ABIOTIC AND BIOTIC FACTORS AFFECTING SIZE-DEPENDENT CRAYFISH 
(*ORCONECTES OBSCURUS*) DISTRIBUTION, DENSITY, AND SURVIVAL

A dissertation submitted 
to Kent State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

by

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CHAPTER 1

INTRODUCTION

Due to their large size and aggressive behavior (Renai and Gherardi 2004), crayfish tend to dominate benthic communities (Griffith et al. 1994). Crayfish are often an important driver of community structure, trophic cascades, organic matter processing, and species replacements and have been considered a keystone species in lotic systems (Nyström et al. 1996) and in some cases, ecosystem engineers (Statzner et al. 2003, Creed and Reed 2004). Due to their omnivorous nature (being both predators and shredders, Parkyn et al. 2001), crayfish contribute to many trophic interactions and are a very important component of aquatic food webs (Lodge and Hill 1994, Lodge et al. 1994). Additionally, they play a large role in ecosystem function, particularly relative to their role in detrital dynamics and nutrient cycling (Usio et al. 2001, Usio and Townsend 2001, Montemarano et al. 2007). Thus, given the potential importance of these organisms in stream ecosystems, characterization of spatio-temporal patterns of movement, dispersal, and distribution is necessary to understand the interplay of abiotic and biotic factors influencing crayfish population structure and their role in streams.

Habitat-specificity of crayfish distribution may be enhanced or inhibited by a broad range of abiotic and biotic factors and these effects may be sex- and/or size-dependent (Englund and Krupa 2000, Usio and Townsend 2000, Flinders and Magoulack
2003, 2007a,b, Clark et al. 2008). Abiotic factors including current velocity (Usio and Townsend 2000, Bubb et al. 2004, Parkyn and Collier 2004, Flinders and Magoulick 2007b, Clark et al. 2008), substrate type (Lodge and Hill 1994, Kershner and Lodge 1995, Usio and Townsend 2000, Flinders and Magoulick 2003, 2005, Clark et al. 2008), and water depth (Englund and Krupa 2000, Usio and Townsend 2000, Flinders and Magoulick 2007a,b), and biotic factors including predation risk (Mather and Stein 1993a,b, Englund and Krupa 2000, Usio and Townsend 2000, Light 2003, Magoulick 2004) and competitive interactions (Garvey et al. 1994, Hill and Lodge 1999, Garvey et al. 2003) may all play a role in altering crayfish distributions (see Fig. 1). Additionally, disturbance events such as flooding can also greatly affect crayfish population dynamics (Parkyn and Collier 2004). Although, some abiotic or biotic factors may play stronger roles than others relative to driving crayfish population distribution, the aforementioned abiotic and biotic factors are not mutually exclusive and the combined effects further influence habitat- and size-specific crayfish population dynamics.

**Current Velocity**

Flowing water is a defining characteristic in streams and can play a dominant role in the spatial distribution of crayfish (Parkyn and Collier 2004, Flinders and Magoulick 2005). Although it is difficult to measure an organism’s perception of current velocity, studies suggest that crayfish can withstand a range of velocities with species-specific tolerances (Maude and Williams 1983, Nakata et al. 2003), but are generally most abundant in habitats with slow current velocities (e.g., pools) (Streissl and Hödl, 2002,
Fig 1. Abiotic and biotic factors potentially affecting distribution patterns of the crayfish *Orconectes obscurus* in stream ecosystems.
Crayfish are particularly vulnerable to flow-induced dislodgment and passive entry into the drift primarily because of their blunt body shape (Clark et al. 2008). However, some crayfish can adjust their posture by lowering their body close to the substrate, aligning their chelipeds in front of their body, and/or adjusting their abdomen appropriately to streamline their body (Maude and Williams 1983). Although these behaviors allow crayfish to temporarily resist dislodgment in elevated currents (Maude and Williams 1983), it is likely energetically costly (Mather and Stein 1993a) and may lead crayfish to select habitats with reduced hydraulic stress.

**Sediment Grain Size and Heterogeneity**

Habitat selection by crayfish can be affected by available substrate particle sizes (Flinders and Magoulick 2005, Pöckl and Streissl 2005) and substrate complexity/heterogeneity (e.g., grain sorting, vegetation) (Neveu 2000, Sint and Fureder 2004). In fact, these effects can be size-dependent (Usio and Townsend 2000, Flinders and Magoulick 2005). Most species of crayfish prefer large, rocky substrate and increase in abundance with increasing particle size (Lodge and Hill 1994, Streissl and Hödl 2002, Parkyn and Collier 2004, Pockl and Streissl 2005). However, most studies of this relationship are correlative and the mechanisms driving substrate choice are unclear. Crayfish may select large rocks as refugia from high current velocities and/or predators. However, the combined effect of substrate and current velocity on distribution patterns has not been well tested. Furthermore, although effects of substrate-predation
interactions on crayfish distribution have been extensively tested using crayfish in lentic systems (Garvey et al. 1994, Hill and Lodge 1994, Kershner and Lodge 1995), they have not been well-tested in lotic systems where current velocity is also a factor and habitat characteristics are extremely dynamic.

*Water Depth*

Large crayfish are often positively correlated with deep water (Usio and Townsend 2000) whereas small crayfish are generally negatively correlated with deep water (Flinders and Magoulick 2007b). However, the majority of studies assessing water depth as a factor affecting crayfish distribution have been mostly correlative, and mechanisms underlying this pattern remain unclear. Although crayfish typically display a “bigger crayfish-deeper water” distribution pattern, it is likely a function water depth, and potentially more importantly, predator avoidance (Englund and Krupa 2000, Magoulick 2004). Since large fish predators (and larger conspecific crayfish) are typically found in deep water habitats, small, vulnerable crayfish avoid these habitats to reduce predation risk (Mather and Stein 1993a, Englund and Krupa 2000). Another possible mechanism driving the “bigger crayfish-deeper water” distribution pattern includes water temperature effects as a function of water depth. In warmer, shallower habitats, juvenile survival is low, but growth rate is high (Mundahl and Benton 1990). Juvenile crayfish may prefer shallow habitats due to increased growth allowing individuals to become successful competitors, reach sexual maturity, and decrease predation risk (Lodge and Hill 1994).
**Predation**

In many aquatic systems, community structure is often driven by predation (Sih *et al.* 1985). However, in streams, the role of predation in determining community structure is not well understood (Mather and Stein 1993a). Abiotic variables (i.e., substrate, current velocity), habitat characteristics (i.e., food resources, log jams), and organismal behavior (i.e., predator avoidance, alarm cues) may strongly influence effects of predation on community structure. In fact, these complex interactions may make it difficult to predict predator-prey outcomes. This becomes apparent when reviewing the predation literature from streams. For example, stream predators may reduce prey abundance (Power *et al.* 1985, Mather and Stein 1993a), fail to affect prey abundance (Allan 1982), reduce species-specific abundances (Flecker 1984), or only have effects in certain streams (Wilzbach *et al.* 1986).

Additionally, predator assemblages in stream ecosystems are often very complex. Crayfish are important prey items for many terrestrial (e.g., raccoons *Procyon lotor* L. and great blue herons *Ardea herodias* L.), semi-aquatic (e.g. North American river otters *Lontra canadensis* Schreber), and aquatic predators (e.g., creek chubs *Semotilus atromaculatus* Mitchill, rock bass *Ambloplites rupestris* Rafinesque, and spiny softshell turtles *Apalone spinifera* L.). In turn, crayfish prey upon fish and invertebrates and can be cannibalistic. Thus, with the many trophic linkages associated with crayfish (Lodge and Hill 1994), it is not hard to imagine their role in structuring communities in streams through being both predators and prey.
Crayfish can be affected by predators either directly through consumption or indirectly through behavioral changes such as alteration in activity patterns (Stein and Magnuson 1976, Robinson et al. 2000) or shifts in habitat use (Mather and Stein 1993a, Englund and Krupa 2000). Juveniles, in particular, are especially vulnerable to predators (Stein and Magnuson 1976, Stein 1977, Didonato and Lodge 1993, Kershner and Lodge 1995). Only when a crayfish reaches a size $\geq 20\%$ of a fish predator’s length, does it become invulnerable to predation (Stein 1977). Further, behavioral changes can also occur under high densities of fish predators, with small crayfish demonstrating increased nocturnal activity, complete inactivity, or increased movement out of high predation risk habitats. For example, *Austropotamobius pallipes* (Lereboullet) displays reduced activity during dawn and daytime due to the presence of visual predators, with increasing activity at dusk (Robinson et al. 2000). Furthermore, when smallmouth bass are present, they reduce the activity of juvenile crayfish (Mather and Stein 1993a).

The presence of predators also affects habitat use by crayfish (Englund and Krupa 2000). In lentic systems, use of cobble, a low predation risk habitat, by *Orconectes rusticus* (Girard) was positively correlated with predator density (Kershner and Lodge 1995). Similarly, predator density affects habitat use in Ohio streams as smallmouth bass (*Micropterus dolomieu* Lacépède) cause *O. rusticus* and *O. sanborni* (Faxon) to move from pools into riffles (i.e., habitats that offer reduced predator densities) (Mather and Stein 1993a,b). Habitat shifts by small *Cambarus bartoni* (Fabricius) and *O. putnami* (Faxon) into shallower areas of pools in the presence of predatory fish have also been observed in Kentucky streams, while large crayfish did not respond to predators (Englund
and Krupa 2000). Thus, habitat shifts appear to be size-dependent and driven by predation risk, implying that for larger crayfish, factors other than predation are more important in limiting their distribution.

**Competition**

Both interspecific (Bovbjerg 1970, Garvey et al. 1994) and intraspecific (Bovbjerg 1970, Mather and Stein 1993a) competition may be important factors affecting crayfish habitat choice and distribution patterns. Furthermore, crayfish may engage in exploitative (using crevices as shelter) and/or interference competition (agonistic behaviors) further impacting distribution patterns. Most studies assessing competitive interactions of crayfish examine interspecific interactions that affect replacement of native by invasive crayfish species via competitive exclusion (Garvey et al. 1994, Hill and Lodge 1999, Garvey et al. 2003) or species-specific differences in predation risk (Fortino and Creed 2007). Furthermore, most studies assessing exploitative competition focus on competition for shelter (Bovbjerg 1970, Garvey et al. 1994, Hill and Lodge 1999, Bishop et al. 2008), whereas studies assessing competition for food resources are lacking.

Agonistic behaviors seem to be common for crayfish and they tend to display this behavior when defending shelter or during food acquisition. For example, large crayfish outcompete smaller crayfish for rock crevices (Bovbjerg 1970), with larger conspecifics forcing smaller individuals into shallow water habitats (Mather and Stein 1993a). Although crayfish can be very competitive both interspecifically and intraspecifically,
their competitive interactions with fishes are not well known. Most studies regarding crayfish and fish interactions focus on fishes as predators (Rabeni 1992, Kershner and Lodge 1995, Englund and Krupa 2000) or crayfish as predators on various life stages of fishes. Not only do crayfish consume live fishes, crayfish can be egg predators (Miller et al. 1992, Dorn and Mittelbach 1999) and therefore have the ability to reduce fish reproductive success (Dorn and Mittelbach 1999). In turn, benthic fish competitors (e.g., sculpins *Cottus* spp. and darters *Etheostoma* spp.) can affect foraging success of crayfish (Miller et al. 1992, Stelzer and Lamberti 1999). For example, slimy sculpin (*C. cognatus* Richardson) and crayfish engaged in interference competition, ultimately reducing the number of fish eggs crayfish consumed (Miller et al. 1992). Similarly, the presence of darters can also result in reduced crayfish consumption of benthic invertebrates (Stelzer and Lamberti 1999). In that study, crayfish and darters may have engaged in interference competition, reducing the amount of time crayfish spent foraging (Stelzer and Lamberti 1999). Furthermore, crayfish eat darters (Rahel and Stein 1988, Stelzer and Lamberti 1999) and consumption of darters may have decreased time spent foraging on invertebrate prey (Stelzer and Lamberti 1999). Furthermore, crayfish energetic needs may have been met through feeding on darters, causing decreased consumption of benthic invertebrates by crayfish. Other studies investigating crayfish-fish interactions have focused on how predator-prey interactions induce competitive interactions, focusing on competition as an indirect effect (Rahel and Stein 1988, McNeely et al. 1990) where crayfish tend to outcompete benthic fishes for shelter (Rahel and Stein 1988, Miller et al. 1992).
Flooding

Due to the dynamic nature of streams, organisms are subjected to flood events of varying frequency, duration, and intensity. High velocities resulting from flood events can shift sediments, dislodge invertebrates (including crayfish: Robinson et al. 2000, Clark et al. 2008) and cause their entry into the drift (Maier 2001). While high flows can lead to crayfish mortality (Robinson et al. 2000, Light 2003) and declines in abundance in following years (Light 2003, Parkyn and Collier 2004), moderate flow and periodic floods may only sweep crayfish downstream (Momot 1966, Light 2003).

Oftentimes, crayfish are able to maintain their position within the stream, avoiding entrainment (Robinson et al. 2000). Intense flooding in forested streams has shown no effect on crayfish density when measured three weeks post-flood (Parkyn and Collier 2004). In contrast, work reported in Chapter 4 of this dissertation shows that there is a dramatic effect of flooding on crayfish density when observed at a short post-flood time-scale. While entrainment resulting from flooding can be significant, many lotic organisms also display behavioral adaptations to increased current velocity such as posture changes (Maude and Williams 1983) and movement into areas of decreased current velocity, including lateral floodplains (Ross and Baker 1983), pools and backwaters (Matthews 1986), downstream areas (Williams and Hynes 1976, Horwitz 1978), hyporheic zones (Dole-Olivier et al. 1997), and interstitial spaces (Clark et al. 2008). Behavioral changes such as these may allow for rapid recolonization post-flood and/or resistance to flooding by crayfish.
Organismal Distribution

As noted above, lotic systems, are extremely dynamic and prone to disturbance (i.e., flooding, seasonal drying) causing a frequently changing ‘landscape’ for biota. Furthermore, flow can alter habitat characteristics (i.e., substrate), organism dispersal (active and passive), predation events, competitive interactions, and resource acquisition (Hart and Finelli 1999). In small, headwater streams, which are often intermittent, abiotic factors most likely have the greatest effects on community structure (Creed 2006) and organismal distribution. However, in larger, permanent streams, abiotic conditions tend to be less severe and there tends to be an increase in trophic complexity allowing biotic interactions to also play a role (Creed 2006). Understanding the interplay of abiotic and biotic factors and how their role in affecting organismal distribution change temporally, is important in understanding general population dynamics in streams.

Dissertation Organization

The overall theme of this dissertation focuses on the major abiotic and biotic factors that affect habitat- and size-specific crayfish (*Orconectes obscurus*) distribution and survival in a permanent 4th-order stream. The dissertation is organized into six main research chapters focusing on the effects of seasonality, current velocity, water depth, substrate, predation, and competition in order to tease apart the key drivers of crayfish population dynamics and distribution (see Fig. 1). Chapter 2 focuses on size-dependent effects of the Visible Implant Elastomer (VIE Northwest Marine Technology™) tagging...
system on growth, survival, and tag retention of crayfish. This tagging system is used for mark-recapture purposes in Chapter 4 that assesses the size-specific responses of crayfish to a major flood event. Size-specific crayfish responses to interactions among current velocity, sediment grain size, and grain sorting are examined in Chapter 3. Chapter 5 focuses on the effects of habitat-specific interactions associated with grain size, currently velocity, water depth, and predation risk on size-dependent lotic crayfish distribution. The behavioral responses of crayfish to fish predators and competitors are assessed in Chapter 6. Chapter 7 focuses on habitat overlap and competition for food resources between crayfish and the stream fish assemblage. Last, Chapter 8 represents a brief synthesis of research from Chapters 2-7 to explain the main factors contributing to habitat- and size-specific crayfish distribution and survival.
**References**


CHAPTER 2

SIZE-DEPENDENT EFFECTS OF VISIBLE IMPLANT ELASTOMER MARKING ON CRAYFISH (*ORCONECTES OBSCURUS*) GROWTH, MORTALITY, AND TAG RETENTION

(This chapter was published March 2006 in *Crustaceana*)

Abstract

Many methods have been used to mark crustaceans. However, crustaceans often lose tags during the molting process and some tags may interfere with growth rate, behavior, and mortality of the organism. In this study, four Visible Implant Elastomer (VIE) colors (red, orange, green, and yellow) were used to tag adult [average CL = 25.0 ± 0.6 mm (1SE)] and juvenile [average carapace length (CL) = 13.2 ± 0.6 mm (1SE)] crayfish, *Orconectes obscurus* Hagen, to assess long-term tagging effects on size-specific growth, mortality, and tag retention. The number of molts, average growth per molt, and total growth were not significantly different between tagged and control groups. Additionally, mortality between tagged and control crayfish was not significantly different in adults or juveniles. There was 100% retention of tags and all remained visible until death or the end of the experiments. Although some tag fragmentation
occurred, VIE appears to be an effective tagging technique for adult and juvenile *O. obscurus*.

**Introduction**

Characterization of spatio-temporal patterns of organismal movement, dispersal, and habitat use is an important aspect of population and community ecology (Turchin 1998). In order to evaluate these patterns in the field, populations and/or individuals must be identifiable upon recapture. Mark-recapture techniques using either internal or external tags are commonly used to address these types of questions/objectives.

Many methods of marking and tagging have been used with crayfish, including radio transmitters (Robinson *et al.* 2000, Bubb *et al.* 2002, 2004), microchips (Wiles and Guan 1993), carapace branding (Abrahamsson 1965), color coded rods (Weingartner 1982), pleural clips (Hazlett *et al.* 1979, Light 2003), colored ink (Black 1963), fluorescent pigment (Brandt and Schreck 1975), external plastic tags (Gherardi *et al.* 1998, 2000), uropod, telson, and pleura punches (Guan 1997), coded wire tags (Isley and Eversole 1998), and visible implant alpha tags (Isley and Stockett 2001). Unfortunately, many of these techniques are not practical for use in the field and some require expensive equipment.

Additionally, tagging can be problematic when used with organisms that possess an exoskeleton, such as crustaceans (e.g., crayfish). These organisms go through multiple molts during their lifetime in order to grow and often lose their tags during this process (Brandt and Schreck 1975, Guan 1997; Gherardi *et al.* 1998, 2000, Isely and
Additionally, tags may interfere with organismal behavior (Guan 1997) and movement, and may also influence growth rates (Guan 1997, Brown et al. 2003) and mortality (Brandt and Schreck 1975, Brown et al. 2003). Thus, evaluation of both tag retention and effects of tagging on the study organism is necessary before using a given marking technique.

Visible implant elastomer (VIE, Northwest Marine Technology™) has been used to mark fish, amphibians, reptiles, and crustaceans [including lobster (Homarus gammarus L., Uglem et al. 1996, Linnane and Mercer 1998; Jasus edwardsii Hutton, Woods and James 2003), shrimp (Penaeus vannamei Boone, Godin et al. 1996), freshwater prawns (Macrobrachium rosenbergii De Man, Brown et al. 2003), crayfish (Cherax destructor Clark, Jerry et al. 2001), and blue crab (Callinectes sapidus Rathbun, Davis et al. 2004)] (Table 1). It is a non-toxic compound that cures into a pliable, biocompatible solid. It is injected under translucent or transparent tissue for maximum visibility and small marks can be applied to tag small and large organisms. The product is available in fluorescent and non-fluorescent colors that can be used to individually mark a large number of organisms.

The objective of this laboratory study was to evaluate long-term retention of VIE tags and effects on growth and survival for juvenile and adult crayfish, Orconectes obscurus Hagen, for future use in field experiments. Although VIE has been previously tested in crayfish (Jerry et al. 2001), my study differed in that I assessed two age groups, measured growth effects, and tested multiple colors for visibility. Additionally, my study
Table 1. Studies using VIE in crustaceans (Codes for colors used: R = red, O = orange, G = green, Y = yellow, L = lime, B = blue)

<table>
<thead>
<tr>
<th>Study organism</th>
<th>Life stage</th>
<th>Study period</th>
<th># of molts</th>
<th>Colors used</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Callinectes sapidus</em></td>
<td>juvenile</td>
<td>~7 weeks</td>
<td>not recorded</td>
<td>R, O</td>
</tr>
<tr>
<td>(Davis et al. 2004)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Orconectes obscurus</em></td>
<td>juvenile</td>
<td>6 months</td>
<td>2-5</td>
<td>R, O, G, Y</td>
</tr>
<tr>
<td>(this study)</td>
<td>adult</td>
<td>9 months</td>
<td>0-4</td>
<td>R, O, G, Y</td>
</tr>
<tr>
<td><em>Cherax destructor</em></td>
<td>juvenile</td>
<td>10 weeks</td>
<td>3-5</td>
<td>R</td>
</tr>
<tr>
<td>(Jerry et al. 2001)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Homarus gammarus</em></td>
<td>juvenile</td>
<td>----</td>
<td>3</td>
<td>R</td>
</tr>
<tr>
<td>(Linnane and Mercer 1998)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Homarus gammarus</em></td>
<td>juvenile</td>
<td>~4 months</td>
<td>3</td>
<td>----</td>
</tr>
<tr>
<td>(Uglem et al. 1996)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Machrobrachium rosenbergii</em></td>
<td>larval</td>
<td>70 days</td>
<td>2.41</td>
<td>R</td>
</tr>
<tr>
<td>(Brown et al. 2003)</td>
<td>postlarval</td>
<td>99 days</td>
<td>7.24</td>
<td>R</td>
</tr>
<tr>
<td><em>Penaeus vannamei</em></td>
<td>juvenile</td>
<td>10-14 weeks</td>
<td>17-23</td>
<td>R, O, G, L, B</td>
</tr>
<tr>
<td>(Godin et al. 1996)</td>
<td>adult</td>
<td>10-14 weeks</td>
<td>5-7</td>
<td>R, O, G, L, B</td>
</tr>
<tr>
<td><em>Jasus edwardsii</em></td>
<td>juvenile</td>
<td>6 months</td>
<td>21-22</td>
<td>O</td>
</tr>
<tr>
<td>(Woods and James 2003)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
had a longer duration allowing for a long-term assessment of growth, mortality, and tag retention.

**Methods**

Adult and juvenile *Orconectes obscurus* were collected from the West Branch of the Mahoning River at Jennings’ Woods in Ravenna, Ohio (41°09′41″N 81°11′50″W) on 23 July 2004 and 9 October 2004, respectively. All crayfish were housed in aerated 40 x 28 x 15 cm Rubbermaid® tubs throughout the experiment. Each tub was lined with a layer of gravel and water temperature was maintained at ambient room temperature (~20°C). Two halved polyvinyl chloride pipe sections were also placed in the tubs as shelter for experimental crayfish. During the experiments, crayfish were fed fish food, shrimp pellets, macroinvertebrates, and *Elodea canadensis* Planch, and stream-conditioned red maple (*Acer rubrum* L.) leaves.

For each age group, two crayfish of comparable carapace length (within 0.1-2.5 mm of each other) were assigned to a given tub to minimize aggressive behavior. Male-female combinations were used in each adult treatment tub with the exception of one control group, whereas all juveniles were males. Because of this, different abdominal marking locations were used for juvenile males housed together to distinguish them. Upon arrival in the lab, adults were given a two-day acclimation period while juveniles were given four days before injection of VIE. Four adults and two juveniles were assigned to each VIE color, and an additional four adult and two juvenile crayfish were used as controls.
Experimental procedure

Before injection, initial carapace lengths (CL) of all crayfish were measured with Vernier calipers. Carapace lengths averaged 25.0 ± 0.6 mm (1SE, N = 20) for adults and 13.2 ± 0.6 mm (1SE, N = 10) for juveniles. Adults were injected with VIE on 4 August 2004 and juveniles were injected on 25 October 2004. The colors red, orange, green, and yellow were evaluated for visibility in both age classes. A small amount of VIE was injected into ventral abdominal tissue of each crayfish using a 1cc syringe (Fig. 2). Crayfish were checked four-five times weekly for molts, tag retention, and survival. The study period ended on 25 April 2005 for juveniles (experiment duration = six months) and 5 May 2005 for adults (experiment duration = nine months). Final carapace lengths (mm) were measured following crayfish death or at the end of the experiment. Growth rates were calculated using the average growth rate per molt and total growth was calculated using the difference between initial and final carapace lengths.

Statistical analyses

Student’s t-tests were used to test for differences in the number of molts and growth rates between control and tagged crayfish in each size class. A Chi-square test of goodness of fit was used to test for differences in mortality between control and tagged individuals in each size class. For all statistical analyses, crayfish tagged with different VIE colors were pooled to allow tagged vs. control comparisons.
Fig. 2. Abdominal somite of a male *Orconectes obscurus* tagged with red Visible Implant Elastomer (VIE).
Results

Adult crayfish molted an average of $1.65 \pm 0.26$ (1SE) times (range: 0 to 4 molts per crayfish) during the nine month experiment. Juveniles molted an average of $3.40 \pm 0.34$ (1SE) times (range: 2 to 5 molts per crayfish) during the six month experiment (Table 2). The number of molts between control and tagged crayfish was not significantly different for adults ($P = 0.464$) or juveniles ($P = 0.587$) (Fig. 3A). Three female crayfish (one control, one red-tagged, and one yellow-tagged) did not molt before death and were left out of growth analyses. The average growth per molt was not significantly different between control and tagged adults ($P = 0.055$) or juveniles ($P = 0.578$) (Fig. 3B). The nearly significant result for adults may have been driven by the fact that two out of the three adult control crayfish were female, which generally have slower growth rates than males. Total growth during the experiment was $1.94 \pm 0.43$ (1SE) mm CL for adults and $7.49 \pm 0.87$ (1SE) mm CL for juveniles. Total growth was not significantly different between control and tagged adults ($P = 0.157$) or juveniles ($P = 0.983$).

One week after VIE injection, there was 100% survival for all adult and juvenile crayfish, regardless of treatment (Table 2). Although mortality did occur during the experiments, it was not significantly different between control and tagged individuals for adults ($P = 0.109$) or juveniles ($P = 0.564$). Only three crayfish died within the first month of the adult and juvenile study periods, with one being a control and two being tagged. All three were adult females that did not molt and had black shell disease lesions. All mortalities during the experiments were linked to an outbreak of black shell disease.
Table 2. Mean number (± 1SE) of molts, percent survival, and percent retention of VIE in each treatment and size class of crayfish, *Orconectes obscurus*.

