ENVIRONMENTAL AND SOCIAL FACTORS INFLUENCE COMMUNICATION USED DURING CRAYFISH AGONISTIC INTERACTIONS

Michelle Elizabeth Cook

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Committee:
Paul A. Moore, Advisor
Sheryl L. Coombs
Daniel M. Pavuk
AGONISTIC interactions are mediated, in part, by communication between conspecifics. For crayfish, both chemical and visual communication have been shown to regulate aggression. Within each of these sensory systems are factors that influence communication used in agonistic interactions. Environmental factors such as toxins in aquatic ecosystems have the ability to disrupt chemical communication used during aggressive encounters. In the visual system, potential bystanders can extract relevant visual cues from interacting conspecifics and use them in future encounters.

This thesis addresses two factors that influence chemical and visual communication used during crayfish agonistic encounters. In the first study, chemical communication was found to be impacted by sublethal exposure to metolachlor, a herbicide used in northwest Ohio. This research indicates that crayfish exposed to 80 ppb metolachlor were less likely to initiate and win encounters against naïve conspecifics. High sublethal concentrations of metolachlor may be interfering with the ability of crayfish to receive or respond to chemical signals and thus affect certain agonistic behaviors. In the second study, visual communication was investigated in the broader context of a communication network and related to the role of previous social history in shaping crayfish aggression. In the field, visual cues are important for crayfish as dyadic interactions often occur in the vicinity on non-participating individuals. By observing fights, these bystanders have the potential to extract relevant information about future opponents. Results indicate that previous social history was more influential in determining fight outcome than information gathered via visual signals. Crayfish use a combination of sensory cues,
including chemical signals, during social interactions. Thus, visual information independent of other sensory cues may not provide the proper context for information use in subsequent interactions. Crayfish use both chemical information and visual information during agonistic encounters and by examining how each sensory system shapes crayfish aggression, the mechanisms can be further understood.
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Animals across all taxa must solve life’s basic problems in order to survive and reproduce. These problems include avoiding predators (Rottman and Snowdon 1972; Marler 1973; Sherman 1977), finding mates (Bernstein 1976; Baker 1983; Ryan and Wilczynski 1988), locating food (Menge 1972; Krebs et al. 1978), and allocating energy between these tasks (Dijkstra et al. 1990). Communication between individuals can play an important role in these behaviors by providing information to others. Generally, communication is an action on the part of one organism that alters the probability pattern of behavior in another organism in a fashion adaptive to either one or both of the participants (Wilson 1975). Bradbury and Vehrencamp (1998) further described communication as an exchange of information that is beneficial to both sender and receiver. Finally, Maynard Smith and Harper (2003) made the distinction between who benefits from an information exchange. Signals are traits that are specialized for the purpose of communication, in that the release of the signal and the receiver’s response to the signal have evolved for the purpose of communication. In this case, both the sender and the receiver benefit from the information transfer (Krebs and Davies 1997; Bonnie and Earley 2007). In contrast, cues are not specialized for communication, in that the sender cannot directly control who receives information (Maynard Smith and Harper 2003). However, cues still guide animal behavior, and include information from physical sources such as hydrodynamic, chemical, celestial, magnetic, and visual sources in addition to information from senders (Bonnie and Earley 2007). For example, animals orient to food based on chemical cues in their environment (lobsters- McLeese 1973; crayfish- Keller et al. 2001; Tomba et al. 2001; brown bullheads-
Both signals and cues contribute to forming and maintaining relationships between animals in social behavior.

Communication in a social setting provides information about conspecifics to other members of the same species. Types of information provided include: dominance status of individuals (birds- Fugle et al. 1984; Rohwer 1985; crustaceans- Karavanich and Atema 1998a; Zulandt Schneider et al. 1999), predator location (ground squirrels- Sherman 1977; vervet monkeys- Seyfarth and Cheney 1980; prairie dogs- Hoogland 1996), reproductive receptivity (treefrogs- Murphy 1994; Gerhardt et al. 1996; insects- Leslie 2005), and kin recognition (birds- Collias and Collias 1967; ungulates- Halpin 1991; isopods- Linsenmaier 1987). For example, dominance status is acquired through a series of agonistic interactions, or aggressive encounters in which animals engage each other over access to resources (Hazlett et al. 1975; Wilson 1975; Drews 1993; Zulandt Schneider et al. 2001). Encounters generally begin with a threat display and then progress through a ritualized suite of behaviors increasing in intensity, until one animal wins the encounter and the other loses and withdraws. These relationships can be reinforced, and a dominance hierarchy forms. Generally, being dominant indicates that the animal will have an increased access to resources (i.e. food, habitat space, mates; Wilson 1975; Söderbäck 1991; Hill and Lodge 1994; Rutherford et al. 1995). In some cases, dominance hierarchies in populations can reduce aggression within a population, allowing animals to utilize energy for other activities (Francis 1988; Drews 1993). Accepted animal models of dominance hierarchies include hen flocks (Guhl and Allee 1944; Cloutier et al. 1996), birds (Popp 1988; Drummond and Osorno 1992), fish (Thines and Heuts 1968; Frey and Miller 1972; Beachman and Newman 1987),
hyenas (Tilson and Hamilton 1984), and crustaceans (Bovbjerg 1953; Barki et al. 1991; Goessmann et al. 2000; Gherardi and Daniels 2003).

Crayfish are an established model for aggression (Dingle 1983; Hyatt 1983). They engage in highly ritualized fighting behavior (Bovbjerg 1953), which leads to the establishment of a stable dominance hierarchy (Bovbjerg 1953; Bovbjerg 1956; Zulandt Schneider et al. 2001; Bergman et al. 2003). Fighting behavior begins with an initial encounter between individuals and proceeds through a stereotypical series of events that ends with a winner and a loser. Crayfish that continue to be successful in winning agonistic encounters become dominant in a social hierarchy (Bergman et al. 2003). Agonistic behavior is mediated by a number of factors including communication (Bruski and Dunham 1987; Breithaupt 2001; Zulandt Schneider et al. 2001; Bergman et al. 2003), prior social experience (Goessmann et al. 2000; Daws et al. 2002; Bergman et al. 2003), fighting ability (Stein 1976), possession of resources (Fero et al. 2007), size (Garvey and Stein 1993; Ranta and Lindström 1993; Pavey and Fielder 1996), and changes in neurochemistry (Edwards and Kravitz 1997; Yeh et al. 1997). Some of these factors interact in order to produce aggression in crayfish and social information use has been proposed to link the mechanisms of social learning and social eavesdropping in a network environment (Bonnie and Earley 2007).

Recently, studies have begun to examine the broader social context which includes dyadic interactions and bystanders that could potentially observe these dyadic interactions (Oliveira et al. 1998; Earley and Dugatkin 2002; Peake et al. 2006). In communication networks, signals are transmitted further than the average spacing between individuals such that many potential signalers and receivers may be within the range of one another (Oliveira et al. 1998; McGregor and Peake 2000; Peake et al. 2002). Therefore, if individuals are spaced close enough
together in order to perceive and transmit signals, a network environment can be created
(McGregor and Peake 2000). Social information theory has been proposed as a unifying concept
that links the different consequences (i.e. eavesdropping- Oliveira et al. 1998; Doutrelant and
McGregor 2000; Peake et al. 2005; priming- Oliveira et al. 2001; Matos et al. 2003; and reverse
priming- Earley et al. 2005; Zulandt et al. 2008) in communication networks and the effects of
previous social history, and each operates through distinct mechanisms (Bonnie and Earley
2007).

Social learning, in the form of winner and loser effects help contribute to the formation
and maintenance of dominance hierarchies (Dugatkin 1997; Hsu and Wolf 2001; Bergman et al.
2003; Dugatkin and Earley 2004). Animals that repeatedly win fights have a higher probability
of winning encounters in the future and are more likely to become dominant (Dugatkin 1997;
Hsu and Wolf 1999; Mesterton-Gibbons 1999). Loser effects occur when individuals given
losing experiences are more likely to continue losing and become subordinate (Dugatkin 1997;
Hsu and Wolf 1999; Johnstone and Dugatkin 2000). Winner and loser effects have been
documented in several species of fish: blue gouramis (Trichogaster trichopterus, Frey and Miller
1972), green swordtails (Xiphophorus helleri, Beaugrand and Cotnoir 1996), and pumpkinseed
sunfish (Lepomis gibbosus, Beachman and Newman 1987). Winner and loser effects have also
been described in crayfish (Goessmann et al. 2000; Daws et al. 2002; Bergman et al. 2003). In
addition to social learning, several elements of communication networks must be considered.

Eavesdropping involves gathering information on the part of a bystander (Peake et al.
2002; Peake et al. 2005) and priming and reverse priming involve physiological changes in the
bystander (Clotfelter and Paolino 2003; Matos et al. 2003; Earley et al. 2005). Social
eavesdropping occurs when bystanders extract relevant information from aggressive encounters
and use that information to change their behavior towards one of the observed combatants (Oliveira et al. 1998; Peake et al. 2001). Social eavesdropping occurs in teleost fish (Oliveira et al. 1998; Peake et al. 2006) and in birds (Peake et al. 2001; Peake et al. 2002; Peake et al. 2005). In priming, bystanders experience a change in the physiological regulators of aggression and are more likely to win future encounters (Oliveira et al. 2001; Clotfelter and Paolino 2003). Contrastingly, in reverse priming, bystanders are more likely to lose future encounters as a result of changing physiology (Earley et al. 2005; Zulandt et al. 2008). The different consequences of communication networks have been well studied in birds and teleost fish, but there has been little work completed on invertebrates.

