective of this study was to investigate the influence potential increasing suspended sediment loads due to global climate change could have on *Orconectes virilis* chemical communication. Experiments were designed to determine if suspended sediment triggers an increase in the quantity of chemical stress signals that crayfish release voluntarily under stressful environments. As well as determine if crayfish behavior is altered when exposed to chemical stress signals and damage-induced signals under turbid water conditions. In order to determine if suspended sediment triggers an increase in urine quantity crayfish were catheterized and exposed to clear water as well as turbid water in separate treatments. The influence of suspended sediment on the behavior of conspecifics exposed to both stress signals and alarm signals was determined by monitoring behavior in a Y-maze experimental stream. This Y-maze consisted of two separated arms to allow for the simultaneous input of clear and turbid with holding tanks for conspecifics to release chemical signals. First, crayfish exposed to turbid water conditions released urine quantities that were similar to that of crayfish exposed to clear water conditions. The behavior of conspecifics exposed to damage-induced alarm signals in turbid water showed no difference from the behavior of conspecifics exposed to these chemicals in clear water conditions. This indicates that *Orconectes virilis* disturbance chemical communication is tolerant to turbid water conditions for at least short durations of time.

Introduction

As the concentration of carbon dioxide in the atmosphere continues to increase many projections have forecasted drastic alterations to the earths climate. Depending on the scenario used mean surface air temperature is expected to increase by about 1.3°C-1.8°C by the middle of the century (Meehl et al. 2007). These changes in temperature are likely to alter the amount and distribution of the worlds freshwater, due to changes in key processes such as evaporation, water vapor transport and precipitation (Carpenter et al. 1992). In addition there will be increases in the intensity of precipitation events, due to the increased water holding capability of a warmer atmosphere (Fowler and Hennessey 1995).

If the intensity of rain events does in fact increase in the future as a result of climate change, increased runoff and subsequent sediment deposition is to be expected. Simulations have also suggested that the amount and intensity of rainfall are the primary factors influencing erosion (Nearing et al. 2004). Considering both of these factors are expected to increase as a result of climate change erosion is predicted to increase approximately 1.7% for each 1% change in annual rainfall (Nearing et al. 2004). Using the Universal Soil Loss Equation (USLE) models have predicted that the national average for sheet and rill erosion of cropland could increase by 2 to 16 percent (Phillips et al. 1992). While there is variation among scenarios, most suggest that average annual runoff will increase in higher latitudes and decrease in mid-latitude regions (Arnell 1999). From a local or regional point of view this erosion may result in increasing sediment loads in streams across the United States.

As early as 1990 siltation was identified as the leading cause of stream pollution in terms of impaired stream miles affected (Waters 1995). The negative impacts of suspended sediment have been well documented for a wide variety of aquatic organisms. Fishes experience reductions in production and diversity, due to the disruption of normal reproduction and destruction of the food supply (Berkman and Rabeni 1987). Fine sediment has the potential to block light from penetrating the water column, resulting in a reduction in productivity at the base of the food chain (Wood and Armitage 1997). In some situations sedimentation reduces benthic habitat by filling interstitial spaces in the substrate when particles settle out of the water column (Waters 1995). While suspended sediment has been documented to reduce the fitness of numerous organisms, there is still much to be learned about its affects on ecological interactions and behavior.

In aquatic organisms the exchange of information is essential and chemosensory signals are the main source of this information (Moore and Crimaldi 2004). Chemical communication in aquatic organisms plays a significant role in feeding, reproduction, navigation and kin recognition (Bronmark and Hansson 2000). Organisms not only use chemical substances to communicate with conspecifics, but other taxa have also been documented to respond to each other's chemical substances (Bronmark and Hansson 2000). This concept is especially apparent in damage-induced chemical alarm pheromones that are often released by the prey item when attacked by a predator. For example, it is hypothesized that minnow alarm substances released when a minnow is injured attract additional predators such as pike (Mathis et al. 1995). While chemosensory signals have been described in many taxa, invertebrates are an ideal group of organisms for study because they are extremely sensitive to even minute concentrations of chemicals (Krieger and Breer 1999).