<table>
<thead>
<tr>
<th>Age group</th>
<th>VIE color</th>
<th>Mean number of molts ± 1SE</th>
<th>% survival at 1 week</th>
<th>% survival at 6 mo.</th>
<th>% survival at 9 mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult (N = 4)</td>
<td>None</td>
<td>1.25 ± 0.48</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Juvenile (N = 2)</td>
<td></td>
<td>3.00 ± 1.00</td>
<td>100</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>Adult (N = 4)</td>
<td>Red</td>
<td>1.25 ± 0.48</td>
<td>100</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Juvenile (N = 2)</td>
<td></td>
<td>4.50 ± 0.50</td>
<td>100</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Adult (N = 4)</td>
<td>Orange</td>
<td>1.0</td>
<td>100</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Juvenile (N = 2)</td>
<td></td>
<td>4.0</td>
<td>100</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>Adult (N = 4)</td>
<td>Green</td>
<td>3.00 ± 0.71</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Juvenile (N = 2)</td>
<td></td>
<td>3.00 ± 1.00</td>
<td>100</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Adult (N = 4)</td>
<td>Yellow</td>
<td>1.75 ± 0.63</td>
<td>100</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Juvenile (N = 2)</td>
<td></td>
<td>2.50 ± 0.50</td>
<td>100</td>
<td>50</td>
<td>-</td>
</tr>
</tbody>
</table>
Fig. 3. A) average number of molts; and, B) average growth (mm CL) per molt of control and tagged juvenile and adult *Orconectes obscurus* Hagen. Crayfish tagged with different colors are pooled within age groups. Black bars represent control crayfish and gray bars represent tagged crayfish. Reported statistics are from Student’s *t*-tests.
**Number of molts (±1SE)**

<table>
<thead>
<tr>
<th>Number of Molts</th>
<th>Juveniles</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>N=2</td>
<td>N=8</td>
</tr>
<tr>
<td>1</td>
<td>N=4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>N=16</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
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</tr>
</tbody>
</table>

**Growth (mm CL)/molt (±1SE)**

<table>
<thead>
<tr>
<th>Growth</th>
<th>Juveniles</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td></td>
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</tr>
<tr>
<td>1.0</td>
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<tr>
<td>1.5</td>
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<tr>
<td>2.0</td>
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<tr>
<td>2.5</td>
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<tr>
<td>3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.5</td>
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<td></td>
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</tbody>
</table>

**P-values**

- Juveniles: \( P = 0.587 \)
- Adults: \( P = 0.464 \)

- Juveniles: \( P = 0.578 \)
- Adults: \( P = 0.055 \)
All tags were retained and were visible with and without ultraviolet light at the end of the experiments or at death, regardless of VIE color. While all tags remained visible, in one adult, the elastomer tag moved anteriorly into the thorax approximately 12 days after injection. Tag fragmentation within the abdomen (with the exception of the one crayfish above) occurred in 30% of adult and 75% of juvenile crayfish.

**Discussion**

Overall, VIE appears to be an effective tagging technique for adult and juvenile crayfish. All tags were retained, remained visible, and did not affect growth or mortality in either adults or juveniles. Relative to tag visibility, previous studies have found that VIE remains highly visible (Godin *et al.* 1996, Uglem *et al.* 1996, Jerry *et al.* 2001, Woods and James 2003), but can show reduced visibility over time (Brown *et al.* 2003, Davis *et al.* 2004).

As in this study, previous studies have found that VIE tag retention through molting is very high. In lobsters (juveniles and adults, Linnane and Mercer 1998; juveniles, Uglem *et al.* 1996) and juvenile spiny lobsters (Woods and James 2003), there was 100% retention through three molt cycles and 21-22 molt cycles, respectively. Similarly, shrimp had 99.9% retention in juveniles and 100% in adults, molting 17-23 times and 5-7 times, respectively (Godin *et al.* 1996). While not 100%, tag retention was still high for juvenile crayfish with 92% retention through 3-5 molts (Jerry *et al.* 2001), and for prawns, with 80% retention in larvae and 90% in postlarvae after 70 days (Brown
et al. 2003). In juvenile blue crabs, tag loss was high but was due to loss of the limb associated with the tag rather than loss through the injection site (Davis et al. 2004).

Growth and mortality of adult and juvenile *O. obscurus* were not affected by tagging with VIE. Similarly, growth was not affected by VIE in blue crabs (Davis et al. 2004) or spiny lobsters (Woods and James 2003). However, growth was affected in both larval and postlarval prawns (Brown et al. 2003). As with *O. obscurus*, tagging did not affect mortality in lobsters (Linnane and Mercer 1998), shrimp (Godin et al. 1996), blue crabs (Davis et al. 2004), or postlarval prawns, whereas mortality of tagged larval prawns was significant (Brown et al. 2003).

As noted, VIE tagging is reliable in terms of retention through molting. When used in crayfish abdominal somites, it did not appear to interfere with movement and would not be visible to predators. The tag is small enough that it can be effectively used in juveniles and adults and many colors are available to allow for individual identification. Due to the quickness of mixing and applying VIE, it can be used efficiently with large populations in field situations. VIE is also relatively inexpensive compared to other tagging methods, such as radiotelemetry. In general, VIE tag retention through multiple molt cycles is quite high, unlike fluorescent pigment (Brandt and Schreck 1975), external plastic tags (Gherardi et al. 1998, 2000), uropod, telson, and pleura punches (Guan 1997), and coded wire tags (Isely and Stockett 2001). Finally, it does not appear to negatively influence organismal behavior, movement, growth rate, or mortality.
Disadvantages of VIE are primarily associated with tag fragmentation and migration, which may make individual identification problematic when tagging specific abdominal somites or using color combinations. This result was not surprising given that in other studies that used VIE to tag juvenile crustaceans, tag fragmentation (Linnane and Mercer 1998, Jerry et al. 2001, Woods and James 2003) and migration (Linnane and Mercer 1998, Davis et al. 2004) also occurred. In this study, tag fragmentation was 45% lower in adult crayfish than in juveniles. Therefore, VIE may be a more successful tagging technique for adults than juveniles when individual identification is required. Despite tag fragmentation, the benefits of using VIE as a tagging method outweigh the disadvantages, allowing its use in studies of organismal movement, habitat use, behavior, growth, and mortality.

Acknowledgments

I would like to thank Ben and Laura Leff for their help with adult crayfish collection, Justin Montemarano and Joel Mulder for their help with juvenile crayfish collection and experiment maintenance, and Ferenc de Szalay for use of equipment.
References


CHAPTER 3

GRAIN SIZE AND SORTING EFFECTS ON SIZE-DEPENDENT RESPONSES
BY Lotic Crayfish TO HIGH FLOWS

(This chapter was published May 2008 in *Hydrobiologia* )

Abstract

I examined how sediment grain size and sorting mediate flow-induced dislodgment, movement (i.e., time spent crawling), and postural changes of small [10.5-19.9 mm carapace length (CL)], medium (20-29.9 mm CL), and large (30-42.4 mm CL) crayfish (*Orconectes obscurus*) subjected to incrementally increased flows in a laboratory flume. I also compared size-specific distributions of *O. obscurus* among three habitat types (deep pools, shallow pools, and riffles) with different current velocities and grain properties in a 4th-order stream in northeastern Ohio, USA. Number of crayfish dislodged increased with current velocity and with crayfish body size on all grain sizes (small pebble, large pebbles, small cobbles) and all sorting treatments (well, moderately, poorly sorted) in the flume. Small crayfish were dislodged at significantly higher average current velocities than medium and large crayfish on all grain sizes and sorting treatments, in part because they were small enough to crawl into stone interstices to avoid
high hydraulic stress. Movement by all crayfish decreased as current velocity increased on all grain sizes and sorting treatments. However, activity responses differed in a size-specific fashion. All sizes of crayfish flattened their bodies against grains, flattened or tucked their telson under their abdomen, and attempted to use their chelipeds to grasp grains to resist dislodgment or aid in movement on the grain patch at increased velocities. Field collections showed that small crayfish occupied riffle habitats characterized by faster current velocity and larger grain size, whereas larger crayfish occupied deep and shallow pools characterized by slow current velocity and smaller grain sizes. My results imply that body size is a critical factor in determining crayfish habitat distributions and resistance to high-flows in streams with stony beds.

**Introduction**

Spatiotemporal variation in current velocity and associated hydraulic forces (e.g., shears, turbulence, lift), can greatly affect spatial distributions (e.g., Hart and Finelli 1999, Parkyn and Collier 2004, Flinders and Magoulick 2005) and relative abundances (e.g., Bouckaert and Davis 1998, Kilbane and Holomuzki 2004, Parkyn and Collier 2004) of the stream benthos. Flooding is an obvious source of elevated current velocities, as it can mobilize bed sediments and wash away individual organisms often causing their death (e.g., Giller *et al.* 1991, Dodds *et al.* 1996, Holomuzki and Biggs 1999, Matthaei *et al.* 2000, Robinson *et al.* 2000). However, spatial heterogeneity across channel landscapes creates physical patches that are differentially affected by hydraulic forces, such that some patches act as refugia by mitigating detrimental effects of flow. Flow
Refugia are patches characterized by stable sediments and low hydraulic stress, and where flow-induced losses of benthic organisms are comparatively small (Rempel et al. 1999). Refugia can vary greatly in type and spatial scale. For example, large-scale geomorphic features such as lateral floodplains (Ross and Baker 1983), pools, and backwaters (Matthews 1986), downstream areas (Williams and Hynes 1976, Horwitz 1978), and hyporheic zones (Dole-Olivier et al. 1997), or smaller-scale sediment properties of the streambed, such as grain size (boulders, e.g., Matthaei et al. 1999), arrangement (imbrications, embeddedness, e.g., Biggs et al. 1997, Matthaei et al. 2000), sorting (i.e., range of grain sizes present, Holomuzki and Biggs 2003), and shape (Holomuzki and Biggs 2003), can also mitigate hydraulic stress. Even at baseflow, these geomorphic features and sediment properties may provide suitable flow conditions for species with rather large or blunt bodies that increase form drag and hinder maneuverability in fast currents, or for smaller, immature life-stages of species that are particularly vulnerable to hydraulic stress (DiStefano et al. 2003, Flinders and Magoulick 2005). Thus, in addition to potentially promoting population persistence in flood-prone streams, spatial refugia may also contribute to size-dependent species distributions within streams.

Lotic crayfish occupy a wide range of physical habitats and current velocities (Maude and Williams 1983, Nakata et al. 2003), but are generally most abundant in habitats with slow current velocities (e.g., pools) (Streissl and Hödl 2002, DiStefano et al. 2003, Light 2003, Nakata et al. 2003). Moreover, crayfish abundance is often positively associated with grain size (Lodge and Hill 1994, Streissl and Hödl 2002, Parkyn and Collier 2004, Pockl and Streissl 2005) and grain diversity (Flinders and Magoulick 2003),
suggesting that architectural properties of the grain population may be an important factor influencing within-stream distributions. Because crayfish are large and highly mobile organisms (Momot 1966), they may be particularly vulnerable to flow-induced dislodgment and drift entry. While some crayfish can resist drag by lowering their body close to the substrate, aligning their chelipeds in front of their body, or adjusting their abdomen appropriately to streamline their body (Maude and Williams 1983), they often do so with relatively high energetic costs (Mather and Stein 1993a). It is less clear how different size classes behave when exposed to current velocity and how grain properties mediate size-dependent crayfish responses to high flows. Understanding the effects of flow on crayfish is relevant to understanding benthic community structure, given that crayfish are ecosystem engineers (Nyström et al. 1996) and play a disproportionately important role in affecting community composition and the flow of energy and nutrients in streams (Creed and Reed 2004).

Here, I examine how grain size and sorting affect dislodgment velocities and movement of different sizes of the lotic crayfish, *Orconectes obscurus* (Hagen), subjected to incrementally increased current velocities in a laboratory flume. This crayfish is relatively abundant in rocky streams in southeastern Ontario, New York, Pennsylvania, eastern Ohio, West Virginia, northern Virginia, and western Maryland in North America (Hobbs 1974). I also quantify crayfish size distributions in a northeastern Ohio USA stream and relate these distributions to current velocity and grain size and sorting. I hypothesized that large grains and increased sorting would increase sediment patch stability and hence provide better refugia for all crayfish sizes during elevated flows. I
also predicted smaller, presumably weaker, immature stages that are less able to resist drag would decrease their activity more than larger crayfish during high flows.

Methods

Flume experiments

Grain size effects.—Dislodgment velocities and movement (i.e., crawling activity) of small [10.5-19.9 mm carapace length (CL)], medium (20-29.9 mm CL), and large (30-42.4 mm CL) *O. obscurus* were recorded in a laboratory flume, as used (and drawn) in Kilbane and Holomuzki (2004). The flume (2.3 m long, 0.4 m wide) was constructed of 16-mm plexiglass and recirculated ~360 L of dechlorinated tap water (~19°C) with a 2.2 kW motor in a closed loop. Water first circulated through a plastic collimator (1 x 1-cm mesh) then over a 0.4 x 0.7-m patch of sediment grains. Grains in patches were tightly packed monolayers of either small pebbles (16-32 mm), large pebbles (32-64 mm), or small cobbles (64-128 mm).

Both sediment grains and male *O. obscurus* were collected from the West Branch of the Mahoning River, a 4th-order stream in Ravenna, Portage County, Ohio (41°09′41″N 81°11′50″W), during September-December 2004 and March-May 2005. Grains were picked free of macroinvertebrates, scrubbed thoroughly to reduce attached biofilm, and separated into size categories based on the Wentworth scale (Wentworth 1922). Only male crayfish were used so as to minimize confounding effects of gender size differences. Individual crayfish (separated by size class) were temporarily kept in
aerated 40 x 28 x 15 cm plastic tubs filled with dechlorinated tap water (~20°C) and lined with a layer of gravel. Experimental trials began within 48 h of crayfish collection. For each trial, a single crayfish was placed in the middle of the flume’s grain patch and given 20 min to acclimate to a current velocity of 0.1 m/s (measured 1 cm above patch). A single crayfish was used per trial to avoid any inter-individual effects (e.g., aggressive behavior) on movement or dislodgment. Current velocity, as controlled by a rheostat, was then increased by 0.1 m/s increments at 5-minute intervals until the crayfish was dislodged from the grain patch or a maximum current speed of 1.5 m/s was reached. During each 0.1 m/s velocity interval, the amount of time (s) each crayfish was active (i.e., crawling or climbing) was recorded with a stopwatch. I also recorded direction of crayfish movement (i.e., upstream, downstream, and across stream) and burrowing activity, as well as the number of grains entrained during each velocity interval. Foraging was not observed during any of the trials. While the size class of each crayfish was known before each trial, actual crayfish size (CL) was measured using Vernier calipers following each trial (to minimize pre-trial handling). A different crayfish was used for each trial and entrained stones were replaced. Eight replicates were run for each crayfish size class on each of the three grain size treatments resulting in a total of 72 trials.

Grain sorting effects.—Crayfish movement and dislodgment velocity were examined using the same procedure as described before, except grain patches contained either well, moderately, or poorly sorted grains. The well sorted treatment consisted of 100% small pebbles [sorting index (s.i.) = < 0.5 (Gordon et al. 2004)], the moderately sorted
treatment was approximately half small pebbles and half large pebbles (s.i. = 0.5-1.00), and the poorly sorted treatment was a third small pebbles, a third large pebbles, and a third small cobbles (s.i. > 1.00). These fractions were based on the weight of each grain size category, where the cumulative weight of each grain size category within the sorting treatment was equal. Before grains were added to the flume, all sediment grains within a given sorting treatment were placed in a box and shaken to thoroughly mix them. Eight trials were run for each of the three crayfish size classes in each sorting treatment (total \( n = 72 \)).

Statistical analyses.—I used the Cox Proportional Hazard Model (Cox 1972) to compare dislodgment profiles of the different crayfish size classes for each grain size and sorting treatment. As noted in previous studies (e.g., Englund and Krupa 2000), this model not only uses actual losses from the population (in my case, dislodgments), but also uses data from “censored observations.” Censored observations for my study included crayfish that were not dislodged during the velocity range (\( \leq 1.5 \text{ m/s} \)) used in these experiments. Regression slopes for number of grains entrained over current speeds from 0.6 m/s to 1.5 m/s were used in separate one-way ANOVAs to compare bed movement among grain treatments (SAS 8.01, SAS Institute Inc., 2000). To analyze the effects of current velocity on crayfish size-specific activity levels for each grain size and sorting treatment, and take into account that less active crayfish tend to dislodge at higher velocities, I used a repeated measures linear mixed model followed by Tukey’s post-hoc tests (SAS 8.01, SAS Institute Inc., 2000). For the mixed model, hypothesis testing was done using the
AIIC criterion; and chosen covariance structures were heterogeneous toeplitz (toeph) for grain size data and toeplitz (toep) for grain sorting data.

*Crayfish size distributions in the field*

*Crayfish size, current velocity, and grain characteristics.—* I assessed size-specific crayfish distributions in four deep pools (water depth ≥ 51 cm), four shallow pools (maximum water depth = 50 cm), and four riffles (maximum water depth = 12 cm with noticeable breaks in the surface water) (12 sample habitats total) in the West Branch of the Mahoning River from 8 June to 20 July 2005 to compare with results of the flume experiments. Sample habitats (ea. ≤ 10 m long) were enclosed by upstream and downstream block nets, and crayfish were collected using a four-pass removal method (Zippin 1958) with a backpack electroshocker. Electroshocking can be an effective technique to sample crayfish populations (Rabeni et al. 1997) and this method met the assumptions (Zippin 1958) required for population estimation. Many studies have successfully sampled crayfish using electroshocking in combination with pass removal methods (Usio and Townsend 2000; Hicks 2003; Seiler and Turner 2004). During each pass, crayfish were collected by two netters walking upstream in a zig-zag pattern. All crayfish collected during each pass were counted and measured (CL, mm) using Vernier calipers. Lengths and widths of each sample habitat were measured in order to calculate an area (m$^2$) measurement to be used in density estimation. To determine grain characteristics in each sample habitat, a Wolman pebble count was done using a random
transect method (Wolman 1954). The b-axis (i.e., grain width) of 100 grains was measured in each habitat (except one where 50 grains were measured) with Vernier calipers. For sand, the b-axis was assumed to be 1 mm, while clay was assigned a diameter of 0.001 mm. Nearbed (1 cm above sediments) current velocity in each sample habitat was measured during baseflow conditions using a Marsh-McBirney® flow meter at fifteen randomly selected points.

Statistical analyses.— To calculate the average grain size and sorting index for each sample habitat, measured grains were separated into size classes in 1Φ intervals (Φ = - log₂D; D = b-axis in mm) (Moore 1999). A fractional grain size curve, which gives the proportion of grains within each size class, was obtained by dividing the number of grains in each size class by the total number of grains (Moore 1999). Average grain size [graphical mean (MZ); MZ = (Φ₁₆ + Φ₅₀ + Φ₈₄)/3; e.g., average of the median and ± 1 standard deviation from the median] and sorting index [graphical standard deviation (σ₁); σ₁ = (Φ₁₆ − Φ₈₄/4) + (Φ₅ − Φ₉₅/6.6); e.g., the average distance between ± 1σ and 2σ] (Folk and Ward 1957) were calculated from each fractional grain size curve.

To calculate crayfish density, a depletion method was used (Zippin 1958); this method allows density to be estimated by plotting cumulative total catch against catch per unit effort (CPE), fitting a regression line through the points, and solving for CPE = 0 to determine the population estimate for each sample habitat. The population estimate was then divided by the area calculated for each sample habitat. To calculate a density estimate for each size class within each sample habitat, the total density of crayfish for a
given channel unit was multiplied by the proportion of crayfish captured within each size class for a given sample area. Habitat- and crayfish size-specific densities were analyzed using two-way full factorial ANOVA and Tukey’s *post-hoc* tests (*α* = 0.05) (SAS 8.01, SAS Institute Inc., 2000). Habitat-specific differences in average grain size, sorting indices, and crayfish CL were analyzed using one-way ANOVA and Tukey’s *post-hoc* tests with *α* = 0.05 (SAS 8.01, SAS Institute Inc., 2000). Finally, the non-parametric two-dimensional Kolmogorov-Smirnov test (2-DKS) (Garvey *et al.* 1998) was used to compare crayfish size (CL) to current velocity, average grain size, and sorting index from each sample habitat. The 2-DKS is used to detect relationships in continuous bivariate data (Garvey *et al.* 1998).

**Results**

Flume experiments

*Grain size effects.*—Dislodgment profiles significantly varied with crayfish size on all three grain size treatments (Cox tests: small pebbles $\chi^2 = 4.45$, *P* = 0.035; large pebbles $\chi^2 = 6.76$, *P* = 0.029; small cobbles $\chi^2 = 6.76$, *P* = 0.008), with small crayfish having the lowest rates of dislodgment (Fig. 4a-c). In fact, 75% of all small crayfish were able to resist dislodgment at the maximum current velocity (1.5 m/s) on small cobbles.

Behaviorally, all crayfish flattened their bodies against grains, flattened or tucked their telson under their abdomen, and attempted to use their chelipeds to grasp grains to
Fig. 4. Number of crayfish dislodged at each current velocity (m/s) interval on (a) small pebbles, (b) large pebbles, (c) small cobbles, (d) well, (e) moderately, and (f) poorly sorted grain treatments. Solid circles = small crayfish; gray upside-down triangles = medium crayfish; open squares = large crayfish.
resist dislodgment or aid in movement at increased velocities. However, all small crayfish moved into stone interstitial spaces at velocities >0.3 m/s, particularly on large pebbles and small cobbles. Larger crayfish were unable to fit between small and large pebbles, and only four of eight medium, and one of eight large crayfish, were able to squeeze under small cobbles.

Overall, activity level decreased significantly during velocity rampings (mixed model, $F_{1,85} = 57.95, P < 0.0001$) and differed significantly among size classes (mixed model, $F_{2,95} = 59.09, P < 0.0001$, Fig. 5a-c). The interaction between current velocity and crayfish size was also significant (mixed model, $F_{2,78} = 25.00, P < 0.0001$). In general, activity of all size classes decreased as current velocity increased, but pronounced reductions in activity were particularly evident between ~0.4 to 0.8 m/s in all grain-size treatments (Fig. 5a-c). However, small crayfish had slowest declines in activity with increasing velocity (Tukey’s test, $P < 0.05$) while medium crayfish had the highest activity (Tukey’s test, $P < 0.05$) when grain sizes were grouped. Regardless of body size, 65% of all crayfish generally moved in an upstream direction as current speed increased. Crayfish flattened their bodies, brought their abdomen close to the grain patch, and used controlled telson movements to maintain upstream movement. These postural changes were not displayed by crayfish in a downstream direction.

Coincidentally, crayfish were dislodged 2X more frequently when moving downstream than upstream. Overall, the main effect of grain size on activity level was not significant (mixed model, $F_{2,94} = 2.70, P = 0.0726$, Fig. 5a-c). Additionally, the interaction between
Fig. 5. Average activity time (s) (mean ± 1 SE) of crayfish during each 5-minute current velocity (m/s) interval on (a) small pebbles, (b) large pebbles, (c) small cobbles, (d) well, (e) moderately, and (f) poorly sorted grain treatments.
crayfish size and grain size was not significant (mixed model, $F_{4,94} = 1.70$, $P = 0.1569$). However, interactions of current velocity and grain size (mixed model, $F_{2,82} = 4.59$, $P = 0.0128$) and current velocity, crayfish size, and grain size (mixed model, $F_{4,81} = 2.91$, $P = 0.0264$) were significant.

In no trials did large pebbles or small cobbles become entrained. Some small pebbles (average $= 0.75 \pm 0.63$ grains per current velocity interval) did entrain, but degree of their entrainment did not differ among treatments (ANOVA, $F_{2,34} = 1.67$, $P = 0.204$).

**Grain sorting effects.**—Dislodgment profiles varied with crayfish size in all three sorting treatments (Cox tests: well sorted $\chi^2 = 4.45$, $P = 0.039$; moderately sorted $\chi^2 = 4.99$, $P = 0.026$; poorly sorted $\chi^2 = 7.20$, $P = 0.007$), with small crayfish having the lowest rates of dislodgment (Fig. 4d-f). Regardless of treatment, maximum current velocities at which crayfish were able to resist dislodgment were higher for small crayfish (up to 1.5 m/s) than for medium and large crayfish (up to 1.0 m/s) (Fig. 4d-f).

Crayfish activity depended on current velocity (mixed model, $F_{1,124} = 70.22$, $P < 0.0001$) and differed significantly among crayfish size classes in all sorting treatments (mixed model, $F_{2,138} = 17.84$, $P < 0.0001$; Fig. 5d-f). Additionally, the interaction between current velocity and crayfish size was significant (mixed model, $F_{2,123} = 8.45$, $P = 0.0004$). Only small and medium crayfish showed significant differences in activity level (Tukey’s test, $P > 0.05$) with activity declining more slowly for small crayfish when grouped by grain size (Fig. 5d-f). Overall, the main effect of grain sorting did not
significantly affect crayfish activity levels (mixed model, $F_{2,143} = 1.24$, $P = 0.2934$) and the interaction between current velocity and grain sorting was not significant (mixed model, $F_{2,123} = 1.96$, $P = 0.1456$; Fig. 5d-f). Additionally, interactions of crayfish size and grain sorting (mixed model, $F_{4,140} = 0.50$, $P = 0.7343$) and current velocity, crayfish size, and grain sorting (mixed model, $F_{4,123} = 0.82$, $P = 0.5169$) were not significant.

The same behaviors used to withstand ramped flows in grain size treatments (i.e., flattening body or telson, use of interstitial space) were also observed in the different sorting treatments. Similarly, grains became increasingly more unstable and entrained as current velocity increased [well sorted, $0.75 \pm 0.63$ grains; moderately sorted, $3.46 \pm 2.67$ grains; poorly sorted, $4.08 \pm 2.03$ grains; all values represent average number of grains entrained per current velocity interval ($\pm 1$ SE)] but no statistical difference was detected among sorting treatments (ANOVA, $F_{2,23} = 2.67$, $P = 0.091$).

**Crayfish size distributions in the field**

_Crayfish size, current velocity, and grain characteristics._—Average crayfish size generally decreased with increasing current velocity, but not significantly (2-DKS, $P = 0.077$; Fig. 6a). Average grain size differed among habitat types (ANOVA, $F_{2,9} = 8.98$, $P = 0.0072$) being significantly larger in shallow pools [$31.93 \pm 9.99$ mm (mean $\pm 1$ SE)] and riffles ($37.83 \pm 2.06$ mm) than in deep pools ($4.56 \pm 1.08$ mm) (Tukey’s test, $P < 0.05$). Similarly, sorting indices differed by habitat type (ANOVA, $F_{2,9} = 20.12$, $P = 0.091$).
Fig. 6  Average crayfish carapace length (CL, mm) (mean ± 1 SE) at each average a) current velocity (m/s), b) grain size (mm), and c) sorting index (phi) in each sample habitat from the West Branch of the Mahoning River, Ravenna, Ohio, USA.
0.0005) being significantly higher in deep pools (2.52 ± 0.13 \( \phi \)) than in either shallow pools (1.30 ± 0.31 \( \phi \)) or riffles (0.87 ± 0.02 \( \phi \)) (Tukey’s test, \( P < 0.05 \)). Deep pools had the highest grain sorting (i.e., habitat heterogeneity) because of the presence of a wide range of grain sizes (e.g., clay to boulders). Nearbed velocities were < 1 cm/s in deep pools, 1.0 ± 0.05 cm/s (mean ± 1 SE) in shallow pools, and 23.0 ± 0.21 cm/s in riffles.