In the field, agonistic interactions occur within the presence of other crayfish that could possibly observe these interactions and use communication networks (Bergman and Moore 2003). Furthermore, in a preliminary study on visual communication networks in crayfish, bystander crayfish were shown to be negatively impacted after watching fights against naïve opponents (Zulandt et al. 2008). Crayfish use visual signals during aggressive encounters, but the role of visual signals has rarely been studied (see Bruski and Dunham 1987; Zulandt et al. 2008). Animals with potentially lethal weaponry may spend time displaying to conspecifics before actively engaging them. For example in laboratory studies, visually conspicuous displays by dominant crayfish such as raised posture and meral spreads caused subordinate crayfish to retreat or tail-flip away (Bruski and Dunham 1987). It is interesting to note that these behaviors were only observed under well-lit conditions indicating that visually locating dominants may be important for subordinate crayfish. Furthermore, fight dynamics, such as aggressive levels, are altered as a result of changes in visual information during encounters (Bruski and Dunham 1987). Given the complex social environment that crayfish occupy, other sensory modalities,
such as the chemical (Zulandt *et al.* 2001; Bergman *et al.* 2003) and mechanical senses (Smith and Dunham 1996; Breithaupt 2001; Bergman *et al.* 2005) work to regulate aggression in crayfish populations in addition to visual communication.

Chemical signal transfer between opponents mediates agonistic interactions and helps form dominance hierarchies, especially in crayfish. In aquatic habitats with low light and high turbidity, visual communication is limited, but chemical information is available as chemical information flows around obstacles and is available at large distances (Vickers *et al.* 1991; Dusenbery 1992). In crayfish, dominance status is conveyed through chemical signals released during agonistic encounters and perceived through the antennae and antennules (Zulandt Schneider *et al.* 1999; Zulandt Schneider *et al.* 2001; Gherardi and Daniels 2003). Urine has been hypothesized to convey the dominance status of individuals to opponents during fights (lobsters- Karavanich and Atema 1998a; crayfish- Zulandt Schneider *et al.* 2001; Bergman *et al.* 2005). During aggressive encounters, urine is released through the nephropores from one combatant and directed at its opponent (Bergman *et al.* 2005). Previous studies have shown that when chemical signals are removed from the fight environment by blocking urine release or when combatants are deprived of the ability to detect chemical signals by lesioning chemoreceptors, fight dynamics change (Karavanich and Atema 1998a; Zulandt *et al.* 2001; Bergman *et al.* 2003). For example, the effects of previous social history are rendered ineffective in crayfish that cannot smell as a result of lesioning the antennae and antennules (Bergman *et al.* 2003). Furthermore, when urine release was obstructed, fights were longer and reached higher intensity levels (Zuland Schneider *et al.* 2001). In aquatic habitats, there are a variety of factors that have the potential to impact chemical communication used during aggressive encounters.
Toxins in the environment have the potential to have great impact on social communication in aquatic organisms. Pesticides, heavy metals, and other organics are especially detrimental to aquatic organisms as they are in constant contact with their environment (Saucier et al. 1991; Moore and Waring 1998). Research has shown that exposure to toxicants negatively impacts the physiology (Baldwin et al. 2003; Carreau and Pyle 2005) and behavior of individuals (Saglio et al. 1996; Abgrall et al. 2000; Scott and Sloman 2004), and has potential to influence populations of organisms as well (Scott and Sloman 2004). Crayfish in northwest Ohio are potentially exposed to several pesticides (i.e. metolachlor, atrazine, cyanazine) throughout the year as a result of agricultural run-off and after high rainfall events (Battaglin et al. 2000; Frey 2001). Furthermore, aquatic animals could be exposed for long periods of time in that some herbicides have the ability to persist in aquatic systems for up to 46 days (i.e. metolachlor, Graham et al. 1999). Agricultural herbicides and insecticides are employed heavily in Ohio on corn and soybean fields during spring planting and atrazine and metolachlor are two of the most heavily used herbicides in the Lake Erie Basin region (Frey 2001).

Pesticides (herbicides and insecticides) are used to rid agricultural areas of weeds, pests, and to increase crop yield (Gilliom et al. 2006). Traditionally, studies on toxicants have examined endpoint lethality tests (i.e. LC50 values, USEPA 1993). These studies, while useful for establishing lethal dosage limits, may not portray an accurate representation of ecosystem health as concentrations of toxins rarely reach lethal levels (Scott and Sloman 2004). However, even sublethal concentrations can have detrimental effects on organisms, which would not be observed in traditional lethality tests. Recently, studies have focused on examining sublethal effects of toxicants on animal behavior in fish (McPherson et al. 2004; Scott and Sloman 2004; Carreau and Pyle 2005; Tierney et al. 2006), amphibians (Hayes et al. 2002; Ingermann et al. 2006).
2002; Rohr et al. 2003), and crustaceans (Sherba et al. 2000; Wolf and Moore 2002; Venkateswara Rao et al. 2007).

The use of pesticides and other toxicants have become a topic of concern for aquatic animals as pollutants enter aquatic systems through agricultural, sewage, and mining run-off and cause disruptions in normal olfactory mediated behaviors in fish (Moore and Waring 1998; Baldwin et al. 2003; Sandahl et al. 2004; Scott and Sloman 2004; Sandahl et al. 2006) and in crustaceans (Hebel et al. 1997; Sherba et al. 2000; Wolf and Moore 2002). In regards to aggression and social hierarchy formation, previous studies have shown that sublethal exposure to cadmium can change an individual’s rank in bluegill dominance hierarchies (Henry and Atchison 1979a, Henry and Atchison 1979b). Furthermore, Sloman (2003 a,b) found that rainbow trout exposed to trace amounts of cadmium became subordinate when paired against unexposed fish due to cadmium accumulation in the olfactory apparatus of the fish. Crayfish, like fish, use olfaction to regulate aggressive interactions and are likely to be negatively influenced by exposure to sublethal concentrations of pesticides.

Both chemical and visual communication are needed during agonistic encounters in crayfish. This thesis focuses on factors that influence both chemical communication and visual communication during agonistic interactions. The first project in this thesis addresses the issue of whether exposure to sublethal concentrations of the herbicide metolachlor interferes with olfactory mediated behaviors used during agonistic encounters. The second project investigates the relative roles of visual communication networks and previous social history on shaping crayfish aggression. Crayfish are an established model for aggression, and by investigating various factors that influence chemical and visual communication, the relative roles of the mechanisms responsible for shaping crayfish aggression can be further delineated.
CHAPTER II

THE EFFECTS OF THE HERBICIDE METOLACHLOR ON AGONISTIC BEHAVIOR IN THE CRAYFISH, *ORCONECTES RUSTICUS*

Introduction

Insecticides and herbicides are used regularly to rid agricultural areas of pests and to increase crop yield. In the Midwestern United States, herbicides are the most abundant group of pesticides employed and are used extensively on corn and soybean fields (Gilliom et al. 1999; Battaglin et al. 2000; Frey 2001). Although there are many examples of commonly used herbicides, including chloroacetamides (alachlor, acetochlor, metolachlor, and propachlor) and S-triazines (atrazine, cyanazine, and simazine), atrazine and metolachlor are among the most heavily applied to crops in the Lake Erie Basin (Frey 2001).

Following application to crops, pesticides can enter into aquatic environments from agricultural run-off either through irrigation or precipitation (Gilliom et al. 1999). Since precipitation varies seasonally, the amount of pesticides found within aquatic environments can also vary. Metolachlor concentrations in Ohio rivers have a yearly average of 5 µg/L (ppb), but during the spring and summer concentrations can reach 80 µg/L shortly after rainfall events (Battaglin et al. 2000; Frey 2001). The yearly averages of metolachlor in aquatic systems are typically well below the established lethal levels for aquatic organisms, which range from 3.9 mg/L (ppm) for rainbow trout (*Oncorhynchus mykiss*) to 25.1 mg/L (ppm) for *Daphnia* (Ahrens 1994). These concentrations of contaminants, although below the established lethal limits, may still have sublethal detrimental effects on aquatic organisms (Saglio and Trijasse 1998; Scholz et

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Sublethal levels of pesticides may be high enough to impair the physiology and/or the behavior of aquatic animals (Scott and Sloman 2004). Possible sublethal effects of pesticides include nervous system alteration, biochemical changes, impacts on reproduction, and possible chronic effects, such as a reduction in biomass and dry weight of adults (Sanchez et al. 2000; Schulz and Liess 2000; Kashian and Dodson 2002; Beketov and Liess 2005; Rakotondravelo et al. 2006). It has been hypothesized that exposure to sublethal concentrations of toxicants may be contributing to the worldwide decline of amphibians (Verrell 2000; Hayes et al. 2002; Rohr et al. 2003). Exposure to sublethal concentrations of atrazine had an impact on sexual development in frogs (*Xenopus laevis*) in that exposure caused the demasculinization of the male larynx and hermaphroditism in frogs (Hayes et al. 2002). In salamanders (*Ambystoma macrodactylum*) sublethal exposure to methoxchlor not only caused deleterious effects on larval survival but also impacted predator-prey relationships (Verrell 2000; Ingermann et al. 2002). In addition to amphibians, the behavior of lobsters and crayfish was affected by exposure to contaminants (Abgrall et al. 2000; Sherba et al. 2000; Wolf and Moore 2002). Sublethal exposure to copper and metolachlor impacted the ability of *Cambarus bartonii* and *Orconectes rusticus* to successfully locate a food source (Sherba et al. 2000; Wolf and Moore 2002). Juvenile American lobsters (*Homarus americanus*) exposed to azalmethiphos vacated their shelters in order to avoid exposure to the pesticide. Juvenile lobsters spend most of their time in shelters to avoid predation (Abgrall et al. 2000).