Crayfish are one of the best-documented decapods in North American and the role that chemical communication plays in their behavior has become the focus of much research in recent years (Thorp and Rogers 2011). Crayfish disturbance chemical cues can be divided into avoidance chemicals, alarm chemicals and stress chemicals (Zulandt Schneider and Moore 2000). In recent years experiments on the disturbance signals of crayfish have concluded that urine is in fact the source of these chemical stress cues (Zulandt Schneider and Moore 2000). Crayfish exposed to stressful environments produce significantly larger quantities of urine than non-stressed crayfish and conspecifics exposed to these chemical cues moved farther away from stress crayfish than non-stressed crayfish (Zulandt Schneider and Moore 2000). These stress chemicals decrease the likelihood that conspecifics will enter situations or environments that may have a negative impact on their survival (Hazlett 1985).

In addition to voluntarily released stress signals disturbance chemicals also include involuntarily damage-induced chemicals that are often released during predatory interactions. These damage-induced chemical signals have been documented in a wide variety of taxonomic groups including both vertebrates and invertebrates (Mathis et al. 1994). In the presence of alarm chemicals released by damaged conspecifics as well as congeneric individuals *Orconectes virilis* reduces its activity level and lowers its posture (Hazlett 1994). These alarm signals presumably function to reduce the probability that conspecifics will enter situations that increase the likelihood of predation (Hazlett 1994).

Different aquatic environments have unique physical characteristics that greatly alter the way chemical signals are dispersed into the ecosystem (Moore and Crimaldi 2004). Variations in natural stream environments such as flow rate and substrate composition have been documented to influence the concentration and distribution of these chemical signals (Wolf and Martin 2009). This suggests that the odor landscapes of chemical signals are habitat-specific and can influence the behavior of organisms differently (Wolf and Martin 2009). Considering a large number of North American streams are already experiencing increasing suspended sediment loads it has become relevant to investigate the role suspended sediment may play in aquatic chemical communication.

At this point it is not understood how the changes in the physical characteristics of aquatic environments due to increasing sedimentation that are modeled under many climate change scenarios will impact the fitness and behavior of numerous aquatic organisms. Crayfish chemical communication in particular has been extensively studied under normal clear water conditions, but little is known about how chemical communication functions during periods of high suspended sediment concentrations. The goal of this study was to determine if suspended sediment triggers an increase in the quantity of chemical stress signals that crayfish release in urine. As well as determine if crayfish behavior is altered when exposed to chemical stress signals and damage-induced alarm signals under turbid water conditions.

Materials and Methods

Crayfish collection

Orconectes virilis were captured from Maple Bay of Burt Lake (45°29'13.80"N and 84°42'24.69"W) during the night using nets and underwater flashlights. Once captured the crayfish were transferred to the University of Michigan Biological Station's stream research facility (45°33'50.27"N and 84°45'4.27"W) where they were placed in large horse troughs that were filled with water diverted from the East Branch of the Maple River. Approximately two hundred crayfish were housed communally in two horse troughs. Unfiltered natural stream water fed in to the holding tanks bringing in detritus and macroinvertebrates to sustain the crayfish for the duration of the study. Natural photoperiod was maintained by placing these tanks in an open area far from any structures. The captured crayfish were allowed to acclimate to the new environment for a total of one week before being used in any experiments.

Experimental Set-up

All trials were performed in a Y-maze experimental stream that was 1.38 m (l) x 0.42 m (w) with two elevated holding tanks 0.6 m above the stream (Figure 1 & 2). The Y-maze was constructed from concrete cinder blocks and lined with clear plastic to hold water. The test section of the Y-maze consisted of two areas: the arms of the choice section and the initial start section. Each long arm of the Y-maze was 116.5 cm (l) x 21 cm (w) and the initial start section was 45.3 cm (l) x 42.0 cm (w). This section of the Y-maze was separated by a vertical piece of plywood for 71.2 cm the length of the stream. Pea gravel with a mean particle size of 10.8 mm was used as a substrate over the entire section of the Y-maze.