Average crayfish size (CL) differed significantly among habitats (ANOVA, \( F_{2,969} = 30.36, P < 0.0001 \)) being greater in deep pools [29.0 ± 0.58 mm CL (mean ± 1 SE)] and shallow pools (24.3 ± 0.24 mm CL) than in riffles (22.9 ± 0.33 mm CL) (Tukey’s test, \( P < 0.05 \)). Crayfish showed a corresponding decrease in CL with increasing average grain size (2-DKS, \( P = 0.017 \); Fig. 6b) and an increase in CL as sorting increased (2-DKS, \( P = 0.022 \); Fig. 6c). Similarly, crayfish density differed significantly among habitat type (ANOVA, \( F_{2,9} = 9.51, P = 0.0007 \)) and crayfish size (ANOVA, \( F_{2,9} = 11.86, P = 0.0002 \)) with a significant interaction (ANOVA, \( F_{2,9} = 3.70, P = 0.0159 \)). Overall, densities were significantly higher in shallow pools than in deep pools and riffles (Tukey’s test, \( P < 0.05 \)). Additionally, medium crayfish [deep pools = 0.32 ± 0.03 crayfish/m\(^2\) (mean ± 1 SE), shallow pools = 1.76 ± 0.54 crayfish/m\(^2\), riffles = 0.63 ± 0.11 crayfish/m\(^2\)] had significantly higher densities (Tukey’s test, \( P < 0.05 \)) than small (deep pools = 0.03 ± 0.01 crayfish/m\(^2\), shallow pools = 0.44 ± 0.11 crayfish/m\(^2\), riffles 0.17 ± 0.02) and large (deep pools = 0.34 ± 0.16 crayfish/m\(^2\), shallow pools = 0.38 ± 0.05 crayfish/m\(^2\), riffles 0.03 ± 0.02 crayfish/m\(^2\)) in all habitat types (Tukey’s test, \( P < 0.05 \)). However, there
were no statistical differences between small and large crayfish or between habitat types (Tukey’s test, $P > 0.05$).

**Discussion**

*Crayfish responses to elevated flows in the flume*

My experiments showed that small crayfish had lower rates of dislodgment and higher dislodgment velocities than medium and large crayfish on all grain size and sorting treatments. Small crayfish were able to crawl between pebbles and small cobbles, whereas most medium and large crayfish were too big to use these interstitial spaces. When grain spacing is relatively close, as in all of my grain treatments, flow skims across grain crests instead of between grains, resulting in slow, less-turbulent flows and stable eddies between stones (Davis and Barmuta 1989, Bouckaert and Davis 1998). Small crayfish occupying interstitial spaces in my treatments likely encountered less hydraulic stress (e.g., turbulence, shear forces) than did larger crayfish that had a relatively large proportion of their bodies exposed to flows in the water column. Thus, the relatively high dislodgment rates and low dislodgment velocities of medium and large crayfish probably resulted, in part, from a blunt body shape that increased form drag. Moore (1964) similarly found dislodgment velocities to be higher for small snails than medium- and large-sized snails. Further, Holomuzki and Biggs (2003) reported that cased caddisflies (*Pycnocentrodes*) were more prone to dislodgment than dorsoventrally flattened mayflies and cone-shaped gastropods because caddisflies tended to remain
epibenthic during ramped flows whereas the other two taxa used deeper, more protective substrates. Shear forces can be quite high on exposed surfaces during high flow conditions and are likely a significant contributor to macroinvertebrate dislodgment (Borchardt 1993).

Dislodgment velocities of medium and large crayfish on stone surfaces in my experiments were ~0.6 to 1.0 m/s compared to average dislodgment velocities of ~0.3 m/s by similarly-sized *O. obscurus* on exposed, roughened plexiglass (Maude and Williams 1983). This contrast further emphasizes the importance of substrate architecture as a mediator of high-flow effects. In addition, an array of behaviors appears to be important in enabling crayfish to resist high flow forces. Unlike some benthic invertebrates, crayfish do not have morphological adaptations that reduce drag forces and decrease the risk of flow-induced dislodgment and drift. I observed all sizes of crayfish flattening their bodies against grains, flattening or tucking their telson under their abdomen, and attempting to use their chelipeds to grasp grains to resist dislodgment or aid in movement on the grain patch at increased velocities. Maude and Williams (1983) similarly observed crayfish altering body posture to streamline their bodies to apparently minimize drag forces at high current velocities. In addition, I also observed crayfish to decrease their overall movements during elevated flows. A few other studies have similarly reported benthic macroinvertebrates to decrease their movement during high flows [snails (Holomuzki and Biggs 2006), caddisflies (Lancaster *et al*. 2006)]. However, direction of movement may mediate these activity responses. I observed that movement in the upstream direction seemed to be more controlled than movement
downstream. Most crayfish moved in an upstream direction (against the current) in the flume, moving through interstitial spaces, flattening their bodies against the grain patch, and controlling movement with their telson. Crayfish that moved downstream were often dislodged even at lower current speeds. However, at very slow flows, when risk of dislodgment is low, crayfish movement appears to be similar between upstream and downstream directions (Robinson et al. 2000).

Although the relationship between grain size and stability in ramped flows is not completely straightforward (Duncan et al. 1999), stability typically increases with increased grain size (e.g., sand < small pebbles < small cobble) (Gordon et al. 2004). Likewise, in my experiment, large pebbles and small cobbles were more stable than small pebbles. Similarly, more grains were transported in my moderately and poorly sorted treatments than in the well sorted treatment. In contrast, Holomuzki and Biggs (2003) reported that poorly sorted mixtures of gravels and cobbles were more stable than well sorted, spherical gravels or cobbles. Thus, sorting effects on grain stability are likely closely related to an assortment of grain features, including shape (e.g., angularity), packing, and embeddedness. In addition, poor grain sorting can increase grain patch topography (i.e., variation of grain height), which may increase turbulence (Davis and Barmuta 1989), and the risk of dislodgment (Lancaster et al. 2006). In my sorting treatments, stone heights in moderately and poorly sorted treatments were very uneven compared to the monolayers of single grain sizes in the well sorted treatment. However, risk of dislodgment due to added turbulence from uneven bed elements appears to depend on animal size, as suggested by my result of small crayfish having their lowest
dislodgment rate on poorly sorted grains. In turn, stone height in my moderately and poorly sorted treatments could have reduced the risk of dislodgment if sediment grains were large enough to shelter crayfish.

*Crayfish size distributions in relation to grain characteristics in the field*

Small *O. obscurus* tended to occupy riffle habitats characterized by faster current velocities and larger grain sizes, whereas large crayfish were typically found in shallow and deep pools characterized by slow current velocities and small (sand) grains. However, crayfish density did not show a clear pattern related to current velocity or grain characteristics. Field patterns also showed an increase in body size with increased grain sorting. These patterns indicate that habitat heterogeneity may be an important factor in structuring crayfish size-specific distribution. However, while the distribution of small crayfish was positively correlated with refugia size (e.g., sediment grain size), it was not correlated with habitat complexity (e.g., sediment grain sorting), likely due to small crayfish presence in areas with low grain sorting values and large sediment grain sizes. Similarly, distributions of young of year (YOY) *Paranephrops zealandicus* (White) are positively correlated to coarse grain abundance (i.e., % bedrock, cobble, and boulder), whereas adult crayfish are associated with sandy grains and negatively correlated with current velocity (Usio and Townsend 2000). Results from my flume experiments help provide causal mechanisms for these field patterns. Small size may improve a crayfish’s ability to reside and function in fast current because small crayfish are better able to use the large interstitial spaces between the relatively large, poorly sorted stones in these
habitats. Even at baseflow conditions, the current velocity of riffles is high enough at many locations to cause dislodgment of medium and large size crayfish based on results from the flume experiment. During high flows (i.e., flood events in streams), smaller body size may be an advantage to resisting dislodgment, especially when larger grain sizes are present. Small and medium *O. obscurus* can occupy riffles during floods even when current velocity exceeds 1 m/s (Clark and Kershner, unpublished data). However, large crayfish must likely find another means of refugia, such as burrowing into the sediment, taking shelter behind or near large substrate, or moving to habitats with reduced hydraulic stress (i.e., backwaters).

Crayfish abundance is often positively correlated with increasing grain size (Lodge and Hill 1994, Streissl and Hödl 2002, Parkyn and Collier 2004, Pockl and Streissl 2005) and both current velocity and substrate diversity can be important factors affecting crayfish densities and distributions in natural habitats (Payne 1984, Flinders and Magoulick 2003). However, other factors such as predation (Stein 1977, Mather and Stein 1993a,b, Lodge and Hill 1994, Kershner and Lodge 1995, Englund and Krupa 2000, Usio and Townsend 2000, Garvey *et al.* 2003), water depth (Creed 1994, Englund and Krupa 2000, Flinders and Magoulick 2007a,b), and competition (Lodge and Hill 1994, Garvey *et al.* 2003) may also structure size-dependent habitat use by crayfish. For example, fish predators can negatively affect crayfish population structure (Usio and Townsend 2000), causing crayfish to shift into habitats with low predation risk (Mather and Stein 1993a,b). Often, this response is size-dependent, with small crayfish shifting into shallow water habitats and larger crayfish showing no response to predatory fish
(Englund and Krupa 2000). However, terrestrial predators [i.e., raccoons (*Procyon lotor*, L.) and Great blue herons (*Ardea herodias*, L.)] may further influence crayfish distributions in shallow areas of the stream (i.e., shallow pools and riffles) (Englund and Krupa 2000). A variety of aquatic and terrestrial predators occur in my study system, potentially further structuring crayfish populations. Additionally, water depth is often strongly correlated with predation risk of crayfish with small crayfish having higher risk in deeper water (Englund and Krupa 2000, Flinders and Magoullick 2007b) and may be more important than current velocity in structuring crayfish size distributions in some habitat types.

Intraspecific (Lodge and Hill 1994) and interspecific (Lodge and Hill 1994, Garvey *et al.* 2003) competition may also influence size distributions of crayfish subpopulations. For example, small, juvenile crayfish are often competitively excluded from preferred, slower-flowing habitats by aggressive adults (Lodge and Hill, 1994). In lakes, invasive *O. rusticus* and *O. propinquus* (Girard) dominate cobble habitats displacing native *O. virilis* (Hagen), to less favorable macrophyte habitat (Garvey *et al.*, 2003). In my study stream, other crayfish species are absent but there is potential for interspecific competition with fishes (i.e., central mottled sculpin, *Cottus Bairdi*, Girard) in shallow pool and riffle habitats, which could also affect size-specific distributions and potentially lead to increased dislodgment of crayfish. However, my data suggest that current speed and grain properties are also important determinants of crayfish size distributions.
My study suggests that sediment architecture and crayfish body size interact to mediate high flow effects on lotic crayfish. Specifically, small body size facilitates burrowing between stones to decrease the risk of flow-induced dislodgment and drift. All sizes of crayfish also reduced their activity and made postural changes to resist dislodgment in ramped flows. Given that stream flooding can cause considerable mortality in lotic crayfish (Robinson et al., 2000), these behaviors and the use of stony riffles by small crayfish appear critical in facilitating population persistence in flood-prone systems.

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References


CHAPTER 4

SHORT- AND LONG-TERM IMPACTS OF A MAJOR FLOOD EVENT ON CRAYFISH (*ORCONETES OBSCURUS*) IN A FORESTED STREAM

(To be submitted to the *Journal of the North American Benthological Society*)

Abstract

Due to their large, blunt bodies, crayfish are particularly vulnerable to dislodgment at high flows associated with flood events. However, little is known about their resistance/resilience to intense spates. In this study, I examined short- and long-term size-dependent recovery by a lotic crayfish (*Orconectes obscurus*) population following a high discharge flood event associated with Hurricane Katrina (2005). Short-term population recovery was assessed using mark-recapture sampling in a riffle-shallow pool-riffle sequence. Crayfish were marked with Visible Implant Elastomer tags the morning of the rainstorm and recapture attempts occurred 2, 8, 10, and 16 days post-flood. Riffles returned to pre-flood densities within 10-16 days, however, the shallow pool did not. Recovery was size-dependent with small crayfish returning to pre-flood densities within 16 days, while large crayfish did not recover during this time interval. Although crayfish showed short-term effects, this flood event had limited impact on
crayfish densities on a longer time scale (assessed using seasonal density estimates collected on a reach scale). For example, Summer 2005 (~ 2 months pre-flood) and Summer 2006 (9 months post-flood) densities were not statistically different from each other for either small or large crayfish. Small crayfish resilience/resistance may result from movement into areas of lower hydraulic stress to avoid high current velocities, whereas large crayfish may require a complete growing season for recovery. Resistance to flooding by small crayfish may be the key component to long-term population persistence of crayfish populations in flood-prone streams.

**Introduction**

In lotic systems, disturbance by flooding can play a dominant role in determining organismal community structure (Resh *et al.* 1988, Palmer *et al.* 1996, Matthaei and Townsend 2000) and the relative abundance and spatial distribution of individual species (Holomuzki and Biggs 2000, 2003, Kilbane and Holomuzki 2004, Parkyn and Collier 2004). Given that flood events are highly variable with respect to duration, frequency, and intensity, the effects of flooding on the lotic community, stream substrate, and the stream channel hydrology can be very complex. Floods can mobilize bed sediments and dislodge individual organisms often causing their death (e.g., Giller *et al.*, 1991; Dodds *et al.*, 1996; Holomuzki and Biggs, 1999; Matthaei *et al.*, 2000; Robinson *et al.*, 2000). Flooding effects on bed sediments alone can be quite complex, with floods causing scour in some areas, fill in others, and some areas showing no sediment effects (Matthaei *et al.*
Additionally, some flood events alter channel geomorphology (Leopold et al. 1964), potentially having dramatic effects on organism distribution.

In order for organisms to persist in flood-prone streams, they must be resistant and/or resilient to hydraulic stress. It is expected that lotic systems experiencing frequent flooding disturbance will contain biota with high resilience and rapid recolonization abilities (Reice et al. 1990). Typically, these organisms possess morphological, physiological, and/or behavioral adaptations to high flow, which determine the flooding severity and frequency that they can tolerate (Holomuzki and Biggs 2000). Organisms with streamlined bodies, high immigration rates, high vagility, and multivoltine life cycles can tolerate frequent, high velocity flood events. In contrast, organisms with large, blunt bodies, low immigration rates, low vagility, and univoltine life cycles are more strongly associated with systems with infrequent, lower velocity flood events (Townsend and Hildrew 1994, Townsend et al. 1997).

In addition to an organism’s morphology, body size is of key importance when considering effects of flooding on flow-induced dislodgment and population persistence. Ultimately, morphology and body size determine how shear stress affects drag and lift forces on a given individual (Stazner and Holm 1982, 1989) with larger individuals experiencing greater drag and lift forces than smaller individuals (Stazner 1988). Not only can resistance to dislodgment be size-dependent (Poff et al. 1991; Clark et al. 2008), it can also be species- (Poff et al. 1991) and/or system-specific (Parkyn and Collier 2004) adding greater complexity to teasing apart effects of flooding on organisms.
Crayfish, in particular, are not adapted morphologically or physiologically to major flood disturbances (Parkyn and Collier 2004) and in some systems, crayfish abundance is negatively correlated with peak discharge and duration of high intensity flood events (Light 2003). However, crayfish populations can persist in flood-prone streams (Parkyn and Collier 2004), responding via postural changes to deflect flow (Maude and Williams 1983; Clark et al. 2008) and/or by moving into areas of reduced hydraulic stress (i.e., pools, interstitial space; Clark et al. 2008). Crayfish population resilience/recovery may also rely on growth and reproduction (within and outside the affected areas) to reestablish populations.

Many studies have examined effects of flooding on population/community structure over long (i.e., annual) time scales (e.g., Parkyn and Collier 2004; Franssen et al. 2006), however, the short-term response of individual species is not well understood or documented. Thus, in this study, I assessed both short-term and long-term impacts of a high discharge flood event on a crayfish (Orconectes obscurus Hagen) population in a forested stream using a mark-recapture approach (for short-term sampling) and density estimation (for both short- and long-term sampling). Since flooding is not uncommon in this study system, I expected crayfish to have behavioral adaptations to withstand major flood events. However, I predicted that crayfish resistance and recovery would be size-dependent with small crayfish recovering rapidly and larger crayfish recovering more slowly, possibly taking a complete growing season to return to pre-flood population sizes. On a habitat scale, I expected to recapture a larger proportion of marked crayfish in shallow pools than riffles. Although riffle habitats tend to have larger sediments (and
thus, more possible refugia), current velocity is higher potentially making it difficult for crayfish to find refugia relative to shallow pools which have slower velocities and low-flow bank margins even during flooded conditions. Furthermore, due to higher current velocities and a greater degree of turbulent flow, dislodgment of crayfish should occur more frequently in riffle habitats. However, I expected crayfish to rapidly return to pre-flood densities in each habitat as discharge returned to baseflow levels. Additionally, I expected that the flood would not affect population size on a longer term scale. In other words, I anticipated that Summer 2006 crayfish densities would be similar to pre-flood densities during Summer 2005.

Methods

Study site description

This study was conducted in the West Branch of the Mahoning River near Ravenna, Portage County, Ohio (41°09′41″N 81°11′50″W). This forested 4th-order stream is surrounded by mature 2nd-growth riparian vegetation dominated by *Platanus occidentalis*, *Acer saccharum*, and *Fagus grandifolia*. The stream itself is characterized by riffle-pool sequences with substrate particles ranging in size from clay to small boulders. Stream habitat type was characterized based on water depth and current velocity and divided into 3 categories consisting of ‘deep pools’ (water depth ≥ 51 cm, average current velocity < 0.02 m/s), ‘shallow pools’ (maximum water depth = 50 cm, average current velocity < 0.02 m/s), and ‘riffles’ (maximum water depth = 12 cm,
noticeable breaks in the surface water, average current velocity > 0.10 m/s). Short-term impacts of flooding on size-specific crayfish densities were assessed in 1 shallow pool and 2 riffle habitats, whose baseflow conditions were measured prior to the flooding event during Summer 2005 (Table 3). Current velocities and water depths were measured within each channel unit using a Marsh-McBirney® flow meter (Model 2000, FLO-MATE™) and a wading rod, respectively. A grid composed of 1 X 1 m squares was set up across each channel unit and measurements were taken at 15 randomly selected points on the grid in each channel unit. To determine average grain size in each channel unit, a Wolman pebble count and random transect method was used (Wolman 1954, see Clark et al. 2008 for a complete description of methods). Shallow pool average current velocity and grain size was substantially lower than in riffle habitats (Table 3). Additionally, the shallow pool had a deeper average water depth than the riffles. Riffle 2 had a higher average current velocity than Riffle 1 but similar average water depth and grain size (Table 3).

**Short-term assessment of flooding effects on crayfish**

Crayfish (*O. obscurus*) populations were monitored in the three contiguous channel units described above during a 16-d period (30 August-15 September 2005) following a major flood event resulting from rains that tracked across northeastern Ohio as a result of Hurricane Katrina. The flood event began on 30 August 2005 (day 0) reaching current velocities of at least 1.09 m/s², a substantial flood event for this stream. On days 1 (31 August 2005) and 7 (6 September 2005) after the flood began (post-flood),
Table 3. Summary of channel unit characteristics for the shallow pool and riffles 1 and 2 at baseflow during Summer 2005. Data presented generally consist of the mean ± 1SE.

<table>
<thead>
<tr>
<th></th>
<th>Riffle 1</th>
<th>Shallow pool</th>
<th>Riffle 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current velocity (m/s²)</td>
<td>0.15 ± 0.02</td>
<td>0.01 ± 0.005</td>
<td>0.26 ± 0.03</td>
</tr>
<tr>
<td>Grain size (mm)</td>
<td>41.93</td>
<td>21.86</td>
<td>40.79</td>
</tr>
<tr>
<td>Depth (cm)</td>
<td>5.1 ± 0.7</td>
<td>17.1 ± 1.7</td>
<td>6.6 ± 0.6</td>
</tr>
<tr>
<td>Length (m)</td>
<td>17.0 ± 1.0</td>
<td>24.1 ± 0.1</td>
<td>12.4 ± 0.6</td>
</tr>
<tr>
<td>Width (m)</td>
<td>7.8 ± 1.4</td>
<td>10.2 ± 0.9</td>
<td>6.5 ± 0.7</td>
</tr>
<tr>
<td>Total area (m²)</td>
<td>132.6</td>
<td>245.8</td>
<td>81.0</td>
</tr>
</tbody>
</table>
current velocity was measured by suspending a Price type-AA flow meter® from a bridge approximately 200 m downstream of the experimental area. On days 8 (7 September 2005), 10 (9 September 2005), and 16 (15 September) post-flood, current velocity was measured using a Marsh-McBirney® flow meter within the study’s channel units. Current velocity in these channel units was not measured on day 0 due to time and safety constraints and the rapid increases in water depth and current velocity. Prior to the flood event, water levels were at baseflow and therefore, an estimate of discharge at baseflow (0.04 m³/s), typical for this time of year, was used for day 0 of the hydrograph (Fig. 7).

Discharge, was calculated using the midsection method (see Hauer and Lamberti 2006) to create a hydrograph (Fig. 7). Discharge reached at least 9.08 m³/s on day 1 following the onset of the rainstorm (Fig. 7). To assess bedload movement during the flood, a hand-held Helley-Smith Sampler® was used along a randomly chosen transect running across the stream. Measurements were taken for 30 s each at four points spaced 2 m apart.

Bedload movement was measured at the same four locations on day 1 (flood conditions) and day 7 (baseflow conditions). Samples were brought back to the lab, dried at 70°C, and weight (g) and grain size (b-axis, mm) of grains > 1 mm were measured. A one-way ANOVA was used to compare differences in bedload movement (g/30 s) between day 1 and 7 (SAS 8.01/2000, SAS Institute Inc., Cary). The flood event caused a high degree of bedload movement with significantly higher bedload movement during the flood event (mean = 711.77 ± 202.35 g/30 s) than at baseflow conditions (mean = 133.11 ± 9.72 g/30 s) (ANOVA, \( F_{(1,6)} = 8.16, p = 0.0289 \)). The largest grains moved during flood and
Fig. 7. Hydrograph (in discharge; m³/s) of the West Branch of the Mahoning River, Ravenna, Ohio, USA from 30 August-15 September 2005. Labeled days represent when biotic and abiotic data were collected. The day-0 discharge estimate was an average of Summer 2005 measurements and was representative of discharge during baseflow conditions.
baseflow conditions were coarse gravels (largest = 24 mm, b-axis) and sand (largest = ~1 mm, b-axis), respectively.

Crayfish were sampled in each channel unit using a backpack electroshocker (LR-24 Electrofisher, Smith-Root™) on the morning of the approaching storm (day 0; 30 August 2005). One complete electroshocking pass, with two netters moving in an upstream, zig-zag pattern, was used in both riffle habitats. However, as a result of rising water levels, only a partial pass (50% of the channel unit) was completed in the shallow pool. Block nets could not be used to enclose the area due to rising water levels and the associated increasing current velocities. Upon completion of each electroshocking pass, the carapace length (CL, mm) of all captured crayfish was measured using Vernier calipers and their sex was determined. Additionally, all crayfish (shallow pool, \( n = 111 \); riffle 1, \( n = 47 \); riffle 2, \( n = 31 \)) were tagged with Visible Implant Elastomer (VIE, Northwest Marine Technology™) in specific abdominal segments using a location and color scheme that identified crayfish by their original channel unit of capture. Tagging with VIE is an efficient and effective means of marking crayfish for long-term studies, with crayfish retaining these tags for at least 9 months through many molt cycles (Clark and Kershner 2006). Following tagging, crayfish were released in the center of the channel unit of capture.

Recapture efforts took place 2, 8, 10, and 16 days post-flood using one complete electroshocking pass, with two netters moving upstream in a zig-zag pattern. Block nets were placed upstream and downstream to enclose each channel unit. On day 2, shallow pools that were upstream and downstream of the experimental area were sampled along
with the original three channel units. No previously tagged crayfish were recaptured in either of the two additional channel units. Thus, all subsequent sampling/recapture efforts were focused on the original shallow pool and two riffles. Upon capture, all crayfish were sexed and carapace lengths were measured. Previously tagged crayfish that were recaptured during a specific recapture effort were then tagged in a different abdominal segment location with each channel unit of capture having a specific location and color. After all measurements and tagging were completed for a given sampling effort, crayfish were released in the middle of the channel unit of capture.

*Mark-recapture of crayfish during flooding versus baseflow conditions*

To get an estimate of crayfish recapture rates in the same 3 channel units during baseflow conditions (8 days following the flooding event), an additional mark-recapture study was conducted. Channel-specific estimates of crayfish recapture during post-flood and baseline conditions were made at 2 and 8 days following the respective tagging events. Crayfish were sampled, marked with VIE, and released using the same methods as in the post-flood study. Each individual was tagged using an abdominal segment location and color scheme that identified crayfish by their channel unit and date of capture. As noted above for the post-flood tagging event, crayfish were captured and marked on 30 August 2005 (day 0, post-flood tagging; number of crayfish tagged: shallow pool, \( n = 111 \); riffle 1, \( n = 47 \); riffle 2, \( n = 31 \)), with recapture efforts on 1 September 2005 (day 2, post-flood tagging) and 7 September 2005 (day 8, post-flood tagging). For the baseflow tagging event, crayfish were captured and marked on 7
September 2005 (day 0, baseflow tagging; number of crayfish tagged: shallow pool, \( n = 111 \); riffle 1, \( n = 69 \); riffle 2, \( n = 30 \)), with recapture efforts on 9 September 2005 (day 2, baseflow tagging) and 15 September 2005 (day 8, baseflow tagging).

Long-term assessment of flooding effects on crayfish

I assessed size-specific crayfish abundance and distribution in 4 shallow pools and 3-4 riffles during Summer 2005 (June-July, pre-flood), Fall 2005 (October, 1 month post-flood), Summer 2006 (June, 9 months post-flood), and Fall 2006 (October, 13 months post-flood) as part of a larger study assessing the effects of grain size, current velocity, water depth, predatory fish and relative predation risk on size-specific distribution patterns. Due to the dynamic nature of streams, channel units changed over time and the same channel units could not be used for each season. However, the same riffles and shallow pool used in the short-term study described above were sampled during each season represented in the longer-term assessment. For this study, the sampling area (each was \( \leq 10 \) m long) in each channel unit was enclosed by fine-mesh upstream and downstream block nets, and crayfish were collected using a 4-pass removal method (Zippin 1958) with a backpack electroshocker. During each pass, crayfish were collected by two netters walking upstream in a zig-zag pattern. All crayfish collected during each pass were counted and measured [carapace length (CL), mm] using Vernier calipers and sex was determined. Lengths (at both stream edges) and widths (at both upstream and downstream block nets) of each sample habitat were measured in order to calculate the area (m\(^2\)) that was sampled for later use in crayfish density estimation.
Given that my sampling methods have relied primarily on electroshocking, it should be noted that electroshocking can be an effective technique for sampling crayfish (Rabeni et al. 1997). Also, my method met the assumptions required for population estimation using the 4-pass removal method (Zippin 1958). In fact, many studies have successfully sampled crayfish using electroshocking in combination with multiple-pass removal methods (Usio and Townsend 2000; Hicks 2003; Seiler and Turner 2004).

**Analysis of Short- and Long-term Assessment**

Crayfish were divided into 2 size categories based on carapace length (small, CL ≤ 19.9 mm; large CL ≥ 20.0 mm). These two crayfish size ranges are affected differently by flow, having significantly different dislodgment rates during high flows (Clark et al. 2008). Population estimates of the shallow pool and riffles 1 and 2 from Summer 2005 density estimation data were used for comparison with short-term data. Further, population estimates were calculated by dividing the number of crayfish captured during the first pass by the total enclosed sampling area to allow comparison with capture data from days 2, 8, 10, and 16. Similarly, total crayfish density was calculated by dividing the total number of crayfish captured by the total area sampled. “Crayfish captured” refers to crayfish either having or not having a VIE tag, whereas “crayfish recaptured” refers only to crayfish having a VIE tag. For long-term data, I used a depletion method (Zippin 1958) to calculate crayfish density which allows density to be estimated by plotting cumulative total catch against catch per unit effort (CPE), fitting a regression line through the points, and solving for CPE = 0 to determine the population estimate for each
sample habitat. The population estimate was then divided by the area calculated for each sample habitat. To calculate a density estimate for each size class (small and large crayfish) within a sample habitat, the total density of crayfish for a given channel unit was multiplied by the proportion of crayfish captured within each size class for a given sample area. Separate one-way ANOVA’s were used to compare size-specific density estimates for crayfish during summer and fall to assess long-term recovery of crayfish populations (SAS 8.01/2000, SAS Institute Inc., Cary). Additionally, total crayfish density estimates in shallow pools and riffles were pooled to provide an overall summary of population impacts at a reach scale. Separate one-way ANOVA’s were used to compare total crayfish density estimates between Summer 2005 and 2006 or Fall 2005 and 2006. All data were log(x+1)-transformed prior to analysis.