In cyprinids and salmonids, sublethal levels of pesticides can affect a number of different behaviors (Scott and Sloman 2004). Exposure to sublethal concentrations of carbofuran, atrazine,
and diuron, a phenylurea herbicide, impacted swimming patterns, social behavior, and orientation in goldfish (*Carassius auratus*, Saglio *et al.* 1996; Saglio and Trijasse 1998). Chinook salmon (*Oncorhynchus tshawytscha*) had a reduced antipredator response when exposed to diazinon, an organophosphate insecticide (Scholz *et al.* 2000). In addition, diazinon disrupted reproductive capabilities in male Atlantic salmon parr (*Salmo salar*, Moore and Waring 1996). Since some of these behaviors are regulated by olfaction, it is possible that the behavioral detriments result from an impacted olfactory pathway (see Hara 1992).

The olfactory system is thought to be especially susceptible to impairment from pesticides because olfactory receptors are in direct and constant contact with the environment (Saucier *et al.* 1991; Moore and Waring 1996; Saglio *et al.* 1996; Scholz *et al.* 2000; McPherson *et al.* 2004; Carreau and Pyle 2005; Tierney *et al.* 2006; Tierney *et al.* 2007a; Tierney *et al.* 2007b). Long term exposure to sublethal concentrations of copper impaired the olfactory discrimination ability of young rainbow trout in that exposed animals showed no preference between own rearing water against well water or heterospecific water (Saucier *et al.* 1991). Work done by Sandahl and colleagues has shown that sublethal exposure to copper alters the sensory physiology and predator avoidance behavior in juvenile coho salmon (*Oncorhynchus kisutch*, Baldwin *et al.* 2003; Sandahl *et al.* 2004; Sandahl *et al.* 2007). In addition, copper is broadly toxic to the olfactory nervous system in coho salmon indicating that many olfactory mediated behaviors could be impacted (Baldwin *et al.* 2003; Sandahl *et al.* 2004). Reproduction is one of the olfactory mediated behaviors that can be altered by sublethal exposure to copper. Belanger *et al.* (2006) showed that olfactory sensory neurons lesioned with copper sulfate in the round goby (*Neogobius melanostomus*) affected the responses of male gobies to female putative pheromones. Also, adult male Atlantic salmon parr had reduced olfactory responses to prostaglandins F$_{2\alpha}$
following short-term sublethal exposures to diazinon, carbofuran and atrazine (Moore and Waring 1996; Waring and Moore 1997; Moore and Waring 1998). Diazinon impacted olfactory-mediated alarm signal responses and homing ability in chinook salmon (Scholz et al. 2000). Salmon failed to reduce their swimming and feeding activities in response to alarm signals compared to controls. Also, fewer salmon exposed to diazinon returned to their natal stream to spawn, indicating that their homing ability was impaired (Scholz et al. 2000). Also interesting to mention is the work from Tierney et al. (2006) in coho salmon parr showing that both behavioral and physiological aspects of the olfactory-mediated alarm reaction can be altered by environmentally realistic concentrations of the carbamate fungicide IPBC. Although most of this work has focused on fish, chemoreception is important for foraging, mating, and other behaviors in aquatic organisms other than fish (Atema 1980; Ward et al. 1992; Snell and Morris 1993; Estebenet 1995; Snell 1998).

Crayfish, in addition to fish, are ideal animals to investigate the effects of toxicants on olfaction. These animals use olfaction to detect and avoid predators (Hazlett 1985; Hazlett 1990); locate food sources (Dunham et al. 1997; Moore and Grills 1999), find mates (Dunham and Oh 1996; Giri and Dunham 2000; Belanger and Moore 2006), and recognize social status among conspecifics (Zulanldt Schneider et al. 1999; Bergman et al. 2003). Wolf and Moore (2002) studied the effects of sublethal exposure to metolachlor on the ability of crayfish to perceive chemical stimuli by testing the ability of exposed crayfish to locate food and respond to alarm signals. This study concluded that metolachlor interferes with olfactory-mediated behaviors in the crayfish, Orconectes rusticus. After sublethal exposure, crayfish could not successfully locate a food odor and walked towards alarm cues, which is not a typical alarm responses in these animals (see Hazlett 1990).
Crayfish are benthic macroinvertebrates that are ecologically important and are found in a variety of aquatic habitats including lakes, rivers, and streams. They are omnivores, consuming invertebrates, algae, detritus, and macrophytes; and therefore have the ability to influence many different trophic levels in their environment (Lodge et al. 1994; Usio 2000). They are also important for carbon cycling in streams by shredding detritus and releasing energy for other organisms to use (Usio 2000). As a result of their ability to influence many trophic levels and their shredding capabilities, crayfish have a disproportional effect on their communities and have thus been referred to as keystone species (Helms and Creed 2005). Therefore, crayfish have the potential to be used as indicators of stream health during times when agricultural run-off is high.

For crayfish, chemoreception is integral not only to finding food and avoiding predators, but also for success in social situations (Zulandt Schneider et al. 1999; Bergman et al. 2003). Agonistic encounters, or aggressive interactions, are important for establishing dominance, which in turn may determine reproductive success, affect food and shelter acquisition, and influence population dynamics (Hazlett et al. 1975; Zulandt Schneider et al. 2001; Bergman et al. 2003; Bergman and Moore 2003; Fero et al. 2007). Typical agonistic encounters begin with a threat display and escalate in intensity until one animal retreats. This establishes a dominance relationship, which is reinforced during subsequent encounters (Daws et al. 2002). The encounter plays a pivotal role in determining the relative fitness of each crayfish. The purpose of this experiment was to determine whether sublethal exposure to metolachlor alters the fighting abilities of crayfish (Orconectes rusticus). We hypothesize that crayfish exposed to metolachlor will be unable to successfully participate in agonistic encounters against unexposed conspecifics perhaps due to the inability to receive or respond to chemical signals used in agonistic encounters or due to a change in internal aggressive state. These social impacts, such as the
ability to form dominance hierarchies, may subsequently affect fitness and population structure of crayfish exposed to sublethal metolachlor levels in natural aquatic habitats.

Materials and Methods

Animals

Male crayfish, *Orconectes rusticus*, were collected from the Portage River near Bowling Green, OH. Crayfish were housed in an environmental chamber at a constant temperature and light/dark cycle (23°C, 14L:10D). Intermolt form I male crayfish (mean ± SEM, carapace: 3.4 ± 0.04 cm, chelae length: 3.4 ± 0.06 cm, and weight: 14.4 ± 0.58 g) with fully intact sensory appendages were kept socially and physically isolated in a flow through holding tank. Crayfish had no social experience for at least one week prior to experimentation to remove any previous social effects on experimental outcomes (Zulandt Schneider et al. 2001). All crayfish were fed one rabbit pellet three times per week. Crayfish were used only once in these trials.

Chemical preparation and exposure protocol

Crayfish were exposed to 3 environmentally relevant concentrations of metolachlor: 60 ppb, 70 ppb, and 80 ppb (Frey 2001). Concentrations were chosen based on results from Wolf and Moore (2002), which showed that lower concentrations of metolachlor (25 ppb and 50 ppb) did not significantly impair crayfish foraging behavior. Metolachlor was purchased through the Supelco Chemical Company, Bellfonte, PA (Lot # LB274-68B, 96.1% purity). Stock solutions (17 mg/L metolachlor) were kept in the dark at 4°C and were not used after 30 days (Hartgers et al. 1998; Graham et al. 1999; Lin et al. 1999).

Exposure treatments were as follows:

- Treatment 1: 80 ppb – 4.71 mL metolachlor stock: 1 L of de-chlorinated tank water
- Treatment 2: 70 ppb – 4.12 mL metolachlor stock: 1 L of de-chlorinated tank water
Treatment 3: 60 ppb – 3.53 mL metolachlor stock: 1 L of de-chlorinated tank water

Treatment 4: De-chlorinated tank water only (control treatment)

Crayfish were exposed to each treatment for 96 hours before engaging in an agonistic encounter (USEPA 1993; Wolf and Moore 2002). Crayfish were exposed to metolachlor in 1500 mL pots with new water and metolachlor changed each day to ensure a constant concentration. This temporal regime of water and metolachlor should ensure that exposure concentrations remained constant as significant volatilization of metolachlor occurs at temperatures above 30ºC (Lau et al. 1995; Rice et al. 2004). Crayfish were kept at 23ºC during exposure. A sample size of N = 15 was used for each treatment, and a total of 120 crayfish were used for this experiment (1 exposed and 1 tester crayfish for each bout).