In order to deliver specific odors, two elevated holding tanks were constructed at the head of each arm of the Y-maze (Figure 1 & 2). These tanks were each 21.0 cm x 21.0 cm in size and separated with a plywood board. Silicon glue was used to create a watertight seal between the two channels. Suspended sediment was pumped through the stream to test the effectiveness of the separated channels. Constant and controllable flow to the Y-maze was created by elevating two 208.2 liter barrels at the head of the Y-maze. Each barrel was fitted with eight brass hose spigots, which could then be attached to washing machine hoses (14 mm in diameter) to direct flow through the holding tanks and into the Y-maze arms.

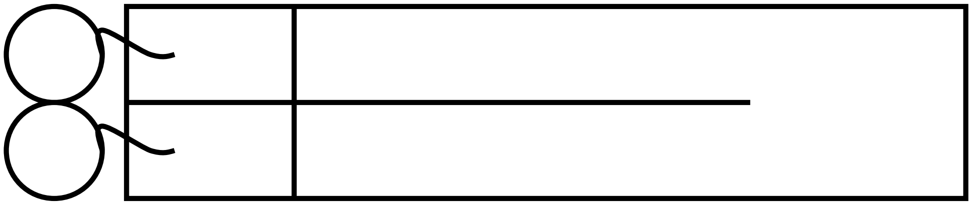

Figure 1. Aerial depiction of the experimental stream with 208-liter barrels draining into the elevated holding tanks and then draining into the separated Y-maze arms.

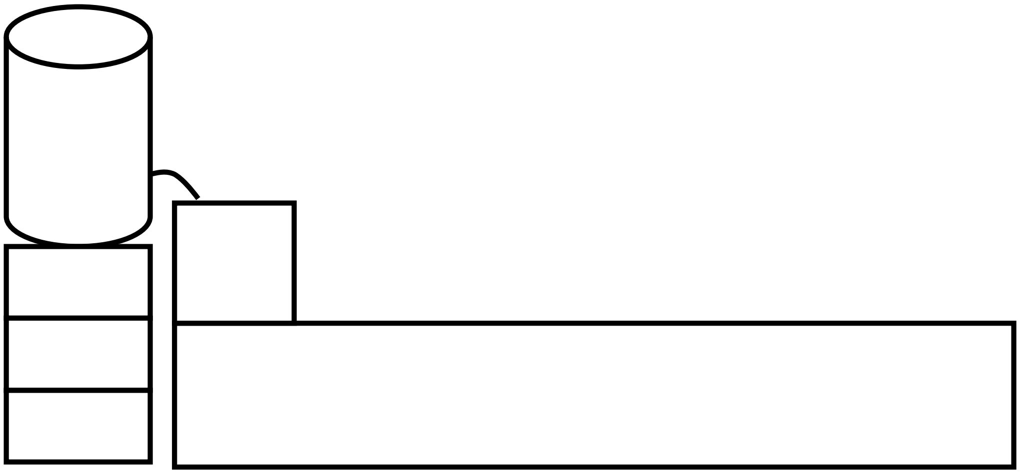

Figure 2. Diagram showing a side view of the experimental stream with the 208-liter barrels and holding tanks elevated above the Y-maze.

Experimental structure

To determine if increased sedimentation altered the release and response to stress and alarm signals, the following treatments were performed by placing conspecifics in the start section of the Y-maze and monitoring behavior for fifteen minutes. In these treatments disturbance chemicals were introduced to the experimental stream by placing crayfish in the holding tanks. For treatments involving damage-induced alarm signals one cheliped was clipped off the crayfish before being placed in the holding tank.

Treatment 1: Y-maze control: Clear water in both arms of the Y-maze (N = 10)

Treatment 2: Animal control: Clear water in one arm, clear water + intact crayfish in other arm (N = 10)

Treatment 3: Turbidity test: Clear water in one arm, turbid water in second arm (N = 10)

Treatment 4: Stress test: Clear water in one arm, turbid water + intact crayfish in other arm (N=10)

Treatment 5: Alarm test: Clear water in one arm, clear water + injured crayfish in second arm (N=8)

Treatment 6: Alarm + Turbidity test: Clear water in one arm, turbid water + injured crayfish in second arm (N=11)