Results

*Short-term assessment of flooding effects on crayfish*

Total crayfish density (# of individuals/m²) decreased from day 0 through day 2 in the shallow pool (Fig. 8A) and in riffle 1, but did not decrease in riffle 2 (Fig. 8B). Total crayfish density returned to pre-flood densities (sampled during Summer 2005) in both riffle 1 and 2 by day 10 and 16, respectively. However, in the shallow pool, total crayfish density had not recovered to pre-flood densities (Summer 2005) within 16 days of the flood event (Fig. 8A). Small crayfish density decreased from day 0 through day 2 in each channel unit (Figs. 9A-C). Density of small crayfish then increased dramatically in each
Fig. 8. Total density of crayfish (individuals/m²) captured via electroshocking from day 0 through day 16 (30 August-15 September 2005) in A) the shallow pool (SP) and B) riffle 1 (R1) and riffle 2 (R2). In panel B, dark and open circles indicate total crayfish density in R1 and R2, respectively. Dashed, labeled lines on each graph represent estimates of baseflow crayfish densities taken earlier during Summer 2005 in each channel unit.
Total crayfish density (individuals/m²)

- **A**
  - Plot of Population Estimate from Summer 2005
  - Line graph showing the trend over days

- **B**
  - Graph comparing R1 and R2 populations
  - Line graphs for R1 and R2 populations over days
Fig. 9. The density (# of individuals/m²) of small (carapace length (CL) < 20.0 mm) and large (CL ≥ 20.0 mm) crayfish captured via electroshocking from day 0 through day 16 (30 August-15 September 2005) in A) R1, B) SP, and C) R2. Dark and open circles represent small and large crayfish, respectively. The large arrow along the y-axis indicates the direction of stream flow across these three channel units.
Crayfish density (individuals/m²)

Stream flow

Day

0 2 4 6 8 10 12 14 16 18

A  
- Small Crayfish
- Large Crayfish

B

C
channel unit from day 2 through day 16 post-flood with a 4-fold increase in riffle 1, an 8-fold increase in the shallow pool, and a 6-fold increase in riffle 2 (Figs. 9A-C). Large crayfish density did not decrease in either riffle 1 or 2 from day 0 through day 2 and stayed relatively constant from day 0 through day 16 (Fig. 9A, C). However, in the shallow pool, large crayfish density decreased 4-fold from day 0 through day 2 and increased only slightly (~0.1 individuals/m²) from day 2 through day 16 (Fig. 9B).

The overall proportion of tagged crayfish that were recaptured in the shallow pool increased steadily from on day 2 to day 16 (Fig. 10). However, in riffles, the proportion of recaptured crayfish was much lower and did not show much change over the 16-d time period (Fig. 10). Population mixing occurred across the 3 channel units, with individuals moving in upstream and downstream directions (Fig. 10). Many recaptured crayfish originally tagged in the riffles did not remain in those riffles and moved upstream or downstream into the shallow pool (Fig. 10). However, crayfish initially tagged in the shallow pool tended to remain there (Fig. 10). The steady increase in the proportion of recaptured crayfish in the shallow pool seems to be from immigration from adjoining riffles. Lastly, only a few crayfish moved out of the shallow pool into adjoining riffles.

*Mark-recapture of crayfish during flooding versus baseflow conditions*

Comparing the proportion of tagged crayfish recaptured on days 2 and 8 immediately post-flood versus baseflow conditions, substantially more crayfish were recaptured on both 2 and 8 days after tagging under baseflow conditions than immediately post-flood (Fig. 11A-B). During post-flood conditions, more crayfish were
Fig. 10. The proportion of tagged crayfish recaptured on post-flood days 2-16 (1-15 September 2005) in A) R1, B) SP, and C) R2. Habitat-specific color/fill codes within each bar represent the channel unit of original capture/tagging (also noted on legend). Proportion of crayfish within an entire panel represent crayfish that have been recaptured within R1 (panel A), SP (panel B), and R2 (panel C) regardless of original channel unit of capture. The large arrow along the y-axis indicates the direction of stream flow across these three channel units.
Fig. 11. The proportion of crayfish recaptured from the original tagging event (day 0) and the baseflow tagging event (day 8) over two distinct time intervals: two days (panel A) following a given tagging event and eight days (panel B) following a given tagging event in riffle 1 (R1), the shallow pool (SP), and riffle 2 (R2). Each bar represents the proportion of crayfish recaptured from each channel unit for a given tagging event. The black bars represent the proportion of crayfish recaptured from the first tagging event during the first two recapture attempts directly post-flood (1 and 7 September 2005). The gray bars represent the proportion of crayfish recaptured from the second tagging event during the first two recapture attempts at baseflow conditions (9 and 15 September 2005).
Proportion of crayfish recaptured

**A**
- Post-flood
- Baseflow

**B**
- R1
- SP
- R2
recaptured on day 8 than day 2 post-flood (Fig. 11A-B). However, a similar proportion of crayfish were recaptured on days 2 and 8 during baseflow conditions (Fig. 11A-B). Across both sampling periods, a low proportion of crayfish were recaptured and more crayfish were recaptured in the shallow pool than in either riffle habitat (Fig. 11A-B).

*Long-term assessment of flooding effects on crayfish*

Overall, small and large crayfish densities were cyclical with small crayfish densities peaking during Fall and large crayfish densities peaking during Summer following typical growth/reproductive cycles (Figs. 12A-C, 13A-B). In riffle 2, Summer (~2 months pre-flood) and Fall (1 month post-flood) 2005 densities appeared to be similar to Summer (9 months post-flood) and Fall (13 months post-flood) 2006 densities for both small and large crayfish (Fig. 12A). In the shallow pool, small crayfish density was similar during Summer 2005 and 2006 (Fig. 12B). However, large crayfish density was slightly higher in the shallow pool during Summer 2005 than Summer 2006 (Fig. 12B). Additionally, Fall 2005 density was lower than Fall 2006 density for both size-classes (Fig. 12B). In riffle 1, Summer 2005 and 2006 densities appeared to be similar for small crayfish (Fig. 12C). However, large crayfish density was much higher in riffle 2 during Summer 2005 than Summer 2006 (Fig. 12C). During Fall, small crayfish density was higher in 2005 than 2006 (Fig. 12C). Large crayfish showed no difference in density in riffle 2 between Fall 2005 and 2006 (Fig. 12C).

In shallow pools, both small and large crayfish had similar densities during Summer 2005 and 2006 (ANOVA, small, $F_{(1,10)} = 3.55, P = 0.1085$; large, $F_{(1,10)} = 0.10$,
Fig. 12. Total crayfish density (# of individuals/m²) (mean ± 1 SE) of small and large crayfish captured via electroshocking for long-term assessment of flooding on the crayfish population in A) R2, B) SP, and C) R1. Collection occurred during Summer 2005 (pre-flood), Fall 2005 (1 month post-flood), Summer 2006 (9 months post-flood), and Fall 2006 (13 months post-flood). Dark and open circles represent small and large crayfish, respectively. The arrow indicates when the flood event occurred.
Stream flow

Crayfish density (individuals/m²)

A

- Small crayfish
- Large crayfish

Flood

B

C

Summer 2005  Fall 2005  Summer 2006  Fall 2006
Fig. 13. Total crayfish density (# of individuals/m²) (mean ± 1 SE) of small and large crayfish captured via electroshocking in 12 channel units for long-term assessment of flooding on the crayfish population in A) shallow pools and B) riffles. Collection occurred during Summer 2005 (pre-flood), Fall 2005 (1 month post-flood), Summer 2006 (9 months post-flood), and Fall 2006 (13 months post-flood). Dark and open circles represent small and large crayfish, respectively. The arrow indicates when the flood event occurred.
Crayfish density (individuals/m²)

A
- • Small crayfish
- ○ Large crayfish

Flood

B

Summer 2005 Fall 2005 Summer 2006 Fall 2006

0 1 2 3 4
\[ P = 0.7637 \) and Fall 2005 and 2006 (ANOVA, small \( F(1,10) = 0.42, P = 0.5402 \); large, \( F(1,10) = 0.42, P = 0.5393 \) (Fig. 13A). In riffles, small crayfish had significantly lower densities in Summer 2006 than Summer 2005 (ANOVA, \( F(1,8) = 15.66, P = 0.0108 \) (Fig. 13B). However, large crayfish had similar densities in Summer 2005 and 2006 (ANOVA, \( F(1,8) = 5.39, P = 0.0679 \) (Fig. 13B). Additionally, both small (ANOVA, \( F(1,8) = 1.76, P = 0.2421 \) and large (ANOVA, \( F(1,8) = 0.12, P = 0.7436 \) crayfish had similar densities during Fall 2005 and 2006 (Fig. 13B). At the reach scale (considered across habitat types), total crayfish densities were similar between Summer 2005 and 2006 (ANOVA, \( F(1,24) < 0.01, P = 0.9483 \) and Fall 2005 and Fall 2006 (ANOVA, \( F(1,24) = 0.11, P = 0.7502 \).

**Discussion**

Overall, high discharge flood events significantly impact crayfish populations on a short-term time scale. Total crayfish densities were substantially lower on day 0 relative to the Summer 2005 population estimates and this pattern was likely driven by crayfish responding to rapidly increasing current velocities resulting from the onset of rain. As current velocity increases (which generally occurs during flooding), crayfish typically decrease activity and retreat into areas of decreased hydraulic stress (Clark *et al.* 2008). In this study, crayfish may have responded to increased flow by moving into areas of reduced hydraulic stress, such as stream margins or under rocks, making capture via electroshocking more difficult. As current velocities returned to baseflow conditions, capture rates of crayfish increased suggesting that crayfish may have decreased use of
refugia (as a function of decreased current velocity) allowing increased capture via electroshocking. In fact, tagged crayfish were recaptured at a higher rate during baseflow than post-flood conditions suggesting that the flood significantly impacted crayfish behaviorally.

Total crayfish densities recovered to pre-flood densities within 10-16 days in both riffle habitats but did not recover as rapidly in the shallow pool. This habitat-specific recovery, however, appears to be dependent on size-specific resistance/resilience to flooding. Essentially, small crayfish seemed to be more resistant/resilient to flooding on a very short time scale with post-flood densities increasing rapidly in all 3 channel units, whereas, large crayfish showed limited short-term recovery. This difference in short-term patterns in habitat-specific recovery was most likely a function of reduced resilience of larger crayfish in response to flooding.

In the 2 riffles, Summer and Fall densities during 2005 and 2006 did not differ substantively, providing some indication that the flood event did not influence long-term population levels in riffle habitats. Furthermore, size-specific density across multiple riffles revealed that there was no significant difference in small or large crayfish density between Fall 2005 and Fall 2006, indicating no negative impact of the flood event on either size class of crayfish. Small crayfish density, however, was lower during Summer 2005 than Summer 2006 but was likely not a function of the flood event as Summer 2005 densities were estimated prior to the flood event. Large crayfish density was also similar across riffles between Summer 2005 and 2006, indicating no negative effects of flooding. This lack of a flooding effect in riffles is likely driven by the fact that large *O. obscurus*
typically avoid riffle habitats, even at baseflow conditions (Clark et al. 2008), explaining their low densities in riffle habitats both pre- and post-flood in this study. In contrast, small crayfish are common in riffle habitats (Clark et al. 2008), and they recovered rapidly in riffles, just as they did in the shallow pool.

In the shallow pool, both small and large crayfish densities decreased dramatically following the flood event. Small crayfish densities, however, increased substantially during the following 16 days returning to pre-flood levels, whereas large crayfish densities did not return to pre-flood levels within the 16-day sampling period. Long-term sampling revealed that both small and large crayfish densities in the shallow pool were lower during Fall 2005 (1 month post-flood) than Fall 2006 (13 months post-flood). This difference is likely a function of both continued population recovery and natural variability, including shifts in channel morphology, reduced predation rates, and increased recruitment of juvenile crayfish during Fall 2006 relative to Fall 2005. However, at the stream reach scale across multiple shallow pools, there was no difference in small or large crayfish densities between Summer 2005 and 2006 or Fall 2005 and 2006, suggesting that size-specific crayfish densities were not negatively affected by the flood event on a long-term temporal scale and a larger spatial scale.

Overall, while flooding had short-term impacts on crayfish populations, there did not appear to be long-term effects of the flood event at the habitat scale for either small or large crayfish. Furthermore, when the data were pooled across habitat types, there were no significant differences in total crayfish densities in either Summer or Fall at the reach scale. Ultimately, both small and large crayfish were resilient to the flood event and
maintained similar cyclical densities pre- and post-flood. These size-specific density cycles are driven by typical stages in crayfish life cycles, including Fall die-offs of large adults and Summer recruitment of juveniles. The flood event did not seem to effect spring/summer recruitment with the population persisting at pre-flood levels in the following Summer and Fall. However, long-term patterns of crayfish resistance and resilience to flooding may be system-specific (Parkyn and Collier 2004). For example, in pasture streams, where spatial refugia is relatively limited, crayfish (*Paranephrops planifrons* White) densities decreased following a high discharge flood event whereas densities were not negatively affected in nearby forested streams characterized by high spatial refugia (i.e., stony substrate and undercut banks) (Parkyn and Collier 2004). Additionally, size-structure of crayfish did not change in forested systems in response to flooding, however, in pastures, no large (30 mm OCL) crayfish persisted post-flood, while 1\textsuperscript{st} and 2\textsuperscript{nd} year crayfish showed no density effects (Parkyn and Collier 2004).

A variety of mechanisms/factors could be associated with short-term flooding effects on crayfish including passive dislodgment and/or flood-related mortality. Although crayfish can hold their position during high current speeds (Kozak *et al.* 2004, Clark *et al.* 2008), passive downstream displacement can occur in response to high intensity spates (Nakata *et al.* 2003, Kozak *et al.* 2004, Kerby *et al.* 2005). Across aquatic organisms, dislodgment can be size-dependent, with smaller individuals having higher resistance to dislodgment than medium and large individuals (e.g., snails, Moore 1964; mayflies, Poff *et al.* 1991; crayfish, Clark *et al.* 2008). Current velocities during this flood event reached levels greater than 1 m/s which are higher than large crayfish can
withstand but still within the range of velocities that small crayfish can withstand (Clark et al. 2008).

Additionally, size-dependent dislodgment may interact with substrate type, arrangement, and stability. Behavioral responses such as use of refugia (e.g., interstitial spaces associated with stony substrate) can be a key resistance mechanism when subjected to high discharge flood events (Holomuzki and Biggs 2003, Parkyn and Collier 2004, Clark et al. 2008). At high flows, interstitial spaces create turbulent eddies in which pockets of zero current are created and when accessible allow crayfish to avoid dislodgment (Bovbjerg 1970). Small-bodied crayfish, in particular, use interstitial spaces and burrow under stony substrate when exposed to high current velocities whereas larger-bodied individuals are typically unable to access these refugia and are dislodged at high flows (Clark et al. 2008). Small and large crayfish within the stream proper may have displayed these size-specific effects in response to high current velocities.

In addition to direct dislodgment resulting from increased flow, bedload movement can lead to organismal dislodgment. The high current velocities and turbulence typically associated with flooding makes reliance on rocks as potential refugia risky due to the ability of these forces to mobilize bed sediments (Wilzbach 1990). Mobilized sediments can wash away individual organisms often causing their death (Giller et al. 1991, Dodds et al. 1996, Holomuzki and Biggs 1999, Matthaei et al. 2000, Robinson et al. 2000). Although the relationship between grain size and stability in ramped flows is not completely straightforward (Duncan et al. 1999), stability typically increases with increased grain size (e.g., stability of sand < small pebbles < small cobble).
(Gordon et al., 2004) and increased degree of embeddedness (Downes et al. 1997; Matthaei et al. 1999a) making larger cobbles and boulders potential refugia during a flood event. While there is a strong positive relationship between substrate stability and invertebrate resistance to flood events (Allan 1995, Imbert et al. 2005), even agitation (shaking) of sediments can trigger passive dislodgment (Gibbins et al. 2007). Ultimately, while there was bedload movement during this flood event, coarse gravels were the largest moving substrate particles detected, indicating that while some crayfish may have been dislodged as a result of bedload movement, larger cobbles and boulders would still have provided refugia.

In addition to small-scale movements to nearby refugia during flood events, some lotic organisms make large-scale movements into lateral floodplains (Ross and Baker 1983), pools and backwaters (Matthews 1986), downstream areas (Williams and Hynes 1976, Horwitz 1978), and hyporheic zones (Dole-Olivier et al. 1997). Crayfish movement among channel units (relative to channel unit of original tagging) may have been the result of many factors, including movement into pools and backwaters, passive downstream movement, and/or attempts to recolonize their initial home range. In this study, crayfish moved both upstream and downstream into adjoining habitats during the post-flood period. Most crayfish species have the potential to move upstream against the flow of water (Momot 1966, Robinson et al. 2000, Bubb et al. 2002, Clark et al. 2008). In fact, upstream movement has been documented for O. nais in an Oklahoma stream following flood events and may be an important mechanism of stream recolonization for crayfish populations (Momot 1966). Recapture rates in the shallow pool were highest
with immigration occurring into this sample area suggesting that this habitat type may provide refugia for crayfish during flood events. Here, current velocity is typically slower (relative to riffles) especially at stream bank margins and may allow crayfish to make small-scale movements to refugia (e.g., under boulders, interstitial space, undercut banks, tree roots, and stream bank margins. Additionally, sand, although subject to mobilization in the top layers, is relatively abundant in shallow pools and may allow for burrowing deep into the sediments. Burrowing by other invertebrates, such as copepods and chironomids, has been documented in response to increasing water velocity (Palmer et al. 1992) and could also be a flood response used by crayfish.

In summary, this study was set up to examine short- and long-term crayfish responses to a major flooding event. During the first few weeks following the flood, crayfish populations, regardless of size distribution and habitat type, were substantially impacted. Small crayfish were resistant to high intensity flood impacts whereas large crayfish did not appear to recover on the short-term scale. However, no long-term negative effects of the flood event on total or size-specific crayfish density were detected. In forested streams, small crayfish resistance likely results from movement into areas of lower hydraulic stress including large-scale refugia, such as shallow pools, backwaters, and the hyporheic zone, and small-scale refugia, such as interstitial spaces and tree roots so as to avoid high current velocities. These habitat patches are common in forested streams and are probably crucial to the resistance of stream organisms to flood events. Large crayfish, however, showed no negative long-term effects of the flood event and may rely on survival/growth of small individuals and reproduction for recovery.
Ultimately, resistance to flooding by small crayfish may be the key component to long-term persistence of crayfish populations in flood-prone streams.

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References


CHAPTER 5

HABITAT-SPECIFIC EFFECTS OF GRAIN SIZE, CURRENT VELOCITY, WATER DEPTH, AND PREDATION RISK ON SIZE-DEPENDENT CRAYFISH DISTRIBUTIONS

(To be submitted to the Journal of the North American Benthological Society)

Abstract

Understanding the role that spatio-temporal variability of biotic and abiotic factors plays in determining crayfish distribution is critical to assessing the impact of this organism in a given aquatic ecosystem. In this study, I assessed the role of habitat (deep pools, shallow pools, riffles), time of year (Spring, Summer, Fall), and predation risk on size-structured distribution patterns of crayfish (*Orconectes obscurus*) in a 4th-order stream using a combination of field surveys, tethering experiments (i.e., predation assays), and stomach content analysis of predaceous fishes. Habitat-specific estimates of current velocity, water depth, average grain size, and the size and density of predaceous fishes were all used to assess these interactions. Additionally, a predation assay was used in combination with grain size manipulations to assess habitat-specific interactions between grain size and predation risk. In general, large crayfish had highest densities in
deep pool habitats and were associated with increased water depth and large fish predators, whereas, small and medium crayfish had highest densities in shallow pool habitats, and were more strongly associated with increased grain size and small fish predators. Overall, crayfish appeared to move from riffles into deep pool habitats as they became larger, showing a distinct shift in habitat use. Predation assays revealed that, although all size classes of crayfish were vulnerable to predation, small crayfish were significantly more vulnerable than larger crayfish. Regardless of size, crayfish in deep pool habitats had significantly lower survival than in shallow water habitats (shallow pools and riffles). However, only small crayfish showed consistent differences in predation risk by habitat type. Habitat-specific abiotic variables did not consistently affect predation risk of crayfish during tethering assays, implying that predation outweighs the effects of abiotic variables. However, the combined grain manipulation/tethering experiment implied that large rocky refugia (cobbles) results in significantly higher survival than on sand plots. Overall, predation appears to be a key mechanism structuring habitat-specific patterns of distribution for small but not medium or large *O. obscurus*. Large substrates (i.e., cobbles and boulders) may be particularly important in shallow pool habitats where both small crayfish density and predation risk are high.

**Introduction**

Lotic ecosystems are dynamic systems possessing a wide range of hydraulic characteristics and geomorphologies. In streams, this variability leads to a broad range of

Predator assemblages associated with stream ecosystems are often quite complex in terms of both density and diversity. In stream reaches with deep and shallow areas, crayfish are important prey items for many aquatic (e.g., creek chubs Semotilus atromaculatus Mitchell; sculpins Cottus spp.; softshell turtles Apalone spp.; other crayfish—both conspecifics and other species), semi-aquatic (e.g., northern river otters Lontra canadensis Schreber), and terrestrial predators (e.g. raccoons Procyon lotor L.; queen snakes Regina septemvittata Say; and great blue herons Ardea herodias L.). In fact, habitat selection by prey species in these systems can be quite complicated in the
face of complex predator assemblages (Power 1987, Scrimgeour et al. 1994) with responses often dependent on prey size (Mather and Stein 1993a, Englund and Krupa 2000). Habitat shifts/selection becomes particularly challenging as different predators occupy very different habitat types. Further, abiotic characteristics such as substrate type and current velocity also differ with habitat type, and may act in both independent and interactive ways to structure organismal distribution in the face of predation risk.

Substrate type and complexity, in particular, can be important factors influencing predator success (Ware 1972, Brusven and Rose 1981) as they determine the availability of prey refugia. Thus, in stream reaches where predators are present, substrate may play an important role in crayfish distribution patterns. While most crayfish species prefer large, rocky substrate, often increasing in abundance with increasing grain size (Lodge and Hill 1994, Pockl and Streissl 2005), crayfish size is often negatively correlated with increasing grain size (Clark et al. 2008) suggesting that small crayfish are more dependent on refugia due to higher size-related predation risk (Stein and Magnuson 1976). When predator density is high, crayfish typically use low predation risk habitat (i.e., cobble) (Kershner and Lodge 1995), actively selecting substrates that offer the most protection in the presence of a predator (Stein and Magnuson 1976).

The purpose of this study was to determine whether abiotic variables and/or predation are major factors contributing to size-specific lotic crayfish distribution patterns and, further, if any abiotic factors interact with predation risk in modifying crayfish distributions. In this study, I assessed effects of water depth, grain size, current velocity, and fish predators (size and density) on size-specific crayfish (Orconectes obscurus)
distribution in deep pool, shallow pool, and riffle habitats in a 4th-order, forested stream. Additionally, I assessed biotic (e.g., predatory fish density) and abiotic effects of habitat on size-dependent predation risk for crayfish through tethering experiments and stomach content analysis of predatory fishes. Lastly, to clarify whether grain size (i.e., gravel and cobble) mediates crayfish predation risk, I manipulated substrate in deep pool, shallow pool, and riffle habitats. In general, I expected small crayfish distribution patterns to be negatively correlated with large fish predators and habitats in which they had low survival. Furthermore, I expected survival of medium and large crayfish to be higher regardless of habitat and did not expect predation risk to affect larger crayfish distribution patterns. More generally, I hypothesized that small crayfish density would be positively correlated with increasing substrate size and negatively correlated with predatory fish size and density and increasing water depth. Furthermore, I hypothesized that larger grain sizes (small cobble) would provide refugia from predation for small crayfish, while predation would be higher on smaller grain sizes (sand). Small crayfish survival was expected to be lowest in deep pools, which typically have relatively high densities of large fish predators. In contrast, medium and large crayfish were expected to be negatively correlated with increasing current velocities but not necessarily with water depth, substrate size, or fish predators (size and density). Medium and large crayfish survival was expected to be relatively high in comparison with small crayfish regardless of habitat type. However, I predicted that medium and large crayfish would have higher mortality in shallower habitats (riffles and shallow pools) than in deep pools. Furthermore, I anticipated faster current velocities would interact with predation risk by
reducing accessibility of crayfish to predators leading to increased survival at tethering locations with high current velocity.

Methods

Field Site and Habitat Descriptions

This study was conducted in the West Branch of the Mahoning River, a 4th-order forested stream, in Ravenna, Portage County, Ohio (41°09′41″N 81°11′50″W). The riparian zone is dominated by sugar maple (*Acer saccharum* Marsh.), American sycamore (*Platanus occidentalis* L.), and American beech (*Fagus grandifolia* Ehrh.). The stream is characterized by pool-riffle sequences and has benthic substrates ranging from clay and sand to gravel, cobble, and boulders. Stream habitat type was first characterized based on visual assessment and divided into three categories consisting of deep pools, shallow pools, and riffles. Habitat types were then confirmed quantitatively, using a variety of abiotic measurements including current velocity and water depth. During Summer 2005, water depth and average current velocity were measured in four deep pools (water depth ≥ 51 cm), four shallow pools (maximum water depth = 50 cm), and four riffles (maximum water depth = 12 cm with noticeable breaks in the surface water) (12 sample habitats total) during baseflow conditions using a wading rod and Marsh-McBirney® flow meter (Model 2000, FLO-MATE™). A grid composed of 1 m X 1 m cells was visualized within a given stream section and depth and velocity measurements were taken at 15 randomly selected points on the grid in each channel unit.
N = 180 estimates for each abiotic variable across all channel units). Additionally, average grain size was determined in each replicate habitat type using Wolman pebble counts (see Clark et al. 2008).

Habitat-specific Abiotic Measurements and Crayfish/Fish Survey

I assessed size-specific crayfish (O. obscurus) and predatory fish abundance and size distribution in four deep pools, four shallow pools, and four riffles (N = 12 channel units, same as described above) during Summer 2005 (June-July), Fall 2005 (October), Spring 2006 (April), Summer 2006 (June), and Fall 2006 (October). Due to the dynamic nature of stream hydrology, channel units used in this study changed over time, as certain channel units could not be used during each season. Additionally, only three riffles were used during Fall 2006, Summer 2006, and Spring 2006 due to limited riffle habitat within the study reach of the stream. Replicate channel units (ea. ≤ 10 m long) used for these sampling efforts were enclosed by upstream and downstream block nets (mesh size = 0.5 cm), and crayfish were collected using a four-pass removal method (Zippin 1958) with a backpack electroshocker. During each pass, crayfish and fish were collected by two netters walking upstream in a zig-zag pattern as a third person operated the backpack electroshocker. Electroshocking is considered be an effective technique to sample both crayfish (Rabeni et al. 1997) and fish populations (Bohlin et al. 1989; Poos et al. 2007) and this method met the assumptions required for this method of population estimation (Zippin 1958). Furthermore, many studies have successfully surveyed crayfish and fish
populations using electroshocking in combination with pass removal (Usio and Townsend 2000; Hicks 2003; Seiler and Turner, 2004).