Fight protocol

Two crayfish (exposed versus naïve tester crayfish or control versus tester) were used in each agonistic encounter. For identification purposes, one crayfish was picked at random to be marked with correction fluid (Liquid Paper®) on the back of the carapace, which did not alter fight dynamics (Bergman et al. 2003). An opaque plexiglas fight arena (40 X 40 X 14 cm) with four removable dividers separated the tank into four equal sections in order to run two trials at once. The fight arena was filled with 15 liters of de-chlorinated tank water. The crayfish were placed into separate sections and allowed to acclimate for 20 minutes. Therefore, exposed crayfish had 20 minutes to recover from their exposure to metolachlor. The divider was then removed and the crayfish were allowed to interact for 15 minutes. After 15 minutes, all crayfish had either won or lost the fight. All trials were recorded from a camera (Panasonic wv-CL350) mounted 1 m above the fight arena onto a VCR (Panasonic AG-1980) and displayed on a monitor (Sony PVM-1351G).
Data analysis and fight evaluation

A blind analysis was performed on all videotaped fight trials. Crayfish agonistic interactions were analyzed using an ethogram, adapted from Bruski and Dunham (1987), as shown in Table 1. Fights were analyzed for duration of the first fight and number of different encounters over the 15 minute trial period. An encounter was defined as when the distance between two crayfish was less than one body length and separate encounters were defined when that distance was greater than one body length or no interaction occurred for 10 seconds (Bergman et al. 2003). The temporal mechanics of fight intensity (i.e. the escalation of the intensity of a fight) were also recorded, along with the initiator and winner of each fight. Winners were defined when opponents (losers) retreated or tail-flipped away. Initiation and winning were analyzed using a contingency table for multiple comparisons of proportions analysis analogous to Student-Neuman-Keuls or Tukey test ($q_{0.05;\infty,4} = 3.63$, Zar 1999). Duration of the initial fight, number of encounters, and times of escalation to different fight intensity levels were analyzed using a one-way MANOVA with Tukey-HSD post-hoc analysis. All data were analyzed using Statistica 6.0 (one-way MANOVA, StatSoft, Tulsa, OK) and Microsoft Excel (contingency table), with significance set at $p < 0.05$.

Results

Initiating and winning

Crayfish exposed to the highest concentration of metolachlor (80 ppb) were significantly less likely to initiate an encounter with an opponent than all other treatment groups ($q > 3.63$, $p < 0.05$; Figure 1). Crayfish in this group initiated fights only 20% of the time. All other treatment groups were not significantly different from one another nor different from the control group. Crayfish exposed to both the control treatment and 70 ppb metolachlor initiated 47% of the time, and crayfish exposed to 60 ppb metolachlor initiated 53% of the time.
Similarly, crayfish exposed to 80 ppb won significantly fewer fights (20%) than all other treatment groups ($q > 3.63, p < 0.05$; Figure 2). Control crayfish won 47% of fights; crayfish exposed to 60 ppb metolachlor won 67% of fights, and crayfish exposed to 70 ppb metolachlor won 53% of fights.

**Time to different intensities, duration, number of encounters**

There were no significant differences in time to different intensities, duration, and number of encounters (one-way MANOVA, $p > 0.05$; Figures 3, 4, and 5). In time to different intensities, for intensities 2, 3, and 4, crayfish exposed to 80 ppb metolachlor averaged 5.3 ± 1.6 s, 14.3 ± 6.1 s, and 60.6 ± 17.0s, respectively ($F_{9, 71} = 1.22, p > 0.05$; Figure 3). For the duration of the first encounter, results were not significant. Crayfish exposed to 80 ppb averaged 281.3 ± 83.6 s for the first encounter ($F_{3, 56} = 1.18, p > 0.05$; Figure 4). Finally, in the number of different encounters within a fight, results were not significant. Crayfish exposed to 80 ppb averaged 8.8 ± 1.0 encounters ($F_{3, 56} = 1.91, p > 0.05$; Figure 5).

**Discussion**

*Possible mechanisms for results*

Sublethal concentrations of metolachlor altered only the two endpoints of aggressive interactions (i.e. the beginning and the final outcome of a fight; Figures 1 and 2) and only at the highest concentration tested (80 ppb). It appears that exposure to metolachlor may be altering the underlying sensory or neural mechanism that dictates the animal’s decision to either initiate an encounter or to win the encounter compared to other fight behaviors. In crayfish, initiating an encounter indicates an individual’s initial aggressive state and winning an encounter establishes an individual’s ability to become dominant within a population (Daws et al. 2002; Bergman et al. 2003). Therefore, animals exposed to 80 ppb metolachlor were less aggressive initially and
less dominant than conspecifics exposed to weaker concentrations of metolachlor and the control crayfish. It is important to note that less aggression and less dominant are not necessarily equivalent (Francis 1988; Drews 1993), and thus there is potential for two separate effects of exposure. One effect could be altering the initial levels of aggression (Figure 1) and a separate effect could be altering the potential for a crayfish to become dominant (Figure 2).

In previous research on crayfish, Wolf and Moore (2002) showed that sublethal exposure to metolachlor altered the sensory ability of crayfish to locate food and to respond to alarm signals; both of these behaviors are mediated by the ability of crayfish to detect and respond to chemical signals in the environment. Not only are chemical signals needed for detection of food and for avoidance of predators, but they are also used in agonistic encounters (Zulandt Schneider et al. 2001; Bergman et al. 2003). Fight dynamics have been altered by removing the ability of crayfish to receive and respond to chemical signals used in agonistic encounters (Zulandt Schneider et al. 2001; Bergman et al. 2003). For example, in agonistic bouts where chemical communication was blocked either by lesioning the chemoreceptors or by blocking urine release, encounters last longer and reach higher intensity levels (lobsters- Karavanich and Atema 1998a; crayfish- Zulandt Schneider et al. 2001; Bergman et al. 2003). Furthermore, in experiments in which sensory intact crayfish were given a winning experience and then paired with an opponent without sensory abilities, the winner effect was absent (Bergman et al. 2003). Bergman et al. (2003) concluded that the changes observed in fight dynamics were due to the inability of crayfish to perceive chemical stimuli rather than changes in aggressive state alone. Although we did not observe any variations among treatment groups for fight dynamics, we did observe changes in initiating and winning (Figures 1 and 2) due to metolachlor exposure. While
Chemosensory abilities may have been impacted, exposure to metolachlor may also have impacted internal changes in aggressive state or baseline motor activity.

Alternatively, metolachlor may alter fight dynamics by impacting the neurochemistry that regulates crayfish internal aggressive state or may have impacted baseline motor activity. Biogenic amines, such as serotonin, octopamine, and dopamine help to regulate aggression in decapod crustaceans (Kravitz 1988; Huber and Delago 1998; Huber et al. 2001). Furthermore, serotonin confers a heightened aggressive state in crayfish (Huber et al. 2001). Crayfish exposed to metolachlor were less aggressive than unexposed conspecifics, and it is possible that metolachlor may interfere with crayfish neurochemistry responsible for regulating aggression. In addition, metolachlor may have an effect on baseline motor activity at the highest dose. Crayfish may have been too lethargic to initiate and win agonistic encounters. Our results are likely resultant from a mixture of the inability to perceive and respond to chemical signals and from changes in aggression state as some aspects of aggression were impacted while others were not.

Possible consequences on social hierarchies

Aggressive interactions between individuals have the potential to result in a dominant relationship when one individual emerges as the winner and the other as the loser and as these roles are reinforced, social hierarchies form. Since exposure to metolachlor alters the probability of winning, it may also impact the formation of social hierarchies in crayfish, just as some pollutants affect hierarchy formation in social groups of fishes (Henry and Atchison 1979a; Henry and Atchison 1986; Sloman et al. 2003a). Henry and Atchison (1986) found that sublethal concentrations of copper affected social hierarchy formation in bluegills (*Lepomis macrochirus*), particularly in relation to an individual’s rank. Sloman et al. (2003a) concluded that any contaminants in salmonid communities have a potential threat to populations, because salmonids,
like crayfish, form social dominance hierarchies. Changes in these hierarchies, induced by sublethal concentrations of metals and herbicides, may have the ability to influence population dynamics. If exposed crayfish are no longer able to win encounters, and become subordinate, they may have reduced access to resources, such as habitat space, mates, and food. Consequently, an individual’s fitness may be reduced within a population.

In aquatic environments, it is likely that both combatants would be exposed to metolachlor. Fight dynamics between two exposed individuals may be different than fight dynamics between two healthy crayfish. This could impact the temporal dynamics of the formation of social dominance hierarchies. In normal populations of crayfish, social hierarchies become stable after 96 hours (Fero et al. 2007). Metolachlor could interfere with the length of the time period over which dominance hierarchies are established as is the case with rainbow trout exposed to cadmium (Sloman et al. 2003a). Populations of rainbow trout exposed to cadmium formed dominance hierarchies at a faster rate than populations with control fish (Sloman et al. 2003a). Changes in formation and maintenance of social hierarchies could contribute to the overall productivity of the population such as the ability of dominant crayfish to maintain their position and retain resources.