Stress signal release in response to turbidity

To test whether increased turbidity resulted in a change in the amount of stress urine release in crayfish, catheterized crayfish were placed in streams with increased turbidity to collect urine. Crayfish urine was collected using a similar catheterization technique as Schneider and Moore (2000). Catheters were constructed from pipette tips, which had the narrow end cut off and attached to the nephropores of the crayfish using superglue and five minute epoxy. Once the pipette tip was glued to the crayfish tubing was attached to the pipette tip. Rather than attaching the tubing to the dorsal side of the carapace the tubing was allowed to float freely in front of the crayfish in order to reduce handling time. Lastly a latex condom was secured with a zip-tie around the tubing in order to collect and store the urine until it could be quantified. Once catheterized crayfish were allowed to accumulate over a 24-hour period before being used in any experiments. Once a crayfish was used in an experiment it was not used in any later trials.

Collection of urine

During each trial one catheterized crayfish was placed in the experimental stream for fifteen minutes. Following each trial the urine was drained from the latex condom and quantified. Throughout these trials water temperature was maintained constant with the holding tanks by mixing water from the East Branch of the Maple River with well water at the stream facility when necessary. Within one week of each trial exposing the crayfish to a stressful environment and collecting the urine tested the catheters glued to each crayfish. To create a stressful environment crayfish were once again placed in the experimental stream while the observer would continually handle the individual for ten minutes.

Sedimentation

For each trial turbidity was held constant by mixing 1.04 kg of clay with 208 liters of water diverted from the East Branch of the Maple River. This clay was manually removed from a location owned and managed by the University of Michigan Biological Station in Emmet County, MI. This resulted in a concentration of 5000 mg/L in each trial. Before each trial the barrel was mixed using a power drill with a mixing attachment. This attachment consisted of a long steel rod with a small computer fan attached at the base. The 208-liter barrel was elevated off the ground in order to create a constant water flow throughout the stream. Clear water was simply diverted from the river and pumped into a separate 208-liter barrel that was also elevated above the live stream at the same height as the other barrel.

Statistical Analysis

Based on the non-normal distribution of the data collected on the amount of urine released under clear and turbid water conditions a Mann-Whitney U test was used to compare trials. To analyze the effects of suspended sediment on crayfish behavior a Tukey multiple comparison test was used.

Results

Urine Collection

The amount of urine collected from crayfish in 15 minute trials under normal clear water conditions resulted in a mean quantity of 0.09 ± 0.03 mL (N=14). While the amount of urine collected from crayfish in 15 minute trials exposed to turbid water conditions resulted in a mean quantity of 0.06 ± 0.02 mL (N=15). Crayfish intentionally exposed to a stressful environment produced a mean urine quantity of 0.28 ± 0.12 (N=10). There was a significant increase in the urine production comparing the turbid and clear water conditions to the stress treatments ($P < 0.05$; Mann-Whitney U-test), but there was no significant difference between the clear and turbid water treatments ($P > 0.05$; Mann-Whitney U-test)

Turbidity and Stress Signals

Turbidity and stress signals did not influence the proportion of times crayfish chose between the two separate arms of the Y-maze. The overall chi-square for the proportion of times the crayfish chose the left or clear water arm in the first four treatment groups showed no significant difference ($x^2=0.48$, $p>0.05$). Choices between turbid and clear water conditions alone with no crayfish present showed no significant difference ($q=1.36$, $p>0.05$). Choices between turbid and clear water conditions with intact crayfish present showed no significant difference ($q=0.66$, $p>0.05$). Choices between turbid water with intact crayfish and clear water with no crayfish showed no significant difference ($q=1.76$, $p>0.05$). Lastly, choices between clear water with intact crayfish and turbid water without crayfish showed no significant difference ($q=0.32$, $p>0.05$).