All crayfish collected during each pass were counted and measured [carapace length (CL), mm] using Vernier calipers, and sex was determined. All fishes were identified to species and measured [total length (TL), cm] using a standard fish measuring board. Lengths (along both stream edges) and widths (at both upstream and downstream block nets) of each sample habitat were measured in order to calculate the surface area (m²) of the channel unit’s benthic zone for later use in crayfish density estimation.

Statistical Analyses

To estimate crayfish density, I used a four-pass removal method where cumulative total catch from the electroshocking efforts is plotted against catch per unit effort (CPE) (Zippin 1958). Next, a regression line is fit to the data points, and the resulting equation is solved for CPE = 0 to determine the population estimate for each channel unit. The population estimate was then divided by the area calculated for each sample habitat. To calculate a density estimate for each size class within each channel unit, total crayfish density for a given channel unit was multiplied by the proportion of crayfish captured within each size class for that sampling area. Only adult fishes (minimum adult size determined from Trautman 1957) were used in predatory fish analyses, as this age group is most likely to consume crayfish. Due to limitations in gape size, young-of-year fishes rarely consume crayfish (Roell and Orth 1993, Rabeni 1992, Dorn and Mittelbach 1999). Depletion was not reached for every electroshocking effort
for predatory fishes, so density was calculated by dividing the total catch from the four passes by the enclosed sample area for all channel units.

To test the main effects of crayfish size class, season, and habitat type on size-specific crayfish density, data were log \((X+1)\)-transformed and a full-factorial three-way ANOVA was used followed by Tukey’s post-hoc tests (JMP 6.0, SAS Institute Inc., 2005). Separate one-way ANOVAs followed by Tukey’s post-hoc tests were used to assess differences in average predatory fish size among deep pools, shallow pools, and riffles for each season (JMP 6.0, SAS Institute Inc., 2005). Predatory fish data were log \((X+1)\)-transformed prior to analysis. Pearson’s correlation analysis was then used to compare size-specific crayfish density with predatory fish density and average crayfish carapace length (CL, mm) with predatory fish total length (TL, cm) (JMP 6.0, SAS Institute Inc., 2005). Data were log \((X+1)\)-transformed prior to correlation analyses.

A correlation-based principal components analysis (PCA) was used to assess associations of total and size-specific crayfish densities with current velocity, water depth, average grain size, fish predator density, and average fish predator total length at all 12 sample sites during Summer 2005 (PC-ORD™ 5, MjM Software Design, 2005). PCA was used to express covariation among variables in a smaller number of composite variables (axes) to allow assessment of relationships among crayfish size classes and habitat variables (McCune et al. 2002). Data were transformed [using an ‘\(\ln(X+1)\)’ transformation] before PCA was run. The percent of variance in the distance matrix was measured using a Euclidean distance measure (see McCune et al. 2002 for further description of PCA).
To calculate the average grain size for each channel unit, grains were measured and separated into size classes in $1\Phi$ intervals ($\Phi = -\log_2 D; D = b$-axis of the grain in mm) (Moore 1999). Sand and clay were given D-values of 1.0 and 0.0001 mm, respectively based on the Wentworth scale (Gordon et al. 2004). A fractional grain size curve, which gives the proportion of grains within each size class, was obtained by dividing the number of grains in each size class by the total number of grains (Moore 1999). Average grain size [graphical mean ($M_z$); $M_z = (\Phi_{16} + \Phi_{50} + \Phi_{84})/3$; e.g., average of the median and ± 1 standard deviation from the median; Folk and Ward 1957] was calculated from each fractional grain size curve for each replicate channel unit of all habitat types.

Survey of Predatory Fish Diets

To identify potential crayfish predators among the fishes sampled in the West Branch of the Mahoning River, fish diets were collected using gastric lavage during all density estimation surveys and more intensively during Summer 2006. Gastric lavage is a non-invasive technique in which mildly pressurized water is pumped into the stomachs of live fishes causing regurgitation of stomach contents (i.e., food items). In this study, I used either a water bottle or garden sprayer depending on the fish’s gape size. Overall, this technique is highly successful in removal of food items (Brosse et al. 2002, Hakala and Johnson 2004) and mortality rate is generally zero (Brosse et al. 2002, Hakala and Johnson 2004). Since I am evaluating seasonal predation risk in this study, permanent removal of predators from the stream to assess diets would likely have biased my data.
relative to crayfish density patterns. Thus, gastric lavage was the best technique to assess fish diets for the study. Diets of *Cottus bairdi* Girard (central mottled sculpin; TL range = 4.3-8.6 cm; N = 228), *Semotilus atromaculatus* Mitchill (creek chub; TL range = 8.4-20.5 cm; N = 79), *Hypentelium nigricans* Lesueur (northern hogsucker; TL range = 12.0-29.2 cm; N = 29), *Ambloplites rupestris* Rafinesque (rock bass; TL range = 10.6-18.7 cm; N = 10), *Catostomus commersoni* Lacépède (common white sucker; TL range = 12.1-32.2 cm; N = 38), *Lepomis cyanellus* Rafinesque (green sunfish; TL range = 7.0-10.6 cm; N = 6), *Moxostoma erythrurum* Rafinesque (golden redhorse; TL range = 21.9-37.6 cm; N = 3), were collected. Stomach contents were sorted under a dissecting scope and identified as crayfish, insects (including aquatic and terrestrial), or other (fish, fish scales, detritus, snails, clams, eggs, collembola, millipedes, spiders, mites, zooplankton, and unknown organisms). Fishes with no stomach contents present upon gastric lavage were characterized as ‘empty’.

The proportions of fish diets containing crayfish parts, insect parts, other items, and empty guts were calculated for each fish species. Data were pooled by season (Spring, Summer, Fall) and all habitat types were pooled together, primarily to increase within-species sample sizes for species that were infrequently captured.

*Predation Assays*

In order to assess seasonal patterns of predation risk, predation assays were run during Summer (August) 2005, Spring (April) 2006, and Summer (August) 2006. Fall 2006 assays were not possible due to extended periods of intense flooding. To assess
size- and habitat-specific predation risk in each season, small (CL < 20 mm), medium
(CL = 20-30 mm), and large (CL > 30 mm) crayfish were collected by electroshocking
and hand-netting in channel units located downstream from survey and experimental
sites. Only males were collected and used in the assays in order to control for sex-
specific differences in predation risk. Furthermore, only small and medium crayfish were
used during Spring 2006 assays due to typical overwinter die-off of large crayfish and
both males and females had to be used due to low overall population levels. Following
capture, crayfish were brought back to the lab, measured using Vernier calipers (CL,
mm), and a black brass barrel snap swivel (size 14) was super-glued to the middle of the
dorsal portion of their carapace. All crayfish were housed overnight in aerated kiddie
pools to assure that all swivels were secure. In the field, 10-meter sections of stream
(with the exception of a 5-meter section for one riffle that was limited in length) were
randomly selected for tethering in the same 12 sample habitats (4 deep pools, 4 shallow
pools, 4 riffles) used during the Summer 2005 survey. A grid composed of 1 m X 1 m
cells was visualized within a given stream section, and 5 crayfish from each size class
were tethered in randomly selected cells on the grid in each of the 12 channel units (for a
total of N = 180 crayfish tethered during a given run of this experiment). Crayfish were
tethered by tying a 17-cm piece of 6-pound test, transparent monofilament fishing line to
their snap swivel (on one end) and to a tent stake (on the other end of the line). Each tent
stake was then hammered into the streambed. Rebar stakes were used instead of tent
stakes in deep pools to increase stake stability in the looser substrate of this habitat.
Mortality was checked daily at each tethering plot for 5 days.
At the end of the experiment, water depth and average current velocity were measured at each point where a crayfish was tethered using a wading rod and Marsh-McBirney® flow meter. Along with average current velocity, nearbed (2 cm above substrate) current velocity was measured using a Marsh-McBirney® flow meter during Spring and Summer 2006. In addition, characteristics of the top layer of substrate were also measured/defined at each tethering point. To do this, a Hess sampler (34 cm diameter, which conveniently corresponded with tether length) was placed down with the stake at its center and percent cover of grains less than or equal to 4 mm was estimated. All grains greater than 4 mm in diameter (b-axis) were measured in the field using Vernier calipers and all grains less than or equal to 4 mm in diameter (b-axis) were brought back to the lab. Once in the lab, organic matter was removed from all samples by soaking them in 3% hydrogen peroxide for 24 hours. Each sample was then rinsed with tap water, dried at 70°C, and analyzed using a Camsizer® particle analyzer (Retsch® Technology). During Summer 2006, to provide a reasonable estimate of available refugia, only the largest grain [maximum grain size (b-axis, mm)] at each tethering plot was measured using Vernier calipers instead of doing a full pebble count.

In the lab, three individual small, medium, and large crayfish (N = 9) were tethered for 5 days in 50-gallon aquaria to determine whether or not the glue would fail during that time interval and also to determine if crayfish were able to clip the monofilament line. All crayfish remained tethered for the full 5 days. Therefore, it was assumed that all missing crayfish in the stream-based predation assays were either eaten or removed by predators. Given that many studies have successfully tethered crayfish
using this technique (DiDonato and Lodge 1993; Kershner and Lodge 1995; Englund and
Krupa 2000; Flinders and Magoulick 2007a), I believe that this is a reasonable
assumption.

**Statistical Analyses**

The Cox Proportional Hazard Model (Cox 1972) was used to compare temporal
patterns of size-specific predation for different crayfish size classes in each habitat type
2000). As noted in previous studies (e.g., Englund and Krupa, 2000, Clark *et al.* 2008),
this model not only uses actual losses from the population, but also uses data from
“censored observations.” Censored observations for my study included crayfish that
molted or died due to reasons other than predation. A predation event was said to occur
when a piece of carapace was left on the snap swivel, the fishing line was cut, or if a
crayfish was partially eaten. Further, pairwise Cox regressions were used as *post-hoc*
tests to test for differences in survival between habitat types, size classes, and seasons

Habitat-specific effects of water depth (measured during Summer 2005 and 2006,
Spring 2006), average current velocity (Summer 2005 and 2006, Spring 2006), nearbed
current velocity (Spring 2006, Summer 2006) average grain size (Summer 2005, Spring
2006), and maximum grain size (Summer 2005 and 2006, Spring 2006) on crayfish
survival were analyzed using Cox regression. All aforementioned abiotic variables were
converted into categorical variables before analysis to allow grouping of survival curves
based upon these variables. Individual survival curves were generated for each abiotic category within each habitat without consideration of crayfish size class. Categories for water depth were based on maximum water depths at the tethering sites and included shallow (≤ 12 cm), intermediate (13-50 cm), and deep (≥ 51 cm). Categories for current velocity (both average and nearbed) were based on field observations coupled with crayfish responses to current velocity presented in Clark et al. (2008) and included low (≤ 0.9 m/s), intermediate (0.1-0.29 m/s), and high (≥ 0.3 m/s). Grain size categories were based on the Wentworth scale (Gordon et al. 2004) and included clay (0.0001 mm), silt (< 0.063 mm), sand (0.125-2 mm), gravel (3-16 mm), pebble (17-64 mm), cobble (65-256 mm), and boulder (> 256 mm). Silt and sand categories were grouped together due to the low occurrence of silt in these samples.

Wolman pebble count and Camsizer® particle analyzer data were combined to calculate the average grain size for each tethering plot. Wolman pebble count and Camsizer® particle analyzer data was weighted by the percent of grains > 4 mm and < 4 mm, respectively. Grain size was calculated using a fractional grain size curve just as with Summer 2005 PCA grain size data. Separate fractional grain size curves were generated for each tethering plot.

**Grain Size Manipulation Experiment**

In order to assess the effects of refugia as a mediator of predation risk, a predation assay where small crayfish were tethered on manipulated substrate patches was conducted during August 2007. The previously described predation assay methods were
used to set up this experiment with a couple of important exceptions. As a result of patterns of size-specific predation risk observed in earlier assays, this experiment focused on only small crayfish and both males and females were used (due to low crayfish densities. Further, grain size was manipulated using three grain size treatments based on the Wentworth scale (Gordon et al. 2004). The grain size treatments were: sand (range = 0.38-0.57 mm), small pebbles (range = 16-32 mm, b-axis), and small cobbles (range = 64-128 mm, b-axis). All grains were collected downstream of the field site and pre-measured with the exception of sand. To measure the grain size of sand, ten samples were brought back to the lab and analyzed with a Camsizer® particle analyzer (Retsch Technology®). Tethering plots in four deep pools, four shallow pools, and four riffles were selected using methods outlined above. Each tethering plot was randomly assigned one of the three grain size treatments such that all three grain treatments were tested in each habitat type. For experimental setup, a Hess-sampler (34 cm diameter) was placed down at each tethering plot and a complete mono-layer of grains associated with the appropriate treatment was placed and distributed across the area enclosed by the Hess-sampler. Each crayfish was then measure (CL, mm) and tethered on a patch of a single grain size treatment using previously described methods. Five crayfish per grain size treatment were tethered in each replicate of each channel unit type in this experiment (for a total of N = 180 tethering plots). Just prior to tethering, water depth (cm) and average and nearbed current velocity (m/s) were measured in each plot.
Statistical Analyses

The Cox Proportional Hazard Model (Cox 1972) was used to compare predation profiles for small crayfish associated with each habitat type and grain size treatment (SAS 8.01, SAS Institute Inc., 2000). The same censored variables and protocol for assessment of a predation event were used as in the predation assays (described above). Survival of male and female crayfish was not significantly different so data were pooled across sex. Pairwise Cox regressions were used as post-hoc tests to test for differences in survival between grain treatments and habitat types (SAS 8.01, SAS Institute Inc., 2000). Additionally, habitat-specific effects of water depth, average current velocity, and nearbed current velocity were tested using Cox regression with these abiotic variables grouped into the same categories as in analyses of prior predation assays.

Results

Habitat-specific Abiotic Measurements and Crayfish/Fish Survey

Crayfish Survey—Regardless of season and habitat type, large crayfish densities were significantly lower than small and medium crayfish densities (ANOVA; $F_{(44,126)} = 11.18$, $P < 0.0001$; Tukey’s test, $P < 0.05$) (Fig. 14). Size-specific crayfish density was also significantly different among seasons (ANOVA; $F_{(44,126)} = 3.38$, $P = 0.0115$), and habitat types (ANOVA; $F_{(44,126)} = 45.12$, $P < 0.0001$) (Fig. 14). Overall, densities were significantly lower in Spring 2006 than in Summer 2005 and 2006, regardless of crayfish
Fig. 14. Average weighted densities (crayfish/m²) (mean ± 1 SE) of small, medium, and large crayfish in deep pools, shallow pools, and riffles during Summer 2005, Fall 2005, Spring 2006, Summer 2006, and Fall 2006. Black bars = small crayfish, light gray bars = medium crayfish, dark gray bars = large crayfish. Tukey’s results are left out for clarity of the figure.
size class and habitat type (Tukey’s test, $P < 0.05$) (Fig. 14). Fall 2005 and 2006 densities were similar to both Summer 2005 and 2006 and Spring 2006 densities (Tukey’s test, $P > 0.05$) (Fig. 14). Regardless of crayfish size class and season, crayfish densities in shallow pools were significantly higher than in deep pools and riffles (Tukey’s test, $P < 0.05$) (Fig. 14). Additionally, the interactions of size class by season (ANOVA; $F_{44,126} = 9.79$, $P < 0.0001$), size class by habitat type (ANOVA; $F_{(44,126)} = 7.19$, $P < 0.0001$), and size class by season by habitat type were significant (ANOVA; $F_{(44,126)} = 2.78$, $P = 0.0007$) (Fig. 14). The size class by season interaction is likely due to seasonal cycles of growth and reproduction whereas, the size class by habitat interaction can be attributed to habitat-specific abiotic or biotic factors affecting distribution patterns. A significant interaction of size class by season by habitat type is likely due to a combination of the aforementioned factors. However, the interaction of season by habitat type was not significant (ANOVA; $F_{(44,126)} = 1.17$, $P = 0.3213$) (Fig. 14).

**Fish Predator Survey**—Average fish predator size was significantly different during Summer 2005 (ANOVA, $F_{(2,21)} = 11.85$, $P = 0.0041$) and Summer 2006 (ANOVA, $F_{(2,21)} = 5.28$, $P = 0.0346$) with fish predators being significantly larger in deep pools than in riffles (Tukey’s test, $P < 0.05$), yet similar in deep and shallow pools (Tukey’s test, $P > 0.05$) (Fig 15). Similarly, fish predator size was significant during Fall 2005 (ANOVA, $F_{(2,21)} = 10.17$, $P = 0.0063$) and Fall 2006 (ANOVA, $F_{(2,24)} = 27.54$, $P = 0.0001$) but not during Spring 2006 (ANOVA, $F_{(2,21)} = 3.85$, $P = 0.0674$) (Fig. 15). During Fall 2005 and 2006, fish predators were significantly larger in deep pools than in both shallow pools
Fig 15. Average predatory fish total lengths (cm) (mean ± 1 SE) in deep pools, shallow pools, and riffles during Summer 2005, Fall 2005, Spring 2006, Summer 2006, and Fall 2006. Capital letters above error bars indicate Tukey’s results. Different letters indicate significant differences ($P < 0.05$).
Average Predatory Fish Total Length (cm)

- **Summer 2005**
  - DP: A
  - SP: AB
  - R: B

- **Fall 2005**
  - DP: A
  - SP: B
  - R: B

- **Spring 2006**
  - DP: A
  - SP: B
  - R: B

- **Summer 2006**
  - DP: A
  - SP: AB
  - R: B
and riffles (Tukey’s test, \( P < 0.05 \)) and fish predator size was similar in shallow pools and riffles (Tukey’s test, \( P > 0.05 \)) (Fig 15).

**Crayfish and Predatory Fish Comparisons**—Correlations of size-dependent crayfish density and fish predator density were not significant for any season with the exception of a significant negative correlation between large crayfish and fish predators during Summer 2005 (Table 4). However, crayfish carapace length and predatory fish total length were significantly and positively correlated during Summer 2005 (\( r^2 = 0.61, P = 0.0047 \)), Fall 2005 (\( r^2 = 0.48, P = 0.0055 \)), Spring 2006 (\( r^2 = 0.47, P = 0.0284 \)), Summer 2006 (\( r^2 = 0.54, P = 0.0098 \)), and Fall 2006 (\( r^2 = 0.71, P = 0.0006 \)) (Fig. 16).

**Summer 2005 Principal Components Analysis**—Axis 1 and 2 explained 65% and 30% of the variance in the crayfish data, respectively (Fig. 17). Large crayfish density was related more strongly to Axis 2, exhibiting strong positive associations with deep pool habitats and increased water depth (Fig. 17). In contrast, large crayfish were negatively associated with riffle habitats and increased current velocity and predator density. Small and medium crayfish densities were more strongly related to Axis 1 exhibiting strong positive associations with shallow pool habitats and increased average grain size and negative associations with increased fish predator size (Fig. 17).

*Represents a significant result (at $P \leq 0.05$).

<table>
<thead>
<tr>
<th>Season</th>
<th>Crayfish Size Class</th>
<th>$r^2$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer 2005</td>
<td>Small</td>
<td>0.07</td>
<td>0.4073</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>0.01</td>
<td>0.7793</td>
</tr>
<tr>
<td></td>
<td>Large</td>
<td>0.37</td>
<td>0.0345*</td>
</tr>
<tr>
<td>Fall 2005</td>
<td>Small</td>
<td>0.04</td>
<td>0.5484</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>0.14</td>
<td>0.2568</td>
</tr>
<tr>
<td></td>
<td>Large</td>
<td>0.10</td>
<td>0.3461</td>
</tr>
<tr>
<td>Spring 2006</td>
<td>Small</td>
<td>0.17</td>
<td>0.2052</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>0.02</td>
<td>0.7175</td>
</tr>
<tr>
<td></td>
<td>Large</td>
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</tr>
<tr>
<td>Summer 2006</td>
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<td>0.5269</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>0.07</td>
<td>0.4161</td>
</tr>
<tr>
<td></td>
<td>Large</td>
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</tr>
<tr>
<td>Fall 2006</td>
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<td>0.04</td>
<td>0.5521</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>0.02</td>
<td>0.6417</td>
</tr>
<tr>
<td></td>
<td>Large</td>
<td>0.04</td>
<td>0.5386</td>
</tr>
</tbody>
</table>
Fig. 16. Comparison of average crayfish carapace length (CL, mm) (mean ± 1 SE) versus average predatory fish total length (TL, cm) (mean ± 1 SE) during Spring (2006), Summer (2005 and 2006), and Fall (2005 and 2006). Dark circles = 2005 and light circles = 2006.
Fig. 17. Correlation-based PCA displaying axis 1 and 2 with small, medium, and large crayfish densities correlated to current velocity, water depth, average grain size, fish predator density, and average fish predator total length in all 12 sample sites during Summer 2005.
Survey of Predatory Fish Diets

No crayfish parts were found in gut samples from *H. nigricans*, *C. commersoni*, *L. cyanellus*, or *M. erythrurum*. However, *L. cyanellus* were observed preying upon crayfish during predation assays and were therefore included in all of the fish predator analyses. Crayfish parts were found in gut samples of *C. bairdi* during Summer (proportion of diets containing the following prey items: crayfish = 0.09, insects = 0.98, other = 0.05, empty = 0.02) and Fall (proportion of diets containing the following prey items: crayfish = 0.01, insects = 0.87, other = 0.08, empty = 0.12), *S. atromaculatus* during Summer (proportion of diets containing the following prey items: crayfish = 0.30, insects = 0.65, other = 0.35, empty = 0.10), and *A. rupestris* during Summer (proportion of diets containing the following prey items: crayfish = 0.57, insects = 0.86, other = 0.43, empty = 0.14) (Fig. 18). Crayfish were not found in the gut contents of any fish during Spring (Fig. 18). Although *C. bairdi* did consume crayfish, they consumed them at lower levels than either *S. atromaculatus* and *A. rupestris* (Fig. 18).

Predation Assays

*Summer 2005*—The main effects of crayfish size class (Cox regression, $\chi^2 = 10.04, P = 0.0015$) and habitat type (Cox regression, $\chi^2 = 17.53, P < 0.0001$) significantly affected crayfish survival during Summer 2005 (Fig. 19). Regardless of habitat type, small crayfish survival was significantly lower than both medium (Cox regression, $\chi^2 = 12.95$, $P = 0.0003$) and large crayfish (Cox regression, $\chi^2 = 7.41, P = 0.0065$) (Fig. 19).
Fig. 18. Proportion of fish diets containing crayfish parts, insect parts, and/or other items or empty guts for A) Cottus bairdi, B) Semotilus atromaculatus, C) Ambloplites rupestris during Spring (2006), Summer (2005 and 2006), and Fall (2005 and 2006). Sample size represents the number of fish in which gut contents were sampled and is indicated for each season. Black bars = crayfish parts, medium gray bars = insect parts, dark gray bars = other items, and light gray bars = empty guts.
Crayfish

Insects

Other

Empty

A

0.0

N=9

N=134

N=85

B

0.0

N=13

N=20

N=46

C

0.0

N=1

N=7

N=2

C. bairdi

S. atromaculatus

A. rupestris

Proportion found in gut

Spring

Summer

Fall
Fig. 19. Average percent survival (mean ± 1 SE) of small, medium, and large crayfish in deep pools, shallow pools, and riffles during Summer 2005. Dark circles = deep pools, light circles = shallow pools, and dark squares = riffles.
However, medium and large crayfish had similar survival (Cox regression, $\chi^2 = 1.23, P = 0.2680$) (Fig. 19). Regardless of crayfish size class, survival was significantly lower in deep pools than in either riffles (Cox regression, $\chi^2 = 14.57, P = 0.0001$) or shallow pools (Cox regression, $\chi^2 = 5.96, P = 0.0146$) (Fig. 19). Survival of crayfish was similar in shallow pools and riffles (Cox regression, $\chi^2 = 2.11, P = 0.1460$) (Fig. 19).

During Summer 2005, post-hoc Cox regressions showed habitat-specific differences in crayfish survival only for small crayfish. Small crayfish had significantly lower survival in both deep pools (Cox regression, $\chi^2 = 12.25, P = 0.0005$) and shallow pools (Cox regression, $\chi^2 = 8.79, P = 0.0030$) relative to riffles (Fig. 19). Survival of small crayfish was similar in deep pools and shallow pools (Cox regression, $\chi^2 = 1.94, P = 0.1642$) (Fig. 19). However, both medium (Cox regression, deep pools vs. shallow pools, $\chi^2 = 1.85, P = 0.1742$; deep pools vs. riffles, $\chi^2 = 3.61, P = 0.0575$; shallow pools vs. riffles, $\chi^2 = 0.20, P = 0.6577$) and large (Cox regression, deep pools vs. shallow pools, $\chi^2 = 3.63, P = 0.0567$; deep pools vs. riffles, $\chi^2 = 0.95, P = 0.3300$; shallow pools vs. riffles, $\chi^2 = 0.95, P = 0.3297$) crayfish had similar survival in each habitat type (Fig. 19).

Post-hoc Cox regressions showed some size-specific differences in crayfish survival by habitat type. Survival of small crayfish was significantly lower in deep pools and shallow pools than both medium (Cox regression, deep pools, $\chi^2 = 7.36, P = 0.0067$; shallow pools, $\chi^2 = 7.54, P = 0.0060$) and large crayfish (Cox regression, deep pools, $\chi^2 = 7.89, P = 0.0050$; shallow pools, $\chi^2 = 8.82, P = 0.0030$) (Fig. 19). However in riffle habitats, survival of small crayfish was similar to both medium (Cox regression, $\chi^2 = 0.88, P = 0.3494$) and large crayfish (Cox regression, $\chi^2 = 0.30, P = 0.5853$) (Fig. 19).
Spring 2006—During Spring 2006, habitat type significantly affected crayfish survival (Cox regression, $\chi^2 = 32.59$, $P < 0.0001$) regardless of crayfish size class (Fig 20a-f). Survival was significantly higher in both shallow pools (Cox regression, $\chi^2 = 22.06$, $P < 0.0001$) and riffles (Cox regression, $\chi^2 = 22.71$, $P < 0.0001$) than in deep pools (Fig. 20a-f). Survival was similar in shallow pools and riffles (Cox regression, $\chi^2 = 2.04$, $P = 0.1530$) (Fig. 20b,c,e,f). However, crayfish size class did not affect crayfish survival as small crayfish survival was similar to medium crayfish survival regardless of habitat type (Cox regression, $\chi^2 < 0.01$, $P = 0.9677$) (Fig 20a-f).

Summer 2006—Similar to Summer 2005, both crayfish size class (Cox regression, $\chi^2 = 11.70$, $P = 0.0006$) and habitat type (Cox regression, $\chi^2 = 9.19$, $P = 0.0024$) significantly affected crayfish survival during Summer 2006 (Fig. 20a-i). Regardless of habitat type, small crayfish had significantly lower survival than both medium (Cox regression, $\chi^2 = 21.41$, $P < 0.0001$) and large crayfish (Cox regression, $\chi^2 = 9.36$, $P = 0.0022$) (Fig. 20a-i). Medium and large crayfish survival did not differ significantly (Cox regression, $\chi^2 = 2.84$, $P = 0.0920$) (Fig. 20a-i). Regardless of crayfish size class, crayfish survival was significantly lower in deep pools than both shallow pools (Cox regression, $\chi^2 = 3.91$, $P = 0.0480$) and riffles (Cox regression, $\chi^2 = 7.93$, $P = 0.0049$) (Fig. 20a-i). Survival in shallow pools relative to riffles was not significantly different (Cox regression, $\chi^2 = 0.68$, $P = 0.4096$) (Fig. 20b,c,e,f,h,i).