**Conclusion and future research**

Crayfish are proposed keystone species in their environments, which makes them ideal indicators of water quality as they have a disproportional effect on their community (Helms and Creed 2005). We have shown that sublethal concentrations of metolachlor interferes with chemical communication used during agonistic interactions and impacts the fighting ability of crayfish, making them less aggressive and less dominant. In this way, metolachlor has the potential to decrease the health of individuals within the population. If severe enough, the
population may be impacted, which could be used as an indicator of relative stream health. Future studies should be conducted to investigate the effects of pulsed herbicide exposures as well as recovery rates. Crayfish may be most susceptible to metolachlor impairment during times when agricultural run-off is high and pulse contamination may occur in streams. However, runoff events are likely to be shorter than 96 hours so future studies should investigate the effects of using pulsed exposures on the order of six hours or less. In aquatic systems, metolachlor may occur with other pesticides. Studies are needed to explore the synergistic effects of metolachlor mixed with other herbicides on animal behavior. Furthermore, additional work should be completed to link changes in an individual’s fitness within a hierarchy to population models and on the formation of social hierarchies in crayfish.
CHAPTER III

SOCIAL HISTORY AND SOCIAL INFORMATION INTERACT TO INFLUENCE THE OUTCOME OF AGGRESSIVE INTERACTIONS IN THE CRAYFISH *Orconectes rusticus*

Introduction

Studies on agonistic behavior have traditionally been limited to analyses of dyadic interactions; however, recent research has started to examine agonistic behavior within the context of the broader social environment (Johnsson and Akerman 1998; Oliveira et al. 1998; Peake et al. 2001; Peake et al. 2002; Peake et al. 2005). The broader social environment includes the active space of signals used during dyadic interactions, bystanders that are within the active spaces, and previous social learning of the combatants (Bonnie and Earley 2007). When agonistic signals have active spaces that potentially encompass several signalers and receivers, a communication network is established (McGregor and Peake 2000; Peake 2005). Potential effects involved in the influence of communication networks on social behavior include eavesdropping (Oliveira et al. 1998; Peake et al. 2001; Peake et al. 2002), priming (Oliveira et al. 2001; Matos et al. 2003), and reverse priming (Earley et al. 2005; Zulandt et al. 2008). In addition to the information transfer in communication networks, social learning, such as winner and loser effects, can also occur (Earley and Dugatkin 2002). The integration of communication network theory with social learning has been termed social information (Bonnie and Earley 2007). Underlying the individual phenomenon contained within social information theory are

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different behavioral mechanisms which can interact to produce different consequences for signalers and receivers in a communication network or winners and losers in social learning.

One of the consequences of a communication network is that bystanders can obtain additional information that can be used in future encounters (McGregor and Peake 2000). Social eavesdropping allows bystanders to obtain information on the quality of potential future combatants such as fighting ability or resource holding potential (RHP; Parker 1974; Oliveira et al. 1998; McGregor and Peake 2000). Both birds (Parus major) and fish (Betta splendens) have shown that relative information about a combatant’s fighting ability (i.e. combatant 1 is more aggressive than combatant 2) rather than absolute information (i.e. combatant 1 is aggressive) caused bystanders to change their behavior in subsequent encounters with one of the former fighters (Oliveira et al. 1998; Peake et al. 2001; Peake et al. 2002; Peake et al. 2005). Male great tits (Parus major) eavesdrop on acoustic signals in order to assess the fighting abilities of potential rivals allowing them to respond differently to losers than winners (Peake et al. 2001; Peake et al. 2002). Male bystanders of Siamese fighting fish (Betta splendens) have been shown to eavesdrop on the visual displays used in agonistic interactions and subsequently respond to observed winners and losers differently (Oliveira et al. 1998; Peake et al. 2006). Bystanders took longer to approach seen winners and spent more time displaying to winners than to losers (Oliveira et al. 1998). Although social eavesdropping has received a larger percentage of attention in regards to communication networks, the social environment in which aggression occurs has the potential to modify interactions as well.

Bystanders, who watch fights, experience physiological changes, called priming and reverse priming that contribute to future fight success (Oliveira et al. 2001). Both priming and reverse priming involve a change in the neuroendocrine modulators of aggression (Bronstein
These modulators include hormones in fish (Oliveira et al. 1996; Oliveira et al. 2001; Oliveira et al. 2002) and neurotransmitters in crustaceans (Huber et al. 1997; Huber and Delago 1998; Kravitz and Huber 2003). In priming, animals experience an up-regulation of social modulators and are more likely to win future encounters (Clotfelter and Paolino 2003; Matos et al. 2003). In contrast, reverse priming involves a down-regulation of social modulators where bystanders are more likely to lose future encounters (Earley et al. 2005; Zulandt et al. 2008). Siamese fighting fish, who watched fights, were more aggressive in future encounters while green swordtails (Xiphophorus helleri) did not experience any change in aggressive behavior after watching fights (Clotfelter and Paolino 2003; Earley et al. 2005). Although the three previous effects all contribute to future fight success, social information use also includes effects from previous social history.

Previous social history, in the form of winner and loser effects, occur when an animal has an altered probability of outcomes in subsequent encounters after experiencing either a winning event or a losing event (Dugatkin 1997; Hsu and Wolf 1999; Hsu and Wolf 2001; Dugatkin and Earley 2003; Hsu et al. 2006). For example, individuals who experience a win increase their probability of winning future fights (Dugatkin 1997; Hsu and Wolf 1999; Bergman et al. 2003). The opposite is true for losers; individuals are more likely to lose future encounters (Dugatkin 1997; Hsu and Wolf 1999; Dugatkin and Earley 2004). Several methods contribute to underlying mechanisms by which previous social history shapes future behavior, and some of these mechanisms are shared with the elements in a communication network. These mechanisms include neuroendocrine changes (Oliveira et al. 1996; Edwards and Kravitz 1997), learning (Karavanich and Atema 1998b; Zulandt Schneider et al. 2001), and changes in fighting skill.
(McDonald et al. 1968; Thines and Heuts 1968). Winner and loser effects are documented in many taxa including insects (Simmons 1986; Adamo and Hoy 1995), fish (Frey and Miller 1972; Francis 1983; Beaugrand et al. 1991; Chase et al. 1994; Hsu and Wolf 1999), birds (Popp 1988; Jackson 1991), and crustaceans (Thorpe et al. 1995; Goessmann et al. 2000; Daws et al. 2002; Bergman et al. 2003).

Within the broader context of social information use, social learning and bystander effects in communication networks (i.e. eavesdropping, priming, and reverse priming) interact in order to influence future fight success (Oliveira 2005). The interaction of social learning and bystander effects can be tested using aggression in crayfish (Daws et al. 2002; Bergman et al. 2003; Zulandt et al. 2008). Previous social history contributes to the formation and maintenance of crayfish dominance hierarchies in the form of winner and loser effects (Goessmann et al. 2000; Daws et al. 2002; Bergman et al. 2003). Furthermore, preliminary work on communication networks in crayfish has shown that bystanders are negatively influenced in future fights with naïve opponents (Zulandt et al. 2008). In the field, agonistic interactions are brief and often occur within the presence of other crayfish, indicating that there is potential for crayfish to use communication networks (Bergman and Moore 2003).

In this study, we chose to expand the scope of social information use in crayfish by examining how social learning, eavesdropping, priming, and reverse priming influence the outcome of crayfish agonistic interactions. Previous research has shown that crayfish alter their behavior after observing agonistic interactions between conspecifics (Zulandt et al. 2008). Our experimental design extends these studies allowing us to examine the relative roles that previous social history and network elements have on regulating aggression in crayfish. By fighting bystanders against both seen and unseen winners and losers, we will be able to separate network
effects from previous social history effects. We predict that if winner and loser effects are the primary factors regulating aggression, we will not observe any differences in the fighting ability of the focal crayfish in our control treatments (unseen winners and losers) versus our experimental treatments (seen winners and losers). However, if bystanders are obtaining and using information from the interaction, then we predict that we will see differences in the fighting ability of focal crayfish in encounters with unseen winners and losers compared to fights with seen winners and losers.

Materials and Methods

Animals

Male crayfish, *Orconectes rusticus*, were collected from the Portage River near Bowling Green, OH. Crayfish were housed in a flow through tank within an environmental chamber at a constant temperature and light/dark cycle (23°C, 12L:12D). Intomolt form I male crayfish (mean ± SEM, carapace: 3.3 ± 0.02 cm, chelae: 3.0 ± 0.03 cm, and weight: 12.3 ± 0.20 g) with fully intact sensory appendages were kept socially and physically isolated from one another. Crayfish had no social experience for at least one week prior to experimentation to remove any previous social effects on experimental outcomes (Karavanich and Atema 1998b; Zulandt Schneider *et al.* 2001). All crayfish were fed one rabbit pellet three times per week. Crayfish were sized-matched to within 10% of carapace and chelae length of opponents to counteract any size impacts on fight outcome.

Fight arena

The fight arena was made of clear Plexiglas (40 x 40 x 14 cm) and was filled with 10 L of de-chlorinated water (4 cm from the top of the tank; Figure 6). Two of the outer sides of the fight arena were clear, the other two opaque. The fight arena was divided into four quadrants,
separated by opaque retractable walls. Two additional small arenas were used as bystander tanks (20 cm x 10 cm x 14 cm) and were placed adjacent to the clear walls of the fight arena. Bystander tanks had three opaque walls and one clear wall so that crayfish in each trial would be within visual range of one another.

**Bystander conditioning fights**

This project consisted of testing two treatments: winner and loser effects. Each treatment consisted of two elements, a bystander conditioning fight followed by the experimental fight. The bystander conditioning fight served dual purposes in that a “winner” and a “loser” crayfish were determined and a bystander observed this fight. The experimental fight was used to measure any behavioral impact of observation by pairing the bystander crayfish against either the winner or loser crayfish from the conditioning fight.