Turbidity and alarm signals

The introduction of injured crayfish to the experimental stream influenced the choice behavior of conspecifics. The overall chi-square calculation for the proportion of times crayfish chose the left arm showed a significant difference ($x^2=11.6$, $p<0.05$). Crayfish exposed to clear water with an injured conspecific chose the opposite arm a significantly greater proportion of the time than crayfish exposed to clear water conditions with no crayfish present ($q=5.5$, $p<0.05$) and crayfish exposed to clear water conditions with intact conspecifics present ($q=6.0$, $p<0.05$) (Figure 3). Crayfish exposed to turbid water with an injured conspecific also chose the opposite arm a significantly greater proportion of the time than crayfish exposed to turbid water conditions with no conspecifics present ($q=5.5$, $p<0.05$) and turbid water conditions with intact conspecifics present (*q*=6.5, $p<0.05$) (Figure 3). There was no difference in the proportion of choices between turbid water conditions with injured conspecifics and clear water conditions with injured conspecifics (*q*=0.18, $p>0.05$) (Figure 3).

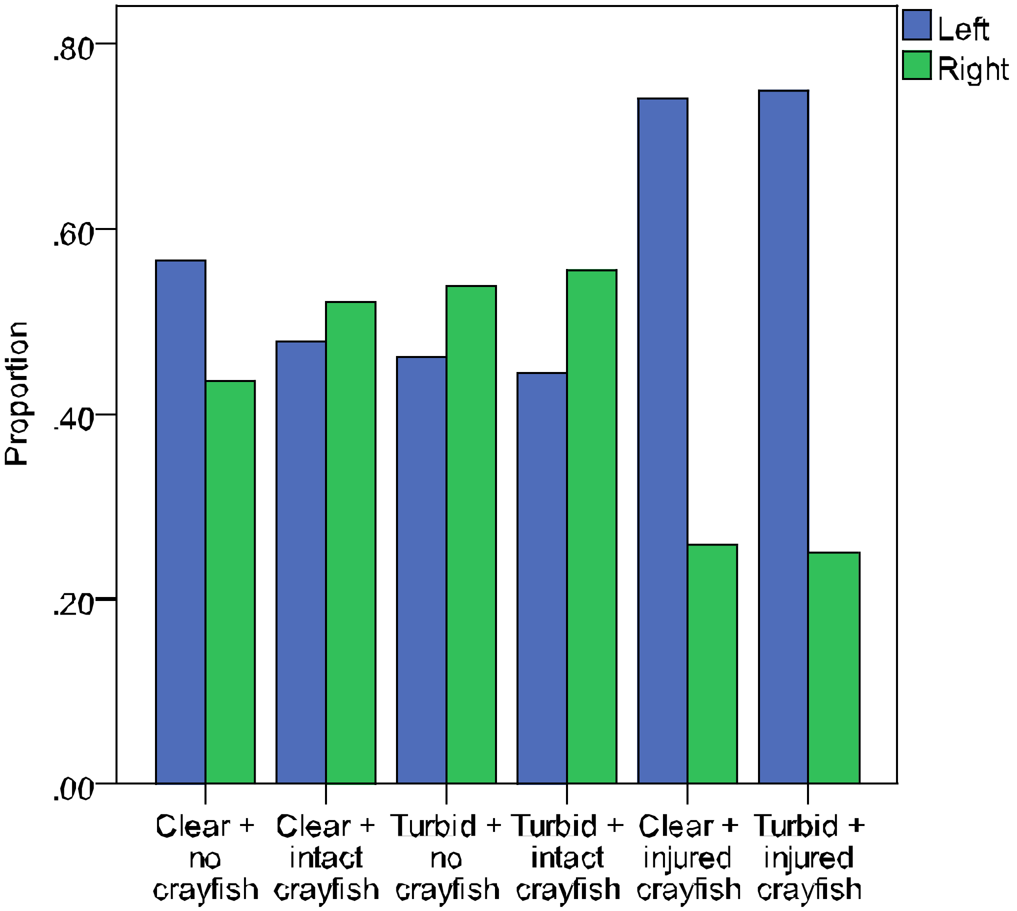

Figure 3. Proportion of times crayfish chose the left or right arm during each treatment.