However, during Summer 2006, post-hoc Cox regressions showed some habitat-specific differences in crayfish survival for small and large crayfish. Small (Cox
Fig. 20. Average percent survival (mean ± 1 SE) of small crayfish in A) deep pools, B) shallow pools, and C) riffles during Spring and Summer 2006, medium crayfish in D) deep pools, E) shallow pools, and F) riffles during Spring and Summer 2006, and large crayfish in G) deep pools, H) shallow pools, and I) riffles during Summer 2006. Dark circles = Spring 2006 and light circles = Summer 2006.
regression, $\chi^2 = 11.03, P = 0.0009$) and large crayfish (Cox regression, $\chi^2 = 4.75, P = 0.0293$) had significantly lower survival in deep pool than riffle habitats (Fig 20a,c,g,i). Medium crayfish survival was not significantly different in deep pools and riffles (Cox regression, $P > 0.05$) (Fig. 20d,f). Similarly, survival was not significantly different in deep pools and shallow pools or shallow pools and riffles for any crayfish size class (Cox regression, all $P > 0.05$) (Fig. 20a-i).

Post-hoc Cox regressions showed some size-specific differences in crayfish survival by habitat type. Survival of small crayfish was significantly lower than medium crayfish survival (Cox regression, deep pools, $\chi^2 = 13.75, P = 0.0002$; shallow pools, $\chi^2 = 7.30, P = 0.0069$) in both deep pools and shallow pools (Fig. 20a,c,d,f). However, small crayfish survival was similar to large crayfish survival in deep pools and shallow pools (Cox regression, all $P > 0.05$) (Fig. 20a,c,g,i). Similarly, medium and large crayfish survival was similar in deep pools and shallow pools (Cox regression, all $P > 0.05$) (Fig. 20d,e,g,h). In riffles, survival of all three crayfish sizes was similar (Cox regression, all $P > 0.05$) (Fig. 20c,f,i).

Seasonal Comparisons—No significant differences in survival occurred between Summer 2005 and 2006 for any crayfish size class in any habitat type (Cox regression, all $P > 0.05$). However, some seasonal differences occurred between Spring and Summer 2006 for small and medium crayfish. During Spring 2006, small crayfish survival was significantly higher in shallow pools (Cox regression, $\chi^2 = 5.69, P = 0.0171$) and riffles (Cox regression, $\chi^2 = 5.57, P = 0.0182$) than during Summer 2006 (Fig 20b,c). However,
there was no seasonal effect on small crayfish survival in deep pools (Cox regression, $\chi^2 = 0.33, P = 0.5633$) (Fig 20a,d).

Medium crayfish survival was similar in both shallow pools (Cox regression, $\chi^2 = 0.79, P = 0.3751$) and riffles (Cox regression, $\chi^2 = 0.42, P = 0.5193$) (Fig. 20e,f) between Spring and Summer 2006. However, in deep pools, medium crayfish survival was significantly lower during Spring 2006 than Summer 2006 (Cox regression, $\chi^2 = 10.41, P = 0.0013$).

*Habitat-specific Abiotic Effects on Survival*—Abiotic variables only affected survival of crayfish only during Summer 2005. Regardless of crayfish size, water depth in shallow pools (Cox regression, $\chi^2 = 4.52, P = 0.0335$) and average current velocity in riffles significantly affected crayfish survival during Summer 2005 (Cox regression, $\chi^2 = 4.89, P = 0.0271$). Survival in shallow water was significantly higher than at intermediate water depths in shallow pools (Cox regression, $\chi^2 = 4.24, P = 0.0394$), whereas no statistically significant differences in crayfish survival occurred between either shallow vs. deep (Cox regression, $\chi^2 = 0, P = 0.9961$) or intermediate vs. deep water depths (Cox regression, $\chi^2 = 0.08, P = 0.7737$).

In riffles, regardless of crayfish size, survival was significantly lower at low than high average current velocities (Cox regression, $\chi^2 = 4.62, P = 0.0316$), whereas, no significant differences occurred between either low and intermediate or intermediate and high average current velocities (Cox regression, all $P > 0.05$). Crayfish survival was not significantly affected by any other habitat-specific abiotic variables during Summer 2005.
(Cox regression, all $P > 0.05$). Furthermore, habitat-specific abiotic variables did not affect overall crayfish survival in any habitat type during Spring 2006 or Summer 2006 (Cox regression, all $P > 0.05$).

**Grain Size Manipulation Experiment**

Habitat type (Cox regression, $\chi^2 = 57.50$, $P < 0.0001$) and grain size (Cox regression, $\chi^2 = 13.56$, $P = 0.0002$) significantly affected survival of small crayfish (Fig. 21). Regardless of grain size, survival in riffles was significantly higher than in both deep pools (Cox regression, $\chi^2 = 41.62$, $P < 0.0001$) and shallow pools (Cox regression, $\chi^2 = 28.76$, $P < 0.0001$) (Fig 21). Additionally, survival of small crayfish was significantly higher in shallow pools than deep pools (Cox regression, $\chi^2 = 6.85$, $P = 0.0089$) (Fig. 21).

Furthermore, survival on cobble plots was significantly higher than on sand (Cox regression, $\chi^2 = 6.66$, $P = 0.0099$) plots, but similar on cobble and gravel plots (Cox regression, $\chi^2 = 0.60$, $P = 0.4383$) (Fig. 21). Small crayfish survival appeared to be higher on gravel than on sand but was not significant (Cox regression, $\chi^2 = 3.13$, $P = 0.0768$) (Fig. 21). There was no effect of habitat-specific abiotic variables (current velocity and water depth) on survival (Cox regression, all $P > 0.05$).
Fig. 21. Average percent survival (mean ± 1 SE) of small crayfish in deep pools, shallow pools, and riffles on sand, gravel, and cobble plots during Summer 2007. Dark squares = sand plots, light circles = gravel plots, and dark circles = cobble plots.
Discussion

Overall, small and medium crayfish were found in highest densities in shallow pool habitats with large crayfish found in highest densities in deep pools. Crayfish appeared to move from riffles into deep pool habitats as they became larger, showing a distinct shift in habitat use. Ontogenetic shifts in habitat use are common in crayfish populations (DiStefano et al. 2003) with large crayfish tending to be more broadly distributed across habitat types than small crayfish (Flinders and Magoulick 2007b). However, in my study, large crayfish were not more broadly distributed than small crayfish. Large crayfish primarily occupied deep and shallow pools and were rarely found in riffles. In turn, small crayfish primarily occupied shallow pool and riffle habitats and were rarely found in deep pools. However, medium-sized crayfish were much more broadly distributed than either small or large crayfish and were found in all three habitat types.

In addition to habitat effects, season played an important role in affecting size-specific crayfish density patterns. Small crayfish were the dominant size class during the fall and spring whereas medium-sized crayfish were dominant during the summer. Although present during each season at low densities, large crayfish are particularly uncommon during spring. Orconectes crayfish typically only live for three years (Holdich 2002) and low spring abundance could be due to fall/winter die-off. Ultimately, seasonal shifts in size-specific crayfish density observed in this study were consistent with the typical life and reproductive cycles for this species of crayfish (Clark, personal observation). In fact, it is likely that the significant interactions of crayfish size class by
season, season by habitat, and crayfish size class by season by habitat can be attributed to
the seasonal cycles of mortality and reproduction for *O. obscurus*.

Furthermore, ontogenetic shifts in habitat use may be governed by differences in
habitat-specific risk of predation for individual crayfish size classes. Although all
crayfish size classes were vulnerable to predation, small crayfish were significantly more
vulnerable than either medium or large crayfish regardless of habitat type. Overall, small
crayfish showed lowest survival in deep and shallow pools, and considerably higher
survival in riffles. Medium and large crayfish, however, had similar predation risk across
all habitat types with the exception of large crayfish having lower survival in deep pools
than riffles during Summer 2006. However, not all streams follow these size- and
habitat-specific patterns. Small, juvenile *O. sanborni* had much higher mortality in deep
pools than in shallow pools and riffles, which shared similarly low juvenile mortality
(Mather and Stein 1993b). In Ozark streams, *O. marchandi* had higher mortality in
deeper water than shallow habitats, but did not show size-selective mortality (Flinders
and Magoulick 2007a). Since size- and habitat-specific mortality are not consistent
across streams, predator types (i.e., aquatic vs. terrestrial) and the complexity of the
predator assemblage (e.g., diversity and abundance) probably play a major role in
affecting size- and habitat-specific crayfish mortality and distribution patterns.

Fish predators were present in each habitat type, but were likely limited in the size
range of crayfish that were susceptible to predation due to fish gape limitations and
relative size. For example, in this system, the abundant central mottled sculpin (abundant
in riffles and shallow riffles) reaches a maximum total length of 10 cm and have gape
sizes capable of consuming only very small crayfish. Based upon diet analyses, central mottled sculpin were weak crayfish predators with only a very small proportion of the population consuming only very small crayfish. While creek chubs were relatively abundant in both shallow and deep pools and sometimes very large, they were also relatively weak predators on crayfish (except during summer). Other predaceous fish, such as green sunfish and rock bass, are more common in deep pools than in shallow-water habitats and are efficient crayfish predators (rock bass, Rabeni 1992; green sunfish, Englund and Krupa 2000). Both of these fish species (uncommon in this stream) were observed preying upon small (but not medium or large) crayfish in deep pools during tethering experiments (as were creek chubs). While green sunfish diets contained no crayfish, rock bass diets during summer commonly contained crayfish, demonstrating their ability as crayfish predators.

Small, juvenile crayfish are generally more vulnerable to predation as many predaceous fish are gape limited (Stein and Magnuson 1976, Stein 1977, Rabeni 1992, DiDonato and Lodge 1993, Kershner and Lodge 1995, Englund and Krupa 2000, this study). Further, even when not gape-limited, predaceous fish tend to prey upon smaller crayfish, reducing handling costs and increasing capture rates as a function of smaller crayfish chelae (Stein 1977). Chela displays are often used by crayfish to deter fish predators (Bovbjerg 1956, Stein and Magnuson 1976), but are likely only a threat from larger crayfish, contributing to their lower vulnerability to fish predation (Rabeni 1992). Ultimately, size-selective feeding exhibited by predaceous fish (Rabeni 1992, Mather and Stein 1993a) may be an important driver of crayfish population dynamics.
Overall, field surveys showed that small and medium crayfish were negatively correlated with large fish predators and these two size classes of crayfish were in lower densities in deep pool habitats where large fish predators were common. Large crayfish, however, were associated with deeper water depths (≥ 51 cm) and thus, positively correlated with large, fish predators. Water depth is often important in structuring crayfish populations (Flinders and Magoulick, 2003, 2005 2007a,b), with larger crayfish generally associated with deep water (Usio and Townsend 2000). However, water depth may only be important when fish predation is a factor, even for small crayfish (Mather and Stein 1993a,b, Englund and Krupa 2000, Usio and Townsend 2000). For example, in Kentucky streams, when fish predators were present, small crayfish occupied shallow water habitats, whereas, in the absence of fish predators, small crayfish were distributed across a broader depth range (Englund and Krupa 2000). Large crayfish, however, did not alter habitat use in the presence of a predator (Englund and Krupa 2000). The pattern of larger fish in deep water and smaller fish in shallow water is common in streams (Schlosser 1982, Power 1984, Harvey and Stewart 1991) and also holds for crayfish populations in a variety of stream systems (Rabeni 1985, Creed 1994, Englund and Krupa 2000, Usio and Townsend 2000, DiStefano et al. 2003, Flinders and Magoulick 2003, 2007b, this study). In this study, fish predator density did not affect size-specific distribution patterns, suggesting that predator size may be more important in structuring crayfish populations than predator density.

Other aquatic predators, such as spiny softshell turtles (*Apalone spinifera* L.), painted turtles (*Chrysemys picta* Schneider), and common snapping turtles (*Chelydra*
serpentina L.), semi-aquatic predators such as North American river otter, and terrestrial predators such as belted kingfishers (Megaceryle alcyon L.), great blue herons, green herons (Butorides virescens L.), and raccoons were common visitors to this system and all can be predators on crayfish. These diving and wading predators are likely culprits (over fish) for predation on medium and large crayfish due to the larger gape sizes of these predators. Half-eaten medium and large crayfish occurred in each of the three habitat types and, in some cases, body parts were scattered around tethering locations, indicating that aquatic, semi-aquatic, and/or terrestrial predators were all preying upon crayfish. Furthermore, cannibalism may play a role in size-specific mortality. In this study, a large female crayfish was observed attacking, killing, and feeding on a small crayfish tethered at a deep pool plot. Thus, it appears that large crayfish have the ability to limit juvenile survival both directly via cannibalism and/or indirectly by suppressing growth and activity (Blake et al. 1994). Ultimately, predation does not seem to be a major structuring force for medium and large crayfish distributions due to the minimal habitat-specific differences in survival for these two size classes. In contrast, predation does have a major impact on small crayfish density as their distribution patterns tend to be negatively related to habitat-specific predation risk.

Although there were no temporal shifts in habitat use by specific crayfish size classes, season did play a role in crayfish survival. Survival of small crayfish was significantly higher in shallow pools and riffles during spring than summer, implying that these shallow water habitats are less risky during spring. Survival of medium crayfish, however, was lower in deep pools during spring than summer and similar to small
crayfish survival, indicating that predation was less size-selective in deep pools during spring. Seasonal effects on size-specific survival appear to be complex and could be due to a variety of factors including seasonal differences in food availability, predator assemblages, and/or activity rates of crayfish -- all of which may alter predation pressure on crayfish. However, there were no crayfish in predatory fish diets during spring, with the most evidence for crayfish consumption being found during the summer. Despite the implication from predatory fish diets that predation risk was lower during spring and fall, predation assays demonstrated that predation risk for crayfish was highest in habitats occupied by large fish predators, leaving open the possibility that predators other than deep water predators (e.g., turtles, otters, or other crayfish) may have been playing a role.

In addition to seasonality, abiotic factors (i.e., current velocity, substrate) can have complex interactions with predation risk. Although crayfish densities were strongly associated with some abiotic variables in field surveys, abiotic-predation interactions were not consistent across tethering assays, implying that predation outweighs the effects of abiotic variables. However, the grain manipulation tethering experiment suggested that large rocky refugia (cobbles) can increase survival with survival on cobble plots being significantly higher than on sand plots. Similarly, mortality was lower for three species of Orconectes crayfish on dense cobble than smaller substrates (Garvey et al. 1994). Interstitial spaces between larger substrates provide better refugia against predation than smaller substrates (Stein and Magnuson 1976).
In fact, small and medium crayfish were both strongly correlated with habitats containing large average grain sizes. In fact, crayfish abundance is often positively associated with increasing substrate size (Lodge and Hill 1994, Streissl and Hödl 2002, Parkyn and Collier 2004, Flinders and Magoullick 2005, Pockl and Streissl 2005) with crayfish densities generally negatively associated with sandy substrate (Kershner and Lodge 1995). Crayfish substrate associations are also size-dependent with juveniles occupying habitat with large particle sizes (e.g., cobbles, boulders) and large crayfish occupying patches with small particle sizes (e.g., sand) (Usio and Townsend 2000, Clark et al. 2008). Large crayfish in this study were associated with habitats characterized by small grain sizes (sand and clay), likely resulting from reduced predation risk and thus, a corresponding decreased need of refugia. Predation is an important source of mortality in early life stages of organisms and can restrict juveniles to refugia while adults may range freely (Stein and Magnuson 1976, Werner et al. 1983a,b, Schlosser 1987).

Large rocky refugia can be limited in some habitats (especially deep pools) (Clark et al. 2008) and while it is important to crayfish survival (Stein and Magnuson 1976, Garvey et al. 1994, Hill and Lodge 1994, Kershner and Lodge 1995, Englund 1999, Englund and Krupa 2000, this study), other refugia including leaf packs and benthic substrate for burrowing may also be important. As anecdotal evidence for this, a single small crayfish survived at a deep pool tethering plot for the full 5-day experiment during Spring 2006. This plot had a leaf pack covering the sediment that the tethered crayfish used as refugia. Given that leaf packs are common in streams, they may represent important refugia for crayfish from predators. Further, one medium crayfish was
burrowed into clay at a deep pool tethering plot, which may also be an important tactic for survival. Crayfish can also be positively associated with woody debris (Usio and Townsend 2000), as it likely represents refuge from predation. Lastly, substrate complexity and heterogeneity (i.e., vegetation and grain sorting) can provide refugia for prey from predators (Stein 1977, Savino and Stein 1982, Holomuzki 1989). Predation typically decreases as structural complexity/heterogeneity increases (Crowley 1981, Holt 1984, Closs et al. 1999) as predator foraging efficiency decreases with decreased visual and tactile cues of prey (Savino and Stein 1982, Gotceitas and Colgan 1989). Given this, it is likely that streams with diverse substrate types may have higher levels of survival.

Although predation plays a major role in affecting distributions of small crayfish, other factors are also important (for all size classes). Intraspecific (Lodge and Hill 1994) and interspecific (Miller et al. 1992, Lodge and Hill 1994, Garvey et al. 2003) competition can influence size distributions of crayfish populations. For example, small, juvenile crayfish are often competitively excluded from preferred, slower-flowing habitats by aggressive adults (Lodge and Hill, 1994). Large crayfish can also competitively exclude smaller crayfish from larger substrate (Bovbjerg 1970). Furthermore, large crayfish can modify the spatial distribution of smaller size classes (Rabeni 1985) and can influence juvenile survival by decreasing juvenile activity and growth (Blake et al. 1994).

Furthermore, small benthic fishes, such as sculpin, commonly share the same food resources and predators (Dorn and Mittelbach 1999) and may have important interspecific interactions with crayfish. In a controlled lab setting, slimy sculpin (Cottus
cognatus) suppressed crayfish feeding rates (O. virilis) (Miller et al. 1992), suggesting that sculpin may outcompete crayfish for food resources. Crayfish may be competitively excluded from optimal habitats by sculpin and forced into a suboptimal diet (Miller et al. 1992). Thus, it is also possible that central mottled sculpin are significant competitors, pushing crayfish out of riffles and into habitats with higher predation risk.

Food availability also affects crayfish distribution patterns (Nyström et al. 2006, Flinders and Magoulick 2007a). In the presence of predators, crayfish prefer substrates that provide the greatest refugia (Lodge and Hill 1994). When predators are absent, crayfish prefer substrates with the greatest food availability (Lodge and Hill 1994). In fact, predation risk causes organisms to occupy habitats that offer greater refuge from predation but diminished food availability (Sih 1982, Werner et al. 1983b, Holbrook and Schmitt 1988, Pettersson and Brönmark 1993, Turner 1997, McIntosh et al. 2004), implying that predation risk trumps food availability as a structuring mechanism. This scenario appears to be borne out for small crayfish in this study.

**General Conclusions**

Although all crayfish sizes were vulnerable to predation, predation was not a major structuring mechanism for medium or large crayfish distributions, however, it did significantly influence small crayfish distributions. Deep pools and shallow pools are highly risky habitats for smaller crayfish; thus, one would predict that these habitats would have low densities of small crayfish. This prediction is borne out in deep pools, however, small crayfish densities were highest in shallow pools. While surprising at first,
tethering experiments demonstrated that large rocky substrates can provide necessary refugia from predators, allowing small crayfish to survive in high risk habitats like shallow pools. Small crayfish densities in riffles were typically much lower than in shallow pools, suggesting that while predation risk is low, riffles are not preferred habitat. Current velocity and/or central mottled sculpin density may be limiting small crayfish densities in riffles. However, in this stream, riffle current velocities were within tolerable limits for this species (Clark et al. 2008) and, therefore, are unlikely to be substantively limiting their distribution. While diet data indicate that sculpin were weak predators, they have relatively high densities in riffles and may play a more significant role as a competitor than as a predator. In contrast, larger crayfish were widely distributed across habitats and had much lower predation risk.

In summary, while predation risk is a major structuring force, it is not the only factor affecting crayfish distribution and probably interacts with substrate availability, intra- and inter-specific competition, and food availability. Although still unclear, the factors affecting medium and large crayfish density may include a combination of current velocity (Clark et al. 2008), food availability, and competitive interactions.

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References


(Orconectes virilis) and the slimy sculpin (Cottus cognatus). Journal of


experiments to estimate Pleistocene tsunami size at Molokai, Hawaii. PhD

Dissertation, University of Washington.

Nyström, P., P. Stenroth, N. Holmqvist, O. Berglund, P. Larsson, W. Graneli. 2006.

Crayfish in lakes and streams: individual and population responses to predation,

productivity, and substratum availability. Freshwater Biology 51:2096-2113.


crayfish populations: flood impacts in pasture and forest streams. Hydrobiologia

527:114-124.


dependent habitat choice and foraging in crucian carp. Oecologia 95:353-357.


quality in running waters? Bulletin Francais De La Peche Et De La Pisciculture

376-377:743-758.

Poos, M. S., N. E. Mandrak, and R. L. McLaughlin. 2007. The effectiveness of two

common sampling methods for assessing imperiled freshwater fishes. Journal of

Fish Biology 70:691-708.


Trautman, M. B. 1957. Fishes of Ohio. The Ohio State University Press, Ohio, USA.


CHAPTER 6

SIZE-STRUCTURED CRAYFISH (ORCONETES OBSCURUS) RESPONSES TO LOTIC FISHES: COUPLING FIELD SURVEYS WITH BEHAVIORAL ASSAYS

Abstract

In many small streams, crayfish and fish follow a pattern of deep water-big crayfish/fish and shallow water-small crayfish/fish. However, mechanisms driving this pattern are not well understood. In this study, I used a field survey to examine size relationships between crayfish (Orconectes obscurus) and fish community in three habitat types in a 4th-order stream. To begin to examine field patterns of size-structured crayfish/fish distributions, I used laboratory-based behavioral experiments to assess size-dependent responses of small [carapace length (CL) ≤ 19.9 mm], medium (CL = 20-20.9 mm), and large (CL > 29.9 mm) crayfish to two species of large-bodied deep water fishes, creek chub (Semotilus atromaculatus, crayfish predator), common white sucker (Catostomus commersoni, non-crayfish predator), two species of small-bodied, shallow water fishes central mottled sculpin (Cottus bairdi, crayfish predator), and fantail darter (Etheostoma flabellare, non-crayfish predator), and large male O. obscurus. Crayfish responses to these fish and crayfish were tested in a flow-through y-maze built to allow a combination of visual and chemical cues to be received by the crayfish. In the field
survey, crayfish size was positively correlated with fish size, regardless of fish species, indicating that fish size (and perceived predation potential) may contribute to size-specific crayfish distributions. Individual fish species had significant correlations of body size with crayfish size. However, these correlations were not consistent across seasons, implying that on a species level, fish populations are dynamic and may not contribute substantively to seasonal crayfish distribution patterns. Furthermore, crayfish did not display either avoidance or attraction relative to any fish species or large conspecific crayfish during behavioral experiments. In fact, the behavioral experiments demonstrated that visual or chemical cues of creek chub, common white sucker, central mottled sculpin, fantail darter, and large conspecifics did not significantly alter size-specific movement or distribution patterns of *O. obscurus*. However, it remains to be determined whether or not crayfish show this lack of response to all predators, particularly northern rockbass (*Ambloplites rupestris*) and green sunfish (*Lepomis cyanellus*).

**Introduction**

In many aquatic systems, predators can influence both prey distribution and abundance (Stein 1977, Power 1987, Schlosser 1987, Harvey and Stewart 1991, Englund 1999). Crayfish, in particular, display dramatic responses to predators, being affected both directly through consumption (Rabeni 1992, Mather and Stein 1993a, Clark, chapter 5) and indirectly through behavioral responses, which are often associated with using refugia (Stein and Magnuson 1976, Garvey et al. 1994), shifting into shallow water
habitats (Mather and Stein 1993b, Englund and Krupa 2000), and reducing activity (Stein and Magnuson 1976, Mather and Stein 1993a, Garvey et al. 1994). Furthermore, field data generally indicate that crayfish abundance is negatively correlated with fish predator abundance (Mather and Stein 1993a, Kershner and Lodge 1995, Englund 1999, Englund and Krupa 2000).

However, many systems, especially streams with shallow areas, can have multiple types of predators, leading to the potential for complex predator-prey interactions (Power 1987, Rahel and Stein 1988, McNeely et al. 1990). Since crayfish are vulnerable to both aquatic and terrestrial predators, complex interactions may occur leading to tradeoffs in habitat choice. Juvenile predators are particularly vulnerable to fish predators (Rabeni 1992, DiDonato and Lodge 1993, Mather and Stein 1993a, Clark, chapter 5) whereas, in many systems, larger adults are released from predation pressure due to their large body size, defensive behavior, and gape limitations of individual fish species. However, larger adults are susceptible to terrestrial predators [e.g., raccoons (Procyon lotor) and great blue herons (Ardea herodias)] and other aquatic or semi-aquatic predators such as North American river otters (Lontra canadensis) and spiny softshell turtles (Apalone spinifera), all of which have much larger gape sizes than most fishes.

In small streams, small crayfish are typically found in shallow habitats whereas large crayfish are found in deeper water (Rabeni 1985, Creed 1994, Usio and Townsend 2000, Clark et al. 2008). This fits the pattern of the big fish-deeper habitat hypothesis developed for stream fishes where larger fishes tend to be associated with deeper water habitats and smaller fishes occur in shallower water habitats, likely to minimize predation.
risk (Power 1987). Many stream fishes follow this pattern (Schlosser 1982, Power 1984, Schlosser 1987, Harvey and Stewart 1991), and in many systems, crayfish also follow this pattern (Mather and Stein 1993a, Englund and Krupa 2000, Clark, chapter 5). However, studies implying that crayfish hold to the “bigger fish-deeper water” are mostly correlative and mechanisms underlying this pattern are not well-understood. Potential mechanisms include past history/encounters with predators, chemical cues, visual cues, avoidance behavior, competitive exclusion by larger conspecifics, and resource availability.

Additionally, interactions between non-predatory fishes and crayfish are not well understood. In some systems, non-predatory fishes can affect crayfish distribution and behavior in the same ways that predatory fishes do (Blake et al. 1994). Therefore, large, non-crackfish fish predators (e.g., suckers, Catostomidae) and small benthic fishes (i.e., darters, Etheostoma sp. and sculpins, Cottus sp.) may play a major role in structuring size-specific crayfish distribution patterns. If crayfish use visual cues to avoid fish predators and do not distinguish between predators and non-predators, fish body size could play a potential role in crayfish distribution patterns, particularly if large fish size is always perceived as being related to increased predation risk. Chemical cues may also alter these interactions. For example, Paraneophrops zealandicus are able to detect chemical cues of native predatory longfin eel but not of exotic brown trout (Shave et al. 1994) suggesting that predator detection may be species-dependent further complicating predator-prey interactions.
In this study, I examine size relationships between crayfish (*Orconectes obscurus*) and the fish assemblage in three habitat types in a 4th-order stream. To potentially provide insight into mechanisms underlying these patterns, I also examined size-dependent responses of crayfish to 2 species of large-bodied, deep water fishes [creek chub (*Semotilus atromaculatus*) and common white sucker (*Catostomus commersoni*)], 2 species of small-bodied, shallow water fishes [central mottled sculpin (*Cottus bairdi*) and fantail darter (*Etheostoma flabellare*)], and large male crayfish (*O. obscurus*) using laboratory behavioral experiments. Creek chub, central mottled sculpin, and larger conspecifics are known crayfish predators (Clark, chapter 5), whereas common white suckers and fantail darters are gape-limited and are not crayfish predators. I hypothesize that 1) crayfish size will be positively correlated with fish (predatory and non-predatory) body size in the field and 2) during behavioral experiments, small crayfish will avoid creek chub, common white sucker, central mottled sculpin, and large crayfish but not fantail darters, whereas medium and large crayfish will not demonstrate avoidance of any fish or crayfish.