During the bystander conditioning fight, a socially naïve crayfish was designated as the bystander and was placed in an adjacent bystander tank (see Figure 6). Additionally, two sized-matched socially naïve crayfish (hence named tester crayfish) were placed on opposite sides of the retractable wall in the fight arena (see Figure 6). All crayfish were allowed to acclimate for 20 minutes. After the acclimation period, the wall was removed and the two tester crayfish were allowed to interact for 15 minutes with the bystander crayfish within visual range of the interaction. During the bystander conditioning fight, a human observer watched the encounter and determined the winner and loser of the fight between the two tester crayfish. The winner was determined using behavioral indicators that have been published elsewhere (Bergman *et al.* 2003).
Experimental fights

After the conclusion of the bystander conditioning fight, a bystander crayfish and a tester crayfish (either a winner or loser) were placed on opposite sides of an opaque wall in a separate identically-sized fight arena. During the experimental fight, both the bystander and tester crayfish were allowed to acclimate for another 20 minutes. The wall was then removed and the two crayfish were allowed to interact for 15 minutes. Bystander crayfish were exposed to one of two treatments (winner or loser) combined with one of four conditions. The four conditions were:

1. 15 minute agonistic encounter with the tester crayfish of the previous fight (fight condition)
2. 15 minute agonistic encounter with a tester crayfish after watching two crayfish that did not interact (i.e. the wall was not removed, visual control)
3. 15 minute agonistic encounter with a tester crayfish after watching an empty tank (handling control)
4. 15 minute agonistic encounter with a tester crayfish after watching the wall being removed with no crayfish present (motion control)

For each of the control treatments, a separate (non-observed) conditioning encounter was performed in order to establish a winner or loser tester crayfish that would be used in the experimental fights. This separate agonistic encounter was run simultaneously as the bystander conditioning control. Each condition group consisted of 20 trials and bystander crayfish were used only once in this experiment. Thus, a total of 160 bystander crayfish were used for two treatments (winner and loser) and four conditions (fight condition, visual, handling, and motion controls).
Data analysis

All fights in this experiment were recorded from a camera (Panasonic wv-CL350) positioned 1 m above the test arena, displayed on a monitor (Sony PVM-1351G), and recorded to a VCR (Panasonic AG-1980). Only agonistic encounters from the experimental fights were used for subsequent analysis. A blind analysis was performed by a person with no prior knowledge of which treatment or condition each crayfish was assigned to for the experimental fight. For each agonistic encounter, the winner and loser of each fight was recorded. Winners were defined when opponents (losers) retreated or tail-flipped away. Also, the temporal mechanics of the fight were recorded, which included time to different fight intensities and duration of the initial encounter. We used a modified ethogram to determine time to different intensities (Bergman et al. 2003; Table 1). To analyze data within each treatment (for bystander effects), initiation and winning were analyzed using a modified chi-square analysis ($\chi^2 = 7.82$, df = 3), followed by a contingency table for multiple proportions ($q_{0.05, \infty, 4} = 3.63$), similar to a Tukey post-hoc analysis (Zar 1999). The temporal mechanics of the fight were analyzed using a one-way MANOVA, followed by a Tukey post-hoc test, if needed. We also performed analyses across the winner and loser treatments (to test for social history effects) using a chi-square analysis ($\chi^2 = 14.07$, df = 7) followed by a contingency table for multiple proportions ($q_{0.05, \infty, 8} = 4.29$, Zar 1999). A 2 x 4 factorial MANOVA, with type of treatment (winner or loser) and bystander condition (fight, visual control, handling control, or motion control), followed by a Tukey post-hoc was used to analyze fight dynamics. In addition, the percent of fights reaching intensity level 4 across the winner and loser treatments was analyzed using a chi-square analysis ($\chi^2 = 14.07$, df = 7) followed by a contingency table for multiple proportions ($q_{0.05, \infty, 8} = 4.29$, Zar 1999). Significance
was set at $p < 0.05$ and all data was analyzed using Microsoft Excel and Statsoft Statistica ver. 6.0.

**Results**

**Initiation and winning**

In winning, significant differences were found across the winner and loser treatments (Figure 7). Bystanders that had fought a winner lost significantly more fights than bystanders that fought losers ($\chi^2 = 49.96$, df = 7, $p < 0.05$; Figure 7). All of the winner conditioning groups were statistically different from the loser conditioning groups. Bystanders in the winner fight condition lost significantly more fights than the loser fight condition, the loser visual control, the loser handling control, and the loser motion control ($q = 9.00$, $q = 11.13$, $q = 9.00$, $q = 11.13$, $p < 0.05$). Also, the visual control winner condition lost more fights than the loser condition, the visual control loser, the handling control loser, and the motion control loser ($q = 9.00$, $q = 11.13$, $q = 9.00$, $q = 11.13$, $p < 0.05$). Bystanders in the winner handling control condition lost more fights than the loser condition, the visual control loser, the handling control loser, and the motion control loser ($q = 6.24$, $q = 8.36$, $q = 6.24$, $q = 8.36$, $p < 0.05$). Finally, bystanders in the winner motion control condition lost significantly more fights than the loser condition, the visual control loser, the handling control loser, and the motion control loser ($q = 10.02$, $q = 12.14$, $q = 10.02$, $q = 12.14$, $p < 0.05$).

We found no significant differences in winning between conditions in the winner treatment ($\chi^2 = 2.26$, df = 3, $p > 0.05$; Figure 7). Bystanders in both the fight condition and the visual control won 25% of fights. Crayfish in the handling control won 40% of fights while crayfish in the motion control won 20% of fights. Within the loser treatment, there were also no statistical differences between conditions ($\chi^2 = 1.25$, df = 3, $p > 0.05$; Figure 7). Bystanders in
the fight condition and the handling control won 75% of fights while bystanders in the visual control and the motion control won 85% of fights.

For initiation within the winner treatment, there were no significant differences among conditions ($\chi^2 = 3.81$, df = 3, p > 0.05; Figure 8). Both the fight condition and the handling control bystanders initiated 50% of fights while the visual control condition initiated 30% of fights and the motion control condition initiated 60% of fights. In the loser treatment, there was no overall significant difference between conditions as well ($\chi^2 = 1.80$, df = 3, p > 0.05; Figure 8). Bystanders in both the handling and motion controls initiated 50% of fights while the fight condition initiated 45% of fights and the visual control initiated 65% of fights. When we compared across the winner treatment and the loser treatment, we found no significant differences in initiation ($\chi^2 = 5.10$, df = 7, p > 0.05; Figure 8).

**Fight dynamics**

The percent of fights that reached fight intensity level 4 was significantly different across the winner and loser treatments (Figure 9). Within the winner treatment, the fight condition reached intensity four 85% of the time, the visual control 70%, the handling control 55%, and the motion control 70%. In the loser treatment, the fight condition reached intensity four 40% of the time; and the visual control, handling control, and motion control all reached intensity four 45% of the time ($\chi^2 = 15.06$, df = 7, p < 0.05; Figure 9). The winner fight condition was statistically different from the loser fight condition, loser visual control, loser handling control, the winner handling control, and the loser motion control ($q = 8.36$, $q = 7.49$, $q = 5.76$, $q = 7.49$, $q = 7.49$, p < 0.05). Also the visual control winner was significantly different than the loser fight condition ($q = 5.28$, p < 0.05), the loser visual control ($q = 4.41$, p < 0.05), the loser handling control ($q = 4.41$, p < 0.05), and the loser motion control ($q = 4.41$, p < 0.05). Finally, the motion control
The winner was different from the loser fight condition ($q = 5.28$, $p < 0.05$), the loser visual control ($q = 4.41$, $p < 0.05$), the loser handling control ($q = 4.41$, $p < 0.05$), and the loser motion control ($q = 4.41$, $p < 0.05$). The winner handling control was only statistically different from the winner fight condition ($q = 5.76$, $p < 0.05$; Figure 9).

When we examined the results from the conditions, we did not find any significant differences in time to different intensities in the winner treatment: time to 2 ($F_{3,76} = 0.524$, $p > 0.05$), time to 3 ($F_{3,76} = 0.813$, $p > 0.05$), and time to 4 ($F_{3,49} = 1.97$, $p > 0.05$). The duration of the fight was also not significant within the winner treatment ($F_{3,76} = 0.677$, $p > 0.05$). In the loser treatment, there were no significant differences in time to different intensities: time to 2 ($F_{3,76} = 0.615$, $p > 0.05$), time to 3 ($F_{3,73} = 0.119$, $p > 0.05$), and time to 4 ($F_{3,31} = 0.529$, $p > 0.05$). The duration of the fight in the loser treatment was also not significant between conditions ($F_{3,76} = 0.035$, $p > 0.05$). Across treatments, data was not statistically different in times to different intensities: time to intensity 2 ($F_{3,152} = 0.923$, $p > 0.05$; Figure 10A), time to 3 ($F_{3,149} = 0.364$, $p > 0.05$; Figure 10B), and time to 4 ($F_{3,80} = 0.918$, $p > 0.05$; Figure 10C). Also, data was not statistically different across treatments in duration ($F_{3,152} = 0.388$, $p > 0.05$; Figure 11).

Discussion

Our results match our first prediction; we did not observe any differences in the fighting ability or fight outcome of bystanders in our control conditions (unseen winners and losers) versus our experimental conditions (seen winners and losers). Bystander crayfish were equally as likely to win their next encounter as crayfish that had watched various control conditions in either the winner treatment or the loser treatment (Figure 7). Therefore, previous social history was more influential in determining fight outcome in that previous winners won over bystanders and previous losers lost against bystanders. If a bystander crayfish fought a previous winner, it
was more likely to lose its next encounter. Along the same line, if a bystander crayfish fought a previous loser then it was more likely to win its next encounter. We also found a trend in the maximum intensity of fights observed between the winner and loser treatments. Fight intensity 4 was the most intensive fight level analyzed, and all conditions in the winner treatment, except the handling control, reached intensity 4 significantly more of the time than conditions in the loser treatment (Figure 9). Therefore, fights were more aggressive in the winner treatment than they were in the loser treatment. In summary, social history is a larger determinant of fight outcome than bystander effects and fights within the winner treatment were more aggressive than fights in the loser treatment (Figures 7 and 9).