Discussion

These data suggest that increasing turbidity in lotic systems does not cause additional release of chemical stress signals in *Orconectes virilis*. Due to the lack of chemical stress release there was also no difference in conspecific behavior. During these treatments crayfish moved freely from one channel to the other at varying rates and posture was similar to that of neutral crayfish. While the influence of turbidity on voluntary stress signals distribution and reception is unknown these data suggest that involuntary damaged-induced alarm signals are not affected by increasing turbidity (q=0.18, p>0.05). During both clear water and turbid water treatments with injured conspecifics observational behavior was similar, with lowered crayfish posture and reduced activity level. In a few trials crayfish would walk to the end of the Y-maze freeze momentarily and then quickly swim in the direction of the arm containing no damage-induced chemicals. These results indicate that *Orconectes virilis* has a high level of tolerance toward increased turbidity in terms of stress and damage-induced chemical communication at least for relatively short durations of time.

If turbidity increases in the future *Orconectes virilis* could possibly be better suited for success in this changing environment than other species of crayfish. For example, *Orconectes propinquus* shows little to no response to disturbance chemical that conspecifics release as well as predatory odors and may rely less on chemical communication than *Orconectes virilis* (Hazlett 1994). This suggests that *Orconectes propinquus* relies on other forms of communication such as visual communication and may be influenced more my increasing turbidity. In streams with both *Orconectes virilis* and *Orconectes propinquus* increased turbidity could possibly favor those individuals that can successfully avoid predation, locate a mate, and find food under these new environmental conditions.

Periods of high turbidity could be particularly important in predatory interactions of crayfish and other freshwater decapods that are less sensitive to chemical communication. These alarm responses such as reduced movement function to reduce the likelihood that a predator will be capable of finding the individual (Hazlett 1994). If in fact aquatic organisms such as *Orconectes propinquus* do rely on visual communication more than chemical communication it is possible that interactions could be greatly altered due to increases in turbidity. Species such as *Orconectes virilis* and *Orconectes rusticus* would presumably be better suited for these conditions as a result of their ability to detect and react to disturbance chemicals (Hazlett 1998). While *Orconectes propinquus* would likely be more susceptible to predation under water conditions that favor organisms that can communicate chemically.

While turbidity may not influence the release and detection of chemical disturbance signals in *Orconectes virilis,* chemical communication among aquatic crustaceans extends beyond disturbance chemicals. Chemicals along with morphology and behavior are used by crustaceans to recognize the opposite sex (Ameyaw-Akumfi and Hazlett 1975). In some species such as the rock shrimp (*Rhynchocinetes typus*) both visual and chemical communication are used simultaneously in mate recognition (Diaz and Thiel 2004). Courtship is similar in the case of the snapping shrimp (*Alpheus heterochaelis*), which uses an open chela display as a visual signal as well as chemical signals (Hughes 1996). Increasing turbidity could hinder the reproductive success of those species that do use visual communication as a portion of their courtship and favor those that rely less on visual communication and more on chemical communication.

With potential changes in predatory interactions and mate recognition due to increased turbidity it is possible that many North American water bodies could experience shifts in crayfish populations. The primary ecosystem process that crayfish provide is the accelerated decomposition of plant matter. Crayfish along with other benthic invertebrates have been estimated to process 20-73% of leaf-litter in headwater streams (Covich et al. 1999). What varies is each species ability to consume rooted macrophytes (Covich et al. 1999). In northern Wisconsin native *Orconectes virilis* were displaced by both *Orconectes propinquus* and *Orconectes rusticus* that removed entire macrophytes beds due to their superior feeding ability (Lodge et al. 1994 and Lodge et al. 1998). If increased turbidity does in fact cause shifts in crayfish species composition changes in macrophyte and detrital decomposition should also be expected.

While this study indicates that turbidity does not alter the ability of *Orconectes virilis* to communicate using disturbance chemicals, visual communication also plays a significant role in behavior of crustaceans as well as a wide range of other aquatic organisms. But as water clarity decreases many animals must rely solely on acoustic and chemical signals to communicate (Moore and Bergman 2005). This suggests that a wide variety of feeding and reproductive interactions could be altered across numerous taxonomic groups possibly resulting in changes to the physical characteristics of the environment. At this point it would be highly beneficial to invest future research towards investigating the impacts of suspended sediment on additional forms of communication in a variety of taxonomic groups.

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