**Methods**

*Size Relationships in the Field*

I collected crayfish (*Orconectes obscurus*) and fishes from the West Branch of the Mahoning River, a 4th-order stream in Ravenna, Portage County, Ohio (41°09′41″N 81°11′50″W). Collections were made in four deep pools (water depth ≥ 51 cm), four
shallow pools (maximum water depth = 50 cm), and four riffles (maximum water depth = 12 cm with noticeable breaks in the surface water) (N = 12 channel units) during Summer 2005 (June-July), Fall 2005 (October), Spring 2006 (April), Summer 2006 (June), and Fall 2006 (October). Due to the dynamic nature of streams, channel unit structure and type changed through time and thus, not all of the same channel units could be used during each season. Additionally, only three riffles were used during Fall 2006, Summer 2006, and Spring 2006 due to limited riffle habitat within the stream reaches where these surveys were done. Sample habitats (ea. ≤ 10 m long) were enclosed by upstream and downstream block nets, and crayfish were collected using a four-pass removal method (Zippin 1958) with a backpack electroshocker. During each pass, crayfish and fishes were collected by two netters walking upstream in a zig-zag pattern. All crayfish collected during each pass were counted and measured [carapace length (CL), mm] using Vernier calipers and sex was determined. All fishes collected were identified to species and measured [total length (TL), cm] using a standard fish measuring board. Ultimately, electroshocking proved to be an effective technique to sample crayfish (Rabeni et al. 1997) and fish populations (Bohlin et al. 1989, Poos et al. 2007) and this method met the assumptions required for population estimation (Zippin 1958). Furthermore, many studies have successfully sampled crayfish and fish using electroshocking in combination with pass removal (Usio and Townsend 2000, Hicks 2003, Seiler and Turner 2004).
Statistical Analyses

Pearson’s correlation analysis was used to compare average crayfish carapace length (CL, mm) and average fish total length (TL, cm) of all fishes (predatory and non-predatory species) collected in each replicate habitat type during each season (JMP 6.0, SAS Institute Inc., 2005). Adult creek chub and common white sucker were the most abundant large-bodied fishes, while adult fantail darter and central mottled sculpin were the most abundant small-bodied fishes. Therefore, these four fish species were chosen as treatment fishes for behavioral experiments described below. Furthermore, creek chub ($F_{(1,50)} = 19.57, P < 0.0001$) and common white sucker ($F_{(1,50)} = 21.45, P < 0.0001$) had significantly higher densities (total abundance/ m$^2$) in deep water habitats (deep pools) than shallow water habitats (shallow pools and riffles) while fantail darter ($F_{(1,50)} = 28.55, P < 0.0001$) and central mottled sculpin ($F_{(1,50)} = 33.11, P < 0.0001$) had significantly higher densities in shallow than deep water habitats (JMP 6.0, SAS Institute Inc., 2005). Pearson’s correlation analysis was also used to compare average crayfish CL and species-specific average fish TL for the most common large-bodied and small-bodied fish species (creek chub, common white sucker, central mottled sculpin, and fantail darter) during each season (JMP 6.0, SAS Institute Inc., 2005). All data were log (x+1)-transformed prior to analysis.
**Y-maze Experiments**

**Experimental Animals**—Individual crayfish, creek chubs, common white suckers, central mottled sculpin, and fantail darters were collected in the same locations in which field survey data were collected during May and August 2008. Crayfish were housed in aerated 40 x 28 x 15 cm plastic tubs lined with a layer of gravel and filled with dechlorinated tap water (ambient water temperature: ~20°C). All crayfish were fed a strict diet of Tetramin® flakes. Similarly, all fish were housed in aerated 10-gallon aquaria. Crayfish and treatment fishes were held in the laboratory for no longer than 30 and 9 days, respectively.

**Crayfish behavioral responses to fish and other crayfish**—Responses of small [carapace length (CL) ≤ 19.9 mm carapace length], medium (CL = 20-29.9 mm), and large (CL > 29.9 mm) crayfish to visual and chemical cues associated with adult creek chub (TL = 7.6-20.0 cm), common white sucker (TL = 25.4-50.8 cm), central mottled sculpin [total length (TL) = 5.3-10 cm], fantail darter (TL = 4.9-7.6 cm) (age class determined from Trautman 1957), and large male *O. obscurus* were assessed in a 61 cm X 29 cm X 31 cm flow-through y-maze filled with dechlorinated tap water (12-14°C). Y-maze construction was identical to that used and drawn in Adams *et al.* (2005). The y-maze was gravity-fed by two 16-gallon reservoirs (one entering each arm of the maze) that were filled with dechlorinated tap water (13-17°C) and aerated using an air pump. One inline flow meter (Manostat Riteflow® #4, 65 mm plain ends, Manostat, Peaquannock, New Jersey) was
installed into tubing on each side leading from the reservoir tank into the arm of the y-maze to set and monitor flow rate during trials. Window screening was installed in the upper 43% of each arm (area measuring 20.5 cm x 25.0 cm), creating a screened-in area to separate treatment fishes/crayfish from experimental crayfish and providing the opportunity for visual and chemical cues from the treatment fishes/crayfish to be received by the experimental crayfish. During each trial, water flowed from the reservoirs (flow rate = 20 ± 0.5 mL/min) through the screened-in treatment area and then to the rest of the y-maze (test arena) allowing for treatment fish/crayfish chemical cues to be carried into the test arena. Water exited the y-maze through 6 exit tubes controlled by pinch clamps at the downstream end. In order to document that the structure of the y-maze was unlikely to significantly influence experimental results, dye trials and crayfish trials (with no cue present) were conducted. The dye trials (using food coloring) confirmed consistent and very similar flow rates and patterns through each arm of the y-maze. Similarly, crayfish exhibited no preference relative to initial arm choice in trials with no cue in either arm ($\chi^2$ test of goodness-of-fit, $\chi^2 = 0.6667, P = 0.4142, N = 6$). The combination of these two results confirms the structural similarity of the two y-maze arms, increasing confidence that confounding effects associated with the structure of the y-maze were limited.

The objective of this experiment was to document size-dependent behavioral responses of crayfish to a combination of visual and chemical cues associated with either individual fish species (adult creek chub, common white sucker, central mottled sculpin, and fantail darter) or large crayfish (male O. obscurus). To do this, an individual
treatment fish or crayfish was placed in the screened-in area of a randomly chosen arm of the y-maze. Once the treatment organism was in place, an experimental crayfish was added to the arena and observed using the following protocol. For each treatment (represented by an individual fish species or the large crayfish), replicate individuals, including five male and five female crayfish from each of the three size class treatments, were used (for a total of 150 trials). A different experimental crayfish was used for each individual trial. A different treatment fish/crayfish was used for each set of 10 trials (10 experimental crayfish) within the same crayfish size class (total of 15 treatment animals).

At the beginning of each trial, crayfish of known sex were measured using Vernier calipers and a small dot of white correction fluid was applied to their carapace to increase the visibility of the crayfish to the observer. Marked crayfish were then placed in the test arena and given an acclimation period of 20 minutes where no visual/chemical cues were present. Following acclimation, crayfish were moved to the rear, downstream end of the y-maze, centered between the two arms, and put under a plastic funnel. Upstream reservoirs were then opened to create the flow-through environment and the treatment fish or crayfish was placed upstream of the experimental crayfish in the screened-in area of a randomly chosen y-maze arm. The other arm of the y-maze received no cue (the screened-in area was empty), serving as a control. The funnel was then lifted out of the test arena and crayfish responses to the introduced stimulus were observed for 10 minutes (following Adams et al. 2005). A camouflage blind was placed between the y-maze and the observer to reduce crayfish responses to human presence. All trials were run under fluorescent lighting. Recorded responses included initial and
final arm choice, time to first arm choice (s), and time spent in each arm (s), with all timed variables being recorded with stopwatches. After each trial, the y-maze was thoroughly flushed and rinsed with tap water to eliminate any cues present in preparation for the acclimation period of the next experimental trial.

Statistical analyses

No statistical differences in initial or final arm choice or proportion of time spent in each arm were observed for crayfish sex and thus, crayfish responses were pooled across sex for all following analyses. A $\chi^2$ test of goodness-of-fit was used to test for differences in initial and final arm choice between each visual/chemical cue treatment and crayfish size class (JMP 6.0, SAS Institute Inc., 2005). Trials in which crayfish did not enter either arm (remaining in the downstream area of the y-maze) were removed from the analysis. Two-tailed paired $t$-tests were used to test for differences in the proportion of time spent in each arm for each visual/chemical cue treatment and crayfish size class (JMP 6.0, SAS Institute Inc., 2005). Proportional data was arcsine-transformed prior to analysis. A two-way ANOVA was used to test for differences in time to first arm choice between each visual/chemical cue treatment and crayfish size class (JMP 6.0, SAS Institute Inc., 2005). This measurement variable allowed for crayfish that did not move to be considered in the analysis. Data for the two-way ANOVA was log(x+1)-transformed prior to analysis. Trials in which crayfish escaped or appeared to be disturbed by activity outside of the y-maze were removed from these analyses [5 large crayfish escaped out of 150 trials (3%)].
Results

Size Relationships in the Field

Crayfish carapace length and fish total length were significantly positively correlated, with crayfish size (CL) increasing with corresponding increases in fish size (TL), during all seasons (correlation analysis results: Fall 2005, $r^2 = 0.5207$, $P = 0.0122$; Spring 2006, $r^2 = 0.5259$, $P = 0.0176$; Summer 2006, $r^2 = 0.8241$, $P = 0.0001$; Fall 2006, $r^2 = 0.8091$, $P < 0.0001$) with the exception of Summer 2005 (correlation, $r^2 = 0.1678$, $P = 0.1860$) (Fig. 22a-e). Species-specific correlations documented that crayfish CL was positively correlated with creek chub TL during Fall 2005 (correlation, $r^2 = 0.4919$, $P = 0.0353$) and Fall 2006 (correlation, $r^2 = 0.6497$, $P = 0.0049$) (Fig. 23a-b) but not during Summer 2005 or 2006 or Spring 2006 (correlation, $P > 0.05$). Crayfish CL was also positively correlated with fantail darter TL during Fall 2005 (correlation, $r^2 = 0.6033$, $P = 0.0238$) (Fig. 24a), negatively correlated during Spring 2006 (correlation, $r^2 = 0.4745$, $P = 0.0401$) (Fig 24b), and not significantly correlated at all during Summer 2005 or 2006 or Fall 2006 (correlation, $P > 0.05$). Additionally, crayfish CL was positively correlated with central mottled sculpin TL during Fall 2006 (correlation, $r^2 = 0.7765$, $P = 0.0017$) only (Fig. 25). There was no significant correlation between crayfish CL and common white sucker TL during any season.
Fig. 22. Average fish total length and crayfish carapace length (mean ± 1SE) in each replicate sampling area during A) Summer 2005, B) Fall 2005, C) Spring 2006, D) Summer 2006, and E) Fall 2006. The fit line represents the correlation between fish total length and crayfish carapace length.
Fig. 23. Average creek chub total length and crayfish carapace length (mean ± 1SE) in each replicate sampling area during A) Fall 2005 and B) Fall 2006. The fit line represents the correlation between creek chub total length and crayfish carapace length.
Fig 24. Average fantail darter total length and crayfish carapace length (mean ± 1SE) in each replicate sampling area during A) Fall 2005 and B) Spring 2006. The fit line represents the correlation between fantail darter total length and crayfish carapace length.
Fig 25. Average central mottled sculpin total length and crayfish carapace length (mean ± 1SE) in each replicate sampling area during Fall 2006. The fit line represents the correlation between central mottled sculpin total length and crayfish carapace length.
Crayfish behavioral responses to fish and other crayfish

There was no significant difference in first arm choice between cue and no cue arms for any combination of crayfish size class and cue treatment (chi-square test, \( P > 0.05 \)) (Fig 26a-c). However, although not significant, medium crayfish tended to make a first choice entering into the arm containing the central mottled sculpin cue over the arm containing no cue in 80% of the trials (chi-square test, \( \chi^2 = 3.60, P = 0.0578 \)) (Fig 26b). There was no significant difference in final arm choice between cue and no cue arms for any combination of crayfish size class and cue treatment (chi-square test, \( P > 0.05 \)) with the following exception (Fig. 27a-c). Medium crayfish were found in the arm containing the large crayfish cue in 70% of the trials, which was significantly higher than final choice in the no cue arm (chi-square test, \( \chi^2 = 7.00, P = 0.0082 \)) (Fig. 27b). Although not significant, small crayfish were found at the end of the trial in the arm containing no cue in 60% of the creek chub cue trials (chi-square test, \( \chi^2 = 3.57, P = 0.0588 \)). Similarly, there was no significant effect of amount of time spent in the cue versus the no cue arm for any combination of crayfish size class and cue (ANOVA, \( P > 0.05 \)) (Fig 28a-c). Additionally, there was no significant effect of crayfish size class, cue type, or the interaction for the time to first movement (ANOVA, \( P > 0.05 \)).

Discussion

Overall, crayfish size was positively correlated with fish size, regardless of fish species, indicating that overall fish size may contribute to size-specific crayfish
Fig. 26. Proportion of trials that A) small, B) medium, and C) large crayfish chose the cue arm or no cue arm as their initial arm choice for each treatment; creek chub (CC), common white sucker (CWS), fantail darter (FTD), central mottled sculpin (CMS), and large male crayfish (LG CF). Black bars represent the cue arm and gray bars represent the no cue arm.
Fig. 27. Proportion of trials that A) small, B) medium, and C) large crayfish chose the cue arm or no cue arm as their final arm choice for each treatment; creek chub (CC), common white sucker (CWS), fantail darter (FTD), central mottled sculpin (CMS), and large male crayfish (LG CF). Black bars represent the cue arm and gray bars represent the no cue arm.
Fig. 28. Average proportion of time spent in the cue arm versus the no cue arm by A) small, B) medium, and C) large crayfish for each treatment; creek chub (CC), common white sucker (CWS), fantail darter (FTD), central mottled sculpin (CMS), and large male crayfish (LG CF). Black bars represent the cue arm and gray bars represent the no cue arm.
distribution patterns. However, while some fish species showed significant correlations of body size with crayfish size, these results were not consistent across seasons indicating that on a species level, fish populations are dynamic and likely not contributing substantively to size-specific crayfish distribution patterns. Although fish size affected crayfish distributions in the field, crayfish did not actively avoid creek chub, common white sucker, central mottled sculpin, or fantail darter during behavioral experiments. This was expected for medium and large crayfish, however, it was expected that small crayfish would avoid both fish predators (creek chub and central mottled sculpin) and large non-predatory fish (common white sucker). Small crayfish, however, did not avoid any fish species and would even sit face-to-face with fishes during many trials.

In most systems, small, juvenile crayfish are particularly vulnerable to predation (Stein and Magnuson 1976, Stein 1977, Rabeni 1992, DiDonato and Lodge 1993, Kershner and Lodge 1995, Englund and Krupa 2000, Clark, chapter 5) and typically avoid fish predators by increasing shelter use (Mather and Stein 1993b, Englund and Krupa 2000), decreasing activity (Garvey et al. 1994, Englund 1999), and shifting in habitat use (Garvey et al. 1994, Englund 1999, Englund and Krupa 2000). Furthermore, in some systems, non-predatory fish can have the same behavioral affects on juvenile crayfish activity and distribution as predatory fish (Blake et al. 1994, Keller and Moore 2000). Overall, *O. obscurus* did not show fish species-specific behaviors during y-maze experiments and it is possible that this population of crayfish does not actively avoid large fish (predators or non-predators). However, responses to all fish species in the system were not tested and avoidance behavior may occur with other fishes that are
strong crayfish predators, such as northern rockbass (*Ambloplites rupestris*; Rabeni 1992) and green sunfish (*Lepomis cyanellus*, Englund and Krupa 2000). Furthermore, not all species of crayfish are intimidated by large fishes. Species such as *O. rusticus* and *O. propinquus* can be aggressive toward large fish predators (largemouth bass *Micropterus salmoides*), often moving toward potentially predaceous fish with their chelae raised in a threatening posture (Garvey et al. 1994).

Although avoidance behavior by crayfish was not observed in this study, a range of behavioral mechanisms may affect size-specific distribution patterns of crayfish observed in the field. For example, some crayfish exhibit defensive behavior in response to chemical cues from fish and disturbed conspecifics (Hazlett 1985, 1990, Blake and Hart 1993), which could also aid in avoidance of high risk habitats. In fact, the combination of visual and olfactory cues associated with fish predators seems to elicit the greatest response in crayfish (Blake and Hart 1993). However, visual cues of fish predators seem to influence crayfish behavior more strongly than chemical cues (Blake and Hart 1993). In fact, fish movement can be an important cue stimulating crayfish defensive behavior (Blake and Hart 1993). These swimming behaviors were likely altered during behavioral experiments and may be necessary for crayfish to detect predators. Furthermore, not all crayfish species respond to chemical cues (Hazlett 1990).

Additionally, intimidation by predators can alter the behavior and distribution of prey (Stein 1979), and is likely size-specific in its impact on crayfish. For example, large crayfish did not use refuge in the presence of predaceous smallmouth bass (*Micropterus dolomieui*), implying that large crayfish were not intimidated (Rahel and Stein 1988).
However, small crayfish exhibited evidence of intimidation as they reduced activity levels and increased shelter use more than large crayfish in the presence of a fish predator (Stein and Magnuson 1976), a pattern also exhibited by lobsters (Smith and Herrnkind 1992, Wahle 1992, Wahle and Steneck 1992). Typically, predator avoidance responses are inversely correlated with body size (Sih 1987), and these size-specific responses may be adequate to explain the spatial distribution of small and large crayfish (Stein and Magnuson 1976, Werner et al. 1983, Schlosser 1987, Brown et al. 1988, Englund and Krupa 2000). However, in some systems both smaller and larger crayfish alter their behavior during encounters with fish predators (Stein and Magnuson 1976, Söderback 1992, Mather and Stein 1993a), suggesting that crayfish assess their vulnerability in a size-dependent fashion and employ behaviors that reduce their risk of predation (Keller and Moore 2000).

In addition to the lack of avoidance of fish by crayfish, no avoidance behaviors toward large male crayfish were displayed by any crayfish size class. Large crayfish can influence juveniles by leading them to reduce activity levels, increase shelter use, and also through cannibalism (Blake et al. 1994). However, avoidance of larger, potentially cannibalistic crayfish may only be important during molting (Capelli 1980). Crayfish used in this study had fully hardened carapaces and no crayfish molted during the experiment.

Due to the lack of species-specific correlations between body size of crayfish and fishes and the lack of avoidance behavior during behavioral experiments, it appears that creek chub, common white sucker, central mottled sculpin, fantail darter, and large
conspecifics do not significantly affect size-specific distribution patterns based on visual or chemical cues alone. Furthermore, *O. obscurus* may use visual and/or chemo-sensory stimuli to avoid fish predators that were not tested appropriately in this study. A variety of factors including intimidation, previous encounters, conspecific alarm cues, and/or visual or chemical cues of other fishes not tested, may be important drivers of this size pattern in the field. Furthermore, it is possible that similar factors (i.e., abiotic characteristics, terrestrial predation) are driving both size-specific fish and crayfish distribution patterns or that patterns are trumped by other factors such as top predators in the system or competitive interactions.

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References


Capelli, G. M. 1980. Seasonal-variation in the food-habits of the crayfish Orconectes propinquus (Girard) in Trout Lake, Vilas County, Wisconsin, USA (Decapoda, Astacidea, Cambaridae). Crustaceana 38:82-86.


Trautman, M. B. 1957. Fishes of Ohio. The Ohio State University Press, Ohio, USA.


CHAPTER 7

HABITAT OVERLAP AND RESOURCE PARTITIONING BETWEEN A LOTIC FISH ASSEMBLAGE AND CRAYFISH (*ORCONECTES OBSCURUS*) IN A FORESTED STREAM

Abstract

In streams, competitive interactions between crayfish and fish for food resources are virtually unstudied. Since many stream fishes are relatively small, they are unable to consume crayfish due to body size and gape limitations. However, they often occupy similar habitats and consume similar resources as crayfish, leading to the potential for fish-crayfish competition. To explore this issue, I assessed habitat overlap among three size classes of crayfish (*Orconectes obscurus*) [small: carapace length (CL) ≤ 19.9 mm, medium: CL = 20.0-29.9 mm, and large: CL ≥ 30.0 mm] and multiple species of young-of-year (YOY) and adult fishes using field surveys conducted during 5 seasons (Summer 2005, Fall 2005, Spring 2006, Summer 2006, Fall 2006). During each season, two-way cluster analysis revealed 2 distinct groups of organisms that possessed substantive within-group overlap in habitat use. Group 1 (shallow-water taxa) included all three size classes of crayfish and most small-bodied fishes. Group 2 (deep-water taxa) included large crayfish, large-bodied fishes, YOY Johnny darters (*Etheostoma nigrum*), and YOY
common white suckers (*Catostomus commersoni*). To gain insight into resource partitioning, stable isotope analysis (natural abundances of $\delta^{15}$N and $\delta^{13}$C) was used to distinguish trophic levels ($\delta^{15}$N) and resource partitioning/overlap ($\delta^{13}$C) among groups. Based on these analyses, all size classes of *O. obscurus* belonged to the same trophic level. Furthermore, crayfish displayed ontogenetic diet switching with larger individuals foraging on more enriched carbon sources than small crayfish. Medium and large crayfish, however, did not partition resources and may compete for food resources in the field. All fishes, with the exception of creek chub (*Semotilus atromaculatus*; which fed at the same trophic level as crayfish), were at a higher trophic level than all sizes of crayfish. Large crayfish overlapped in habitat use and partitioned resources with adult creek chub. Small, medium, and large crayfish overlapped in both habitat and resource use with YOY creek chub and may be competing for food resources in the field. However, food resources are typically abundant in shallow habitats and lack of resource partitioning may be a function of no food limitation. Overall, competition for food resources appears to be a weak interaction among crayfish and stream fishes and does not appear to be a major factor affecting habitat use of crayfish and fish in this system.

**Introduction**

In many aquatic systems, interactions between fish and benthic invertebrates can have dramatic impacts on community structure and ecosystem function. These interactions include top-down effects, such as fish effects on benthic invertebrate distribution and abundance (Stein 1977, Rabeni 1992, Englund 1999), densities (Diehl
1995, Batzer 1998, Dorn and Mittelbach 1999), size-structure (Mittelbach 1988, Mather and Stein 1993a), species composition (Power 1992), and behavior (Lima 1998, Stein and Magnuson 1976), and bottom-up effects, including benthic invertebrate effects on fish diets (Crowder and Cooper 1982), habitat use (Werner et al. 1983), growth rates (Diehl and Kornijów 1998), and abundance (McIvor and Odum 1988). However, many of the aforementioned interactions can depend heavily on the ratio of fish gape size to invertebrate body size. Further, many fish species are size-selective predators (Rabeni 1992) possibly because larger invertebrates provide the highest energy gain for predators (Mittelbach 1981, Persson and Crowder 1998). Since crayfish are among the largest lotic invertebrates and tend to dominate benthic invertebrate communities (Momot 1995), they are often an important prey resource for stream fishes (Rabeni 1992). However, crayfish are highly aggressive and possess a strong, hard carapace (i.e., exoskeleton) making them difficult prey items for some fishes to consume. Furthermore, many stream fishes (e.g., darters, sculpin) are small and unable to consume crayfish due to body size and gape limitations and may serve as prey items for crayfish as well as potential competitors.

In many streams, crayfish are considered opportunistic omnivores (acting as both predators and shredders, Parkyn et al. 2001), consuming a wide range of benthic food resources that are often used by benthic and mid-water fishes. While interactions between predatory fish and crayfish have been well studied (Kershner and Lodge 1995, Englund and Krupa 2000, Usio and Townsend 2000, Magoullick 2004), competitive interactions and resource partitioning between native fishes (benthic and mid-water fishes) and native crayfish are not well understood. Further, competitive interactions for
food resources among conspecific crayfish are not well understood either. Interspecific (Lodge and Hill 1994; Garvey et al., 2003, Dorn and Trexler 2007) and intraspecific (Lodge and Hill 1994) competition can influence size distributions of crayfish populations and likely play an important role in shaping stream community structure.

However, underlying mechanisms, including resource limitation, are difficult to assess in the field (Werner 1986, Schoener 1989). When overlap in habitat use occurs among species feeding at the same trophic level and conspecifics (juveniles and adults) are in high abundance, individual species or size classes may partition resources in order to limit the potential for exploitative competition. Moreover, species-specific ontogenetic diet shifts may occur not only to meet the requirements for growth and reproduction, but also to reduce competition for resources. Limiting competition may allow populations to exist at relatively high densities.

The stable isotope of nitrogen ($\delta^{15}$N) can be used to tease apart trophic level (Minagawa and Wada 1984), whereas the stable isotope of carbon ($\delta^{13}$C) indicates food resources assimilated (Vander Zanden and Rasmussen 2001). Thus, carbon and nitrogen stable isotopes can provide insight into an organism’s feeding history and habitat occupation while feeding (Paterson et al. 2006). Further, stable isotopes can be used to assess resource partitioning/overlap among species (Paterson et al. 2006). Since many crayfish and fish are opportunistic feeders, stable isotopes may be more reliable than gut analysis to assess resource partitioning. Carbon stable isotopes ratios are particularly useful in examining diet over a long time period, whereas gut analysis only provides a snapshot of diet in time (Grey et al. 2001).
In this study, I assessed seasonal trends in the spatial distribution of size-specific crayfish and age-specific fish relative to habitat type in a 4th-order stream to examine overlap in habitat and resource use among groups. I used stable isotope analysis (natural abundances of $\delta^{15}$N and $\delta^{13}$C) to assess food web interactions and to determine the trophic level of three size classes of crayfish (*Orconectes obscurus*) and several young of year (YOY) and adult fish species. Additionally, I used $\delta^{13}$C signatures to quantify resource partitioning between potential competitors (species and size/age classes within a trophic level) in addition to ontogenetic diet shifts by crayfish. I hypothesize that when crayfish size-classes and fish age-classes within the same trophic level overlap in habitat use, resource partitioning (separation of $\delta^{13}$C signatures) will be indicated by stable isotope analysis. Similarly, as crayfish grow, stable isotope analysis (enrichment or depletion of $\delta^{13}$C signature with size) will suggest ontogenetic diet shifts.