The outcome of social encounters is a result of the interaction of a number of different factors, such as previous social experience (Daws et al. 2002; Bergman et al. 2003), motivation (Hazlett et al. 1975), neurochemistry (Edwards and Kravitz 1997; Yeh et al. 1997), carapace and chelae size (Dingle 1983; Rutherford et al. 1995), possession of resources (Peeke et al. 1995; Fero et al. 2007), and communication (Bruski and Dunham 1987; Smith and Dunham 1996; Zulanst Schneider et al. 2001). Within crayfish, the effects of previous social history on agonistic behaviors have been documented (i.e. Daws et al. 2002; Bergman et al. 2003). Crayfish given winning experiences are more likely to win in future encounters and losers will continue to lose in future encounters (Chase et al. 1994; Dugatkin 1997; Hsu and Wolf 1999; Hsu and Wolf 2001). Mechanistic studies have strongly indicated that the behavioral effect is most likely connected to changes in the underlying neurochemistry. For example, corticosteroid levels (stress hormones) are often elevated in animals that have lost a fight (Huhman et al. 1991; Schuett and Grober 2000); and losers become passive and cautious after losing experiences (Hsu et al. 2006). In contrast, individuals with increased levels of testosterone are more aggressive and
frequently are dominant over individuals with lower levels of testosterone (Cardwell and Liley 1991; Higby et al. 1991). Differences in the neurochemistry of winners and losers influences their ability to win or lose future encounters and both reverse priming and priming are mediated by changes in neurochemistry (Yeh et al. 1997; Oliveira et al. 2001; Earley et al. 2005).

Reverse priming, considered a stress response, is a down regulation of the physiological mechanisms responsible for mediating social behavior (Earley et al. 2005; Zulandt et al. 2008). The result is that individuals are less aggressive and are more likely to lose in future fights due to changes in neurochemistry (Earley et al. 2005; Zulandt et al. 2008). In a previous study on crayfish, bystanders were more likely to lose fights against naïve males after watching a previous encounter (Zulandt et al. 2008). The authors hypothesized that reverse priming was contributing to fight outcome. By taking the previous study into account, we theorize that reverse priming is only slightly contributing to our results.

It is possible that both previous social history effects and reverse priming effects are operating through the common mechanism of modulation of the physiological basis of aggression. Of the neurotransmitters, serotonin has received the most attention in crustacean systems (Edwards and Kravitz 1997; Huber et al. 1997). The function of serotonin is opposite in invertebrates compared to vertebrates in that having increased levels of serotonin is usually found in conjunction with heightened levels of aggressiveness (Edwards and Kravitz 1997). The pathways of serotonin are different in dominant versus subordinate animals and can thus be modified by social history (Yeh et al. 1997). In addition, reverse priming could be interacting with the function of serotonin and contributing to fight dynamics as reverse priming is thought to down regulate social modulators (Earley et al. 2005). In conclusion, it is possible that the common neural mechanism underlying both social history and reserve priming is either masking
or overriding any effects that visual communication networks have on modifying crayfish aggression.

In addition, it appears that we can exclude the other two elements of the communication network, eavesdropping and priming, from having influenced our results. If visual eavesdropping were to influence crayfish fights, we would expect that bystanders would either behave differently toward seen winners and losers or have different outcomes of the aggressive interactions. Our results show no difference in initiation, winning, or fight dynamics within conditions for the winner and loser treatments. Therefore, we believe that crayfish are not eavesdropping in these experiments or the effects of visual eavesdropping are minor compared to social history effects. Also, priming appears to be unimportant in this study. If crayfish exhibited social priming, we would predict that bystander crayfish would win against both seen winners and losers more than their respective controls. As our results indicate, we did not observe this and therefore, we conclude priming did not occur in these experiments.

This study has shown that fight outcome is more heavily influenced by previous social history than by visual communication networks. This result could arise from a common mechanism of neurochemistry underlying reverse priming and social learning (Yeh et al. 1997). Additional work is needed on the functioning of hormones, biogenic amines, and other physiological modulators in communication networks.

Social information use has been proposed as a broader framework that includes social experience and communication networks (Bonnie and Earley 2007). Even though we did not observe any effect of visual social eavesdropping, which is in contrast to previous studies on fish, crayfish bystanders may require multiple sensory cues, including chemical cues, for eavesdropping to have any effect. Chemical communication is important during crayfish
agonistic encounters as status recognition of opponents is conveyed most likely through urine release (Breithaupt and Eger 2002; Bergman et al. 2005). Although bystanders could not communicate chemically with opponents during the initial fight, they could during the second fight. Bystanders likely detected the social status of their opponent during the second fight and responded accordingly depending on if the opponent was a previous winner or a loser. Studies have shown that when the ability to detect chemical signals has been removed, winner effects were eliminated, indicating that crayfish require chemical information in order to identify the social status of opponents (Bergman et al. 2003). Crayfish are nocturnal, and are found in a wide variety of habitats including turbid environments, which can limit visual communication between opponents. Therefore, bystanders may need both types of information (visual and chemical) in order for any social eavesdropping to be observed. Future studies are needed on chemical communication networks and the role that social history plays in those.
CHAPTER IV
SUMMARY AND CONCLUSIONS

Communication can be defined as an action on the part of one individual that alters the probability pattern of behavior in another individual (Wilson 1975), and this thesis has explored factors that influence how receivers receive and respond to information generated from senders. The first study investigated how exposure to an environmental toxin disrupted chemical communication used during aggressive encounters. In the second study, visual communication within a communication network was investigated and demonstrated that previous social history was a stronger factor regulating crayfish aggression in that the fighting ability of bystanders was not influenced by watching fights. In summary, this research has shown that environmental and social factors have the ability to manipulate information reception and use in aggressive communication.

Chapter 2 specifically addressed the issue of the effects of exposure to sublethal concentrations of metolachlor on olfactory mediated behaviors used during aggressive encounters. This study indicated that exposure to high sublethal concentrations of metolachlor (80 ppb) negatively impacted the ability of crayfish to initiate and to win fights (Chapter 2; Figures 1 and 2). Crayfish are likely to be most susceptible to negative effects from exposure to metolachlor when agricultural run-off is high, such as during high rainfall events that occur during the spring. After exposure, it is unclear if crayfish would be able to recover olfactory abilities. Fish have the potential to recover olfactory receptor cells due to high cellular turnover while crayfish only replace olfactory receptor cells during the molting process (Zeni et al. 1995). Furthermore, metolachlor may be degraded by cytochrome P450 enzymes found in the
hepatopancreas of crayfish; these proteins are responsible for the biotransformation of xenobiotics, including pesticides and endogenous compounds (Snyder 2000). In aquatic habitats, it is likely that all crayfish in a population would be exposed to the adverse effects of metolachlor.

Although only tested on males, metolachlor may impact the ability of females to successfully engage in agonistic bouts, which occur during the breeding season. In the crayfish, Procambarus clarkii, maternal females aggressively defend their shelters from intruding non-maternal females and males (Figler et al. 2005). Shelter use by maternal females is necessary, as burrows provide protection for females while they extrude their eggs and tend to their offspring. Exposure to sublethal concentrations of metolachlor may impair the ability of ovigerous females to defend their shelters against intruders; and therefore, a loss in fecundity may result. The results from chapter 2 indicate that exposure to metolachlor impacts the ability of exposed male crayfish to win agonistic bouts, and dominance hierarchies are formed through repeated agonistic interactions mediated by chemical signals. Therefore, metolachlor has the ability to impact the formation or stability of dominance hierarchies.

The results from Chapter 2 indicate that exposed crayfish become less aggressive (Chapter 2; Figure 1) and less dominant (Chapter 2; Figure 2) than unexposed counterparts possibly due to an inability to send or receive chemical signals. Crayfish dominance hierarchies are formed and maintained in part by chemical communication between individuals. Previous work has shown that contaminants in aquatic environments have differential impacts on fish populations; in some instances aggression increases while in others it decreases, and the social status of individuals change (Henry and Atchison 1979a; Henry and Atchison 1979b; Henry and Atchison 1986; Sloman et al. 2003a; Sloman et al. 2003b). In contaminated aquatic habitats, if
opponents are unable to perceive social status, social rank will change, which may have the ability to affect resource use, population dynamics, and fitness.

There is a need to move towards interdisciplinary toxicology work that combines behavioral studies with research on the effects on physiology in order to create a holistic view of how contaminants influence individual organisms and populations of organisms. In addition, many aquatic pollutants are understudied in both physiological effects and behavioral toxicology (Scott and Sloman 2004). In aquatic habitats, crayfish are likely to be exposed to a combination of chemicals simultaneously, indicating that synergistic effects are going to impact physiology and behavior as well. By examining physiology, behavior, and synergistic effects, ecological health can be determined. Furthermore, crayfish have been noted as being keystone species in their environments, and therefore they are prime candidates for use as biological indicators of ecological health. Crayfish are found in social populations in the field, and the research in Chapter 2 has examined one component of a crayfish population and provided initial data on how a single toxin impacts the ability of male crayfish to become dominant in a dyadic pairing.