**Methods**

*Habitat-specific crayfish and fish distribution survey*

I assessed size-specific crayfish and fish distribution in four deep pools (water depth $\geq$ 51 cm), four shallow pools (maximum water depth = 50 cm), and four riffles (maximum water depth = 12 cm with noticeable breaks in the surface water) (N = 12 channel units) during Summer 2005 (June-July), Fall 2005 (October), Spring 2006 (April), Summer 2006 (June), and Fall 2006 (October) as part of a larger study assessing the effects of grain size, current velocity, water depth, predatory fish and relative
predation risk on size-specific distribution patterns (Clark, chapter 5). Due to the
dynamic nature of streams, channel units changed over time and the same channel units
could not be used for each season. Additionally, only three riffles were used during
Spring 2006, Summer 2006, and Fall 2006 due to limited riffle habitat within one channel
unit. Sample habitats (ea. ≤ 10 m long) were enclosed by upstream and downstream
block nets, and crayfish were collected using a four-pass removal method (Zippin 1958)
with a backpack electroshocker (LR-24 Electrofisher, Smith-Root™). During each pass,
crayfish and fish were collected by two netters walking upstream in a zig-zag pattern.
Electroshocking is considered to be an effective technique for sampling both crayfish
(Rabeni et al. 1997) and fish populations (Bohlin et al. 1989; Poos et al. 2007) and this
method met the assumptions required for population estimation using a depletion method
(Zippin 1958). Furthermore, many studies have successfully surveyed crayfish and fish
populations using electroshocking in combination with pass removal (Usio and
Townsend 2000; Hicks 2003; Seiler and Turner 2004).

During each pass, collected crayfish were counted and measured [carapace length
(CL), mm] using Vernier calipers and sex was determined. Similarly, all collected fish
were identified to species and measured [total length (TL), cm] using a standard fish
measuring board. Lengths (along both stream banks) and widths (at both upstream and
downstream block nets) of each sample habitat were measured in order to calculate
channel-unit specific areal (m²) measurements to be used in density estimation.
Statistical Analyses

To estimate crayfish density, a depletion method was used (Zippin 1958); this method allows density to be estimated by plotting cumulative total catch against catch per unit effort (CPE), fitting a regression line through the points, and solving for CPE = 0 to determine the population estimate for each sample habitat. The population estimate was then divided by the size of the sampling area estimated for each sample habitat. To calculate size-specific density estimates, crayfish were divided into 3 size classes including small [carapace length (CL) ≤ 19.9 mm], medium (CL = 20.0-29.9 mm), and large (CL ≥ 30.0 mm) crayfish. Size-specific density was calculated within each sample habitat by taking the total density of crayfish for a given channel unit and multiplying that value by the proportion of crayfish captured within each size class for a given sample area. Fish were separated into 2 age categories including young-of-year (YOY) and adult (following Trautman 1957). Depletion did not occur every time during electroshocking for each fish species, so density was calculated by dividing the total catch from 4 passes by the enclosed sample area for all samples.

A two-way hierarchical cluster analysis was used to partition size-specific crayfish and age-specific (referred to as taxa from here on out) fish density data for each season into manageable groups via a dendrogram. Taxa were grouped by size/age class relative to habitat-specific densities (PC-ORD™ 5, MjM Software Design, 2005). In order to group taxa by habitat preferences, I used Ward’s linkage method and the Euclidean (Pythagorean) distance measure for analysis. Each dendrogram was scaled using Wishart’s objective function and converted to a percentage of information.
remaining (see McCune et al. 2002 for a full description of ‘two-way cluster analysis’). For each season, the first division of the dendrogram split taxa into two subgroups which were characterized as groups 1 and 2. Group 1 taxa were typically found in shallow waters (shallow pools and riffles), whereas group 2 taxa commonly occupied deep waters (deep pools). However, some taxa occupied both shallow and deep waters and were occasionally grouped by the dendrogram into both groups 1 and 2. Consequently, a criteria of >25% presence within a group across seasons was set to characterize crayfish and fish size/age classes into group 1 and/or group 2 taxa. Since cluster analysis provided two distinct groups for each season, these two groups were used to assess resource partitioning/overlap.

Descriptions for abbreviations on figures 1-5 are as follows:  DP, deep pool, the number represents the replicate; SP, shallow pool, the number represents the replicate; R, riffle, the number represents the replicate; SM CF, small crayfish; MED CF, medium crayfish; LG CF, large crayfish; CC YOY, YOY creek chub; CC ADULT, adult creek chub; CWS YOY, YOY common white sucker; CWS ADUL, adult common white sucker; FTD YOY, YOY fantail darter; FTD ADUL, adult fantail darter; GSF ADUL, adult green sunfish; HS YOY, YOY northern hogsucker; HS ADULT, adult northern hogsucker; JD YOY, YOY Johnny darter; LMB YOY, YOY largemouth bass; RB ADULT, adult northern rockbass.
Food web interactions between crayfish and fish

**Stable isotope collection**

To assess size-specific trophic level and resource partitioning between crayfish and fish, I collected small, medium, and large crayfish, creek chub (*Semotilus atromaculatus*) (YOY and adult), common white sucker (*Catostomus commersoni*) (YOY and adult), fantail darter (*Etheostoma flabellare*) (YOY and adult), Johnny darter (*E. nigrum*) (YOY), green sunfish (*Lepomis cyanellus*) (adult), northern hog sucker (*Hypentelium nigricans*) (YOY and adult), northern largemouth bass (*Micropterus salmoides*) (YOY), northern rock bass (*Ambloplites rupestris*) (adult), and central mottled sculpin (*Cottus bairdi*) (YOY and adult) on 17 and 20 October 2007 for stable isotope analysis. Whenever possible, 3 male and 3 female crayfish of each size class, and 6 YOY and 6 adult fish of each species and age class were collected via electroshocking from 4 deep pools, 4 shallow pools, and 4 riffles (N = 12 channel units) (for a total of N = 378 organisms). Due to habitat- and size-/age-specific distribution patterns, it was not always possible to obtain all fish and crayfish from each channel unit and/or habitat type. All samples were put on dry ice and transported back to the lab. Samples remained frozen at -15 °C until prepared for analysis.
Stable isotope preparation

All samples were thawed prior to preparation for stable isotope analysis. Abdominal tissue and caudal peduncle muscle tissue was dissected from each crayfish and fish, respectively. Whole bodies were used for some YOY fishes that were too small to remove the desired weight of muscle tissue. All samples were dried at 60°C for 48 h and ground using a GenoGrinder (Spex CertiPrep, Metuchen, NJ) for 3.5 min at 1500 rpm. Samples were analyzed for dual $\delta^{13}$C and $\delta^{15}$N natural abundance by a continuous flow Isotope Ratio Mass Spectrometer (IRMS, UC Davis Stable Isotope Facility, Davis, CA). A total of 18 small [deep pools (DP) = 6, shallow pools (SP) = 6, riffles (R) = 6], 13 medium (DP = 6, SP = 6, R = 1), and 11 large crayfish (DP = 6, SP = 5) were used for analysis. A total of 11 creek chubs (5 YOY, 6 adult), 6 common white suckers (YOY = 3, adult = 3), 11 fantail darters (6 YOY, 5 adult), 6 Johnny darters (6 YOY), 2 green sunfish (2 adult), 5 northern hogsuckers (3 YOY, 2 adult), 1 largemouth bass (1 adult), 1 northern rockbass (1 adult), and 14 central mottled sculpins (5 YOY, 9 adult) were used for stable isotope analysis.

Statistical Analyses

To separate species and size/age classes into distinct trophic levels, a difference of approximately $+3.4 \pm 1.1\%$ in $\delta^{15}$N signatures was used as a criterion to determine that organisms were within different trophic levels (Minagawa and Wada 1984). An individual one-way ANOVA was then used to examine resource partitioning using $\delta^{13}$C.
values for all sizes/ages and species that were within the same taxa group (group 1 or 2) and shared the same trophic level grouping (JMP 6.0, SAS Institute Inc., 2005). For taxa that share habitats, $\delta^{13}C$ can be used to assess overlap in resource use (i.e., with high overlap, $\delta^{13}C$ values would be very similar) or partitioning of resources (i.e., significantly different $\delta^{13}C$ values indicate limited overlap and high partitioning) (Paterson et al. 2006). Additionally, ontogenetic diet switching in crayfish was assessed using Pearson’s correlation analysis to compare $\delta^{13}C$ values with crayfish size (CL), and one-way ANOVA’s to examine resource partitioning.

Results

Two-way cluster analysis of crayfish and fish density data revealed two major groups during each season (groups 1 and 2) (Figs 29-33). Group 1 taxa included small (100%), medium (100%), and large (40%) crayfish, YOY creek chubs (80%), YOY (100%) and adult (100%) fantail darters, YOY northern hogsuckers (80%), YOY Johnny darters (60%), and YOY (100%) and adult (100%) central mottled sculpins. (Note: following each taxon, the parenthetical values represent the percentage of the 5 seasonal density estimates in which that taxon was classified as a member of group 1.) Group 2 taxa included large crayfish (60%), adult creek chubs (80%), YOY (100%) and adult (100%) common white suckers, adult green sunfish (100%), adult northern hogsuckers (75%), YOY Johnny darters (40%), YOY largemouth bass (100%), and adult northern rockbass (100%). (Note: following each taxon, the parenthetical values represent the
Fig. 29. Taxa collected during Summer 2005 clustered into Groups 1 and 2 by density and site. Circles indicate where chaining divided the first 2 subgroups during two-way cluster analysis. Descriptions of codes on the figure can be found within the methods section.
Fig. 30. Taxa collected during Fall 2005 clustered into Groups 1 and 2 by density and site. Circles indicate where chaining divided the first 2 subgroups during two-way cluster analysis. Descriptions of codes on the figure can be found within the methods section.
Fig. 31. Taxa collected during Spring 2006 clustered into Groups 1 and 2 by density and site. Circles indicate where chaining divided the first 2 subgroups during two-way cluster analysis. Descriptions of codes on the figure can be found within the methods section.
Fig. 32. Taxa collected during Summer 2006 clustered into Groups 1 and 2 by density and site. Circles indicate where chaining divided the first 2 subgroups during two-way cluster analysis. Descriptions of codes on the figure can be found within the methods section.
SUMMER 2006

Matrix Coding
■ Presence □ Absence

Information Remaining (%)
Fig. 33. Taxa collected during Fall 2006 clustered into Groups 1 and 2 by density and site. Circles indicate where chaining divided the first 2 subgroups during two-way cluster analysis. Descriptions of codes on the figure can be found within the methods section.
percentage of the 5 seasonal density estimates in which that taxon was classified as a member of group 2.) Based upon $\delta^{15}N$ values and Minagawa and Wada’s (1984) criterion for determination of trophic level, small, medium, and large crayfish are all within the same trophic level ($\delta^{15}N$ difference; small-medium = 0.2‰; small-large = 0‰; medium-large = 0.1‰) (Figs 34 and 35). Additionally, YOY and adult creek chubs overlapped in trophic level with small ($\delta^{15}N$ difference; YOY creek chubs, 0.3‰; adult creek chubs, 0.6‰), medium ($\delta^{15}N$ difference; YOY creek chubs, 0.5‰; adult creek chubs, 0.8‰), and large crayfish ($\delta^{15}N$ difference; YOY creek chubs, 0.3‰; adult creek chubs, 0.6‰) (Figs 34-36). However, adult creek chubs clustered out in group 2, grouping with large crayfish but not small or medium crayfish (Figs 29-33). YOY central mottled sculpin overlapped in trophic level with medium crayfish ($\delta^{15}N$ difference = 2.1‰) (Figs 34 and 35). In contrast, YOY central mottled sculpin were at a higher trophic level ($\geq 2.3‰ \delta^{15}N$) than small and large crayfish (Figs 34 and 35). All other fishes, regardless of group (including YOY and adult common white suckers, YOY and adult fantail darters, adult green sunfish, YOY and adult northern hogsuckers, YOY Johnny darters, YOY largemouth bass, adult northern rockbass, and adult central mottled sculpins) were at a higher trophic level than all three sizes of crayfish (Figs 34-36).

Within group 1, small crayfish [$\delta^{13}C$ range = -26.2 to -28.0‰, average = -27.1 ± 0.1‰ (± 1SE)] partitioned resources with both medium crayfish [$\delta^{13}C$ range = -26.1 to -27.6‰, average = -26.6 ± 0.1‰ (± 1SE); ANOVA; $F_{(1,56)} = 7.79, P = 0.0092$] and large crayfish [$\delta^{13}C$ range = -24.5 to -27.4‰, average = -26.3 ± 0.2‰ (± 1SE); ANOVA; $F_{(1,52)}$]
Fig 34. Average $\delta^{13}$C and $\delta^{15}$N signatures (mean ± 1SE) for individual crayfish size classes and YOY and adult fishes collected in deep pools, shallow pools, and riffles on 17 and 20 October 2007 for stable isotope analysis.
Fig 35. Trophic level overlap of group 1 taxa as indicated by delta N ($\delta^{15}N$). Taxa within a given box belong to the same trophic level.
Fig 36. Trophic level overlap of group 2 taxa as indicated by delta N ($\delta^{15}$N). Taxa within a given box belong to the same trophic level.
YOY largemouth bass

YOY Johnny darter/adult northern rockbass
YOY northern hogsucker

YOY and adult common white sucker/adult green sunfish

large crayfish
adult creek chub

Group 2
Small crayfish had a significantly more depleted δ^{13}C signature than either medium or large crayfish (Fig 37). However, medium and large crayfish overlapped in resource use (ANOVA, $F_{(1,42)} = 1.52, P = 0.2303$) (Fig 37). Additionally, medium crayfish partitioned resources with YOY central mottled sculpins [$δ^{13}C$ range = -24.2 to -26.5‰, average = -25.4 ± 0.3‰ (± 1SE); ANOVA, $F_{(1,38)} = 16.85, P = 0.0006$] (Fig 38). Medium crayfish had a significantly more depleted δ^{13}C signature than YOY central mottled sculpins (Fig 38). Similarly, resource use overlapped between all three sizes of crayfish and YOY creek chubs [$δ^{13}C$ range = -25.4 to -27.9‰, average = -26.4 ± 0.4‰ (± 1SE); ANOVA: small crayfish, $F_{(1,40)} = 3.93, P = 0.0607$; medium crayfish, $F_{(1,30)} = 0.20, P = 0.6587$; large crayfish, $F_{(1,26)} = 0.13, P = 0.7195$] (Fig 39).

Within group 2, large crayfish partitioned resources with adult creek chubs [$δ^{13}C$ range = -23.6 to -25.0‰, average = -24.7 ± 0.2‰ (± 1SE); ANOVA: $F_{(1,28)} = 19.12, P = 0.0005$] (Fig 40). Large crayfish had a significantly more depleted δ^{13}C signature than adult creek chubs (Fig 40).

Crayfish carapace length (CL, mm) was significantly and positively correlated with δ^{13}C signature (correlation, $r^2 = 0.2713, P = 0.0004$) corresponding with resource partitioning results (Fig 41). As crayfish body size (CL) increased, the δ^{13}C signature became more enriched (Fig 41).

**Discussion**

Group 1 taxa were most strongly associated with shallow water habitats (shallow pools and riffles) and included all three size classes of crayfish and most small-bodied
Fig 37. Proportion of small, medium, and large crayfish at each $\delta^{13}C$ value.
Fig 38. Proportion of medium crayfish and YOY central mottled sculpins at each $\delta^{13}\text{C}$ value.
Fig 39. Proportion of small, medium, large crayfish, and YOY creek chubs at each $\delta^{13}$C value.
Fig 40. Proportion of large crayfish and adult creek chubs at each δ¹³C value.
Fig 41. $\delta^{13}$C signatures of crayfish plotted against crayfish carapace length (mm, CL) of individual crayfish.
fishes. In contrast, group 2 taxa were most commonly found in deep water habitats (deep pools) and included only large crayfish and large-bodied fishes with the exception of YOY Johnny darters and YOY common white suckers. Large crayfish, YOY Johnny darter, and YOY northern hogsucker were commonly found in both shallow and deep water habitats and, consequently, belonged to both group 1 and 2 taxa. This pattern of deep water-big fish and shallow water-small fish associations occurs in many stream fish assemblages (Schlosser 1982, Power 1984, Harvey and Stewart 1991) and crayfish populations (Rabeni 1985, Creed 1994, Englund and Krupa 2000, Usio and Townsend 2000, DiStefano et al. 2003, Flinders and Magoulick 2003, 2007b, Clark, chapter 5) and appears to be driven by predation risk. Terrestrial predators that dive and wade, such as birds and raccoons, usually prey on large fish rather than small fish, with reduced foraging efficiency as water depth increases, resulting in large fish demonstrating a preference for deep water habitats to avoid predation (Power 1987). In some streams, crayfish may be choosing shallow water habitats as a refuge from fish predators, which tend to occupy deep water habitats due to terrestrial predation pressure (Englund 1999, Englund and Krupa 2000). In this system, predation appears to be a major structuring mechanism for small crayfish, leading to relatively high overall crayfish densities in shallow pool habitats (Clark, chapter 5); consequently, high densities of crayfish in shallow pool habitats could lead to both intraspecific and interspecific competition for food resources.

*O. obscurus* displayed ontogenetic diet switching, with larger individuals foraging on more enriched carbon sources than smaller crayfish. Although, medium and large
crayfish did not partition resources, both medium and large size classes partitioned resources with small crayfish. Overall, it appears that crayfish change patterns of resource use as they grow from juveniles into adults. Similarly, ontogenetic diet shifts by other *Orconectes* crayfishes have been documented in the literature (Lodge and Hill 1994, Whitledge and Rabeni 1997). Generally, juvenile *Orconectes* require more animal protein for growth, whereas adults eat more plants/detritus than animal material/protein and ontogenetic diet shifts reflect these needs; juvenile crayfish tend to consume greater proportions of animal material relative to plant/detrital material when compared to adult crayfish (Lodge and Hill 1994, Whitledge and Rabeni 1997). However, not all crayfish populations display ontogenetic diet shifts (*O. luteus*, Whitledge and Rabeni 1997; *Pacifastacus leniusculus*, Bondar et al. 2005, Stenroth et al. 2006), and the absence of observed ontogenetic diet shifts may be a function of food availability.

Ontogenetic diet switching may be profitable in some systems to avoid competition with conspecifics and/or to meet energetic costs of growth and reproduction. Further, intraspecific interactions are common in crayfish populations (Hill and Lodge 1994) and may play a major role in crayfish foraging tradeoffs and diet switching patterns. For example, large, aggressive adult crayfish often competitively exclude small, juvenile crayfish from preferred, slower-flowing habitats (Lodge and Hill 1994) and also can indirectly influence juvenile survival by leading to reductions in juvenile activity and growth (Blake *et al.* 1994). In lakes, large male *Astacus astacus* exclude juveniles and females from food-rich habitats, restricting them to resource-poor habitats (Abrahamsson 1966). The resource partitioning between juvenile (small) and adult (medium and large)
*O. obscurus* observed in carbon stable isotope analysis in this study suggests that direct intraspecific competition for food resources between these two age classes is probably limited. However, resource partitioning in this system may also be a consequence of past intraspecific competitive events and/or predator-mediated competition where past competitive events and/or predators have changed size-specific distribution patterns and, consequently, resource use. Medium and large crayfish, however, did not partition resources and if resources are limited in this system, these two size classes may display strong intraspecific competitive interactions. Although resource limitation is difficult to document in the field (Werner 1986, Schoener 1989) and was not documented in this study, resource availability is a limiting factor in many ecological communities (Schoener 1986, Keddy 1989) and could be a factor affecting competitive interactions for crayfish in streams.

All size classes of crayfish were at a lower trophic level than all fish species with two exceptions. Medium crayfish and YOY central mottled sculpin were within the same trophic level. $\delta^{15}N$ differences of 2.3‰ indicate that taxa differ in trophic level (Minagawa and Wada 1984) and medium and YOY central mottled sculpin only had $\delta^{15}N$ differences of 2.1‰, indicating that although these taxa feed within the same trophic level, they probably were not competing strongly for food resources. Furthermore, YOY crayfish did not overlap in trophic level with any other size class of crayfish. Feeding experiments focused on interactions between YOY central mottled sculpin and crayfish need to be done to further tease apart potential competitive interactions. In some systems, the slimy sculpin (*C. cognatus*) is a superior competitor to crayfish (*O. virilis*),
suppressing feeding rates by 50% (Miller et al. 1992). However, interspecific competition was tested for a single food resource (Miller et al. 1992). Since crayfish are considered opportunistic omnivores in most aquatic systems (Huryn and Wallace 1987, Lodge and Lorman 1987, Chambers et al. 1990, Hart 1992, Creed 1994, Lodge et al. 1994, Lodge and Hill 1994, Charlebois and Lamberti 1996, Parkyn et al. 2001), competition for food may decrease between sculpin and crayfish when multiple food choices are available.

Large (adult) crayfish and adult creek chub overlapped in habitat use (deep pools) and were feeding within the same trophic level, while still partitioning resources. Invertebrate biomass, which is a prey resource for both crayfish and creek chub, is typically lower in deep water than in shallow water, whereas detritus is similarly abundant in both habitat types (Flinders and Magoulick 2007a). Thus, invertebrate prey may be limiting in deep pools, where adult creek chub and crayfish may be forced to partition resources.

All three crayfish sizes overlapped in habitat use and fed at the same trophic level as YOY creek chubs. Further, all three size classes of crayfish overlapped in resource use. Although it would be expected for these taxa to partition resources, food resources may not be limiting in shallow pool and riffle habitats. Riffles tend to have a higher abundance of invertebrate prey than pool habitats (Mather and Stein 1993b) and shallow pools tend to have a higher abundance of invertebrate prey than deep pools (Flinders and Magoulick 2007a). Since food resources may be abundant in shallow pools and riffles, resource partitioning between crayfish and YOY creek chub may be limited. However, it
is unclear whether or not resources were abundant or limiting, and interspecific competition may or may not have been occurring between crayfish and YOY creek chub.

Leaves are the predominant food items for crayfish inhabiting forested streams (Whitledge and Rabeni 1997, Parkyn and Collier 2001, Bondar et al. 2005). Furthermore, crayfish often rely on detritus and biofilms and food sources, even though crayfish show increased growth on a diet consisting of a greater proportion of animal material (Gherardi et al. 2004, Bondar et al. 2005). YOY creek chub are generally thought to be predators on invertebrates (Barber and Minckley 1971, Magnan and FitzGerald 1984). However, in this study, creek chubs are similar in $\delta^{13}$C and $\delta^{15}$N isotopic signatures to crayfish, suggesting that creek chub are omnivorous as well. Further, in a small Wyoming stream, creek chubs were opportunistic omnivores, consuming plant material, aquatic and terrestrial insects, gastropods, mussels, and fish (Quist et al. 2006).

In many aquatic systems, intraspecific (Lodge and Hill 1994) and interspecific (Lodge and Hill, 1994; Garvey et al., 2003) competition can influence size distributions of crayfish populations. In this system, *O. obscurus* had very few fish competitors, and competition for food between fish and crayfish did not play a major role in determining habitat use. However, intraspecific competition between medium and large crayfish may play a role in structuring crayfish distribution patterns. Based upon these data, it is unclear how intense these interactions are and what effect they have on size-specific distribution patterns. Thus, it appears that predation (Clark, chapter 5) is likely a more
important driver than competition in determining crayfish distribution and dynamics in this system.

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References


(Orconectes virilis) and the slimy sculpin (Cottus cognatus) Journal of Freshwater 

Minagawa M. and E. Wada. 1984. Stepwise enrichment of N-15 along food-chains-
 further evidence and the relation between delta-N-15 and animal age. Geochimica 
et Cosmochimica Acta 48:1135-1140.

Mittlebach, G. G. 1981. Foraging efficiency and body size: a study of optimal diet and 


Glenden Beach, Oregon, USA.

Momot, W. T. 1995. Redefining the role of crayfish in aquatic ecosystems. Reviews in 
Fisheries Science 3:33-36.

Parkyn, S. M., K. J. Collier, and B. J. Hicks. 2001. New Zealand stream crayfish:

Paterson, G., K. G. Drouillard, G. D. Haffner. 2006. Quantifying resource partitioning in 
Centrarchids with stable isotope analysis. Limnology and Oceanography 51: 
1038-1044.
Persson, L. and L. B. Crowder. 1998. Fish-habitat interactions mediated via ontogenetic
niche shifts. Page 3-23. In Jeppesen E, Søndergaard Ma, Søndergaard Mo,
Christoffersen K (eds). The structural role of submerged macrophytes in lakes.
Springer-Verlag, New York.

Poos, M. S., N. E. Mandrak, and R. L. McLaughlin. 2007. The effectiveness of two
common sampling methods for assessing imperiled freshwater fishes. Journal of
Fish Biology 70:691-708.


Power, M. E. 1987. Predator avoidance by grazing fishes in temperate and tropical
streams: importance of stream depth and prey size. In Kerfoot, W.C. and A. Sih
(eds). Predation: direct and indirect impacts on aquatic communities. University
Press of New England, Hanover, NH, pp 333-351.


Quist, M. C., M. R. Bower, and W. A. Hubert. 2006. Summer food habits and trophic
overlap of roundtail chub and creek chub in Muddy Creek, Wyoming. The
Southwestern Naturalist 51:22-27.

Rabeni, C. F. 1985. Resource partitioning by stream-dwelling crayfish: the influence of

Canadian Journal of Fisheries and Aquatic Sciences 49:1714-1721.


Trautman, M. B. 1957. Fishes of Ohio. The Ohio State University Press, Ohio, USA.


Overall, crayfish (*Orconectes obscurus*) density is higher in shallow pool habitats than either deep pools or riffles in streams. However, crayfish are distributed among deep pools, shallow pools, and riffle habitats in a size-specific fashion. Small crayfish tend to occupy shallow pools and riffles, whereas large crayfish tend to occupy shallow and deep pool habitats. Medium crayfish, however, are distributed across all three habitat types. Based upon the research presented in this dissertation, it appears that multiple mechanisms are structuring the size- and habitat-specificity of patterns of crayfish distribution.

At base flow in this stream, riffles were characterized by shallow water (generally ≤ 12 cm), fast current velocity relative to deep and shallow pools, large rocky substrates (cobbles and boulders), and small benthic fishes. Current velocities in these riffles tend to range from 0.1-0.5 m/s during baseflow. In contrast, deep pools were characterized by deep water (≥ 51 cm), slow average current velocity (~0.01 m/s), small substrate size (mostly sand and clay), and large fishes (including predators and non-predators of crayfish). However, large substrate, including large cobbles and coarse woody debris, occasionally occurred in deep pools and may have served as refugia for medium and large crayfish found in this habitat. On the other hand, shallow pools were characterized
by shallow water (< 50 cm), slow average current velocity (~0.01 m/s), a combination of small and large substrates (cobbles and boulders), and a fish assemblage composed primarily of small fishes with rare occurrences of larger fish. In this habitat, large rocky refugia were readily available for crayfish, especially along the shallow bank margins. Crayfish density was particularly high in these shallow, rocky stream pools. Based upon these characteristics, it appears that shallow pools possess characteristics intermediate between deep pool and riffle habitats and thus, may be an important transition zone for crayfish.

In general, *O. obscurus* seems to prefer habitats with low current velocity. Larger crayfish were negatively associated with current velocity and seemed to be excluded from riffle habitats even at baseflow conditions. In contrast, small and some medium (at the smaller end of the range) crayfish are able to tolerate the higher current velocity of riffle habitats (Clark *et al.* 2008) and occupy them at low densities. Thus, it appears that riffles were not preferred habitat for any crayfish size class, despite the fact that tethering assays demonstrated that predation risk was low for all size classes in riffle habitats. Low crayfish densities in riffles may be related to the presence of abundant central mottled sculpin in riffle habitats, which while it does not seem to be a strong competitor for food resources, may be a stronger competitor for refugia, competitively excluding smaller crayfish from riffle