Given that agonistic interactions occur in the vicinity of other crayfish in the field (Bergman and Moore 2003) and preliminary research (Zulan dt et al. 2008) has shown that crayfish are negatively impacted by observing fights, the second study (Chapter 3) expanded on visual communication networks by investigating the relative roles that visual communication networks and previous social history had on shaping future fight outcome. Previous social history in the form of winner and loser effects have been well documented in crayfish and contribute to the formation of dominance hierarchies (Goessmann et al. 2000; Daws et al. 2002; Bergman et al. 2003; Gherardi and Daniels 2003), and Chapter 3 has expanded on the role of winner and loser effects in a communication network. The concept of winner and loser effects
can be understood in the context of communication networks because animals that have fought in the past will fight in the future with novel opponents in a broad social environment. The results of the study in Chapter 3 indicate that previous social history is a larger determinant of future fight success rather than aspects of a visual communication network (i.e. eavesdropping, priming, and reverse priming). Crayfish who had won or lost the encounter during the bystander conditioning fight continued to win or lose against bystanders during the experimental fight, regardless of whether bystanders had watched the fight or various control treatments (Chapter 3; Figure 7). In addition, this study has shown that fights within the winner treatment were more aggressive than fights within the loser treatment (Chapter 3; Figure 9). However, when the results of Zulandt et al. (2008) are examined along with the results from Chapter 3, the only communication network effect that contributed to results observed was reverse priming. The study from Zulandt et al. (2008) showed that bystanders who watched fights were unable to win future fights against naïve opponents possibly due to the effects of reverse priming. The study in Chapter 3 added the social history component of aggression and showed that any effects of reverse priming were either minor or were being masked by the effects of previous social history. The ability of bystanders to win fights was not dependent on whether they had watched a fight or various control treatments, but on the social history of the opponent. Finally, this study demonstrates that in the context of a social environment, previous social history and reverse priming share a common mechanism in the physiological modulators of aggression, such as biogenic amines.

Biogenic amines, such as serotonin, control aggression in crayfish and appear to modulate certain aspects of aggression in the broader social environment (Edwards and Kravitz 1997; Oliveira 2005). Crayfish neurochemistry, like androgen levels in fish, can be modified by
the social environment. This has adaptive value for combatants if neurochemistry can be altered during and after aggressive encounters depending on the social context. In other words, winner and loser effects are at least partially under physiological control (Yeh et al. 1997). For instance, heightened levels of serotonin are found in dominant animals or animals with increased aggressiveness, and the behavior of dominant animals works in conjunction with neurochemical alterations, creating a positive feedback loop (Edwards and Kravitz 1997). This phenomenon is observed in fights within the winner treatment as these fights reached fight intensity level 4 more than fights in the loser treatment (Chapter 3; Figure 9). In communication networks, the behavioral outcome of winner effects is similar to the effects of priming; and the behavioral outcome of loser effects is similar to the effects of reverse priming. Chapter 3 suggests that changes in neurochemistry link social history to priming and reverse priming in the broader social environment.

Communication networks and previous social history are two factors operating under a larger scope of social information use and each have underlying mechanisms that contribute to their operation (Bonnie and Earley 2007). Social information use represents a more naturalistic view on how social behavior functions in animal systems as animals exist in a network that encompasses many signalers and receivers with the ability to use a variety of sensory signals and cues. For crayfish, it appears that visual information independent of other sensory information may not provide bystanders with enough or appropriate information about opponents. Information available to bystanders and combatants create additional consequences that would not be found in dyadic interactions, and research is moving in the direction of examining each of these consequences and their influence on the behavior of bystanders (e.g. social eavesdropping, priming, reverse priming), but also direct participants (e.g. audience effect). It will be important
to integrate the effects of communication networks with the other factors that influence aggressive interactions, such as previous social history in order to determine the degree to which each factor regulates aggression. For crayfish, future work is needed on chemical communication networks and also on the interaction between visual communication networks and chemical communication networks.

This thesis has examined the broad scope of communication by specifically investigating how an environmental factor and a social factor influenced the ability of receivers to receive and respond to information in agonistic interactions. The first study provided behavioral toxicology data by showing that exposure to toxins negatively influenced chemical communication at high sublethal concentrations. There is increasing concern over the use of pesticides in aquatic systems and the ability to use crayfish as biological indicators has great potential to provide information on relative stream health. Furthermore, since crayfish are found in social populations in the field with the potential to use visual cues available in communication networks, the second study of this thesis examined two factors that regulate aggression and dominance and determined that previous social history was more important than elements of visual communication networks in determining future fight outcome. Further study is needed on the mechanisms of previous social history and how they shape crayfish aggression. More specifically, more work is needed on the role of serotonin and other biogenic amines and how they function during aggressive interactions when chemical communication is used. Crayfish use a variety of communication channels, including chemical, visual, and mechanical systems for aggressive communication. The results from these studies show that crayfish use information from the chemical and visual systems and the use of information can be modified by environmental and social factors present in the environment.
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Table 1. Crayfish Ethogram Codes (Fight Intensity Levels)

<table>
<thead>
<tr>
<th>Intensity Level</th>
<th>Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>-2</td>
<td>Tail-flip away from opponent or a fast retreat</td>
</tr>
<tr>
<td>-1</td>
<td>Retreat – Slowly back away from opponent</td>
</tr>
<tr>
<td>0</td>
<td>No response or threat display</td>
</tr>
<tr>
<td>1</td>
<td>Approach without a threat display</td>
</tr>
<tr>
<td>2</td>
<td>Approach with threat display – Antennal whip, meral spread, or raised posture</td>
</tr>
<tr>
<td>3</td>
<td>Initial claw use – Boxing, pushing, or touching with closed claws</td>
</tr>
<tr>
<td>4</td>
<td>Active claw use – Grab opponent with claws or hold other crayfish with claw</td>
</tr>
</tbody>
</table>
Figure 1. Percent of experimental animals initiating the agonistic encounter (± 90% confidence intervals). Data were analyzed using the contingency table for comparisons of proportions. Crayfish exposed to 80 ppb were significantly different from crayfish exposed to the control, 60 ppb, and 70 ppb (N = 15, q_{0.05,\infty,4} > 3.63, p < 0.05). Letters indicated significant differences.
Figure 2. Percent of experimental animals winning (± 90% confidence intervals). Letters indicate significant differences. Data were analyzed using the contingency table for comparisons of proportions. Animals exposed to 80 ppb were significantly different from the animals exposed to de-chlorinated tank water, 60 ppb, and 70 ppb (N = 15, $q_{0.05,5,4} > 3.63$, $p < 0.05$).
Figure 3. Time (± SEM) to different intensities. Data were analyzed using a one-way MANOVA followed by a Tukey-HSD post-hoc analysis. There were no significant differences for the time to different intensities (N = 15, F = 1.22, p > 0.05).
Figure 4. Fight duration (± SEM) of the first encounter. Data were analyzed using a one-way MANOVA followed by a Tukey-HSD post-hoc analysis. There were no significant differences (N = 15, F = 1.18, p > 0.05).
Figure 5. Number of encounters during the entire trial time (T = 15 minutes, ± SEM).

Data were analyzed using a one-way MANOVA followed by a Tukey-HSD post-hoc analysis. There were no significant differences (N = 15, F = 1.91, p > 0.05).
Figure 6. The observational learning arena. Two trials were run at once in this set-up. The walls next to the bystander tanks were clear allowing crayfish to be within visual range of each other. The other two walls of the fight arena were opaque. Bystander crayfish were placed in the adjacent boxes while two tester crayfish were placed on opposite sides of an opaque wall in the fight arena. The walls were removed in order to allow tester crayfish to interact.
Figure 7. Percent of fights won by bystanders. Darkened bars represent the results of the bystander engaged with winners and the hatched bars are results from the loser treatment. Columns with different letters indicate significant differences using a chi-square test followed by a contingency table ($\chi^2 = 49.96$, N = 20, df = 7, p < 0.05).
Figure 8. Percent of bystanders initiating the fight. Darkened bars represent the results of the bystander engaged with winners and the hatched bars are results from the loser treatment. Results were not statistically different and data was analyzed using a chi-square test ($\chi^2 = 5.10$, $N = 20$, df = 7, $p > 0.05$).
Figure 9. The percent of fights that reached intensity level 4. Darkened bars represent the results of the bystander engaged with winners and the hatched bars are results from the loser treatment. Columns with different letters indicate significant differences using a chi-square followed by a contingency table ($\chi^2 = 15.06$, $N = 20$, $df = 7$, $p < 0.05$).
Figure 10. Time to fight intensity levels (± SEM). Darkened bars represent the results of the bystander engaged with winners and the hatched bars are results from the loser treatment. Data was analyzed using a two-way MANOVA and was not statistically different for time to intensity 2 (A) ($F = 0.923, N = 20, p > 0.05$); intensity 3 (B) ($F = 0.364, N = 20, p > 0.05$); or intensity 4 (C) ($F = 0.918, N = 20, p > 0.05$).
Figure 11. Duration of the experimental fight (± SEM). Darkened bars represent the results of the bystander engaged with winners and the hatched bars are results from the loser treatment. Data was not significantly different and was analyzed using a two-way MANOVA (F = 0.388, N = 20, p > 0.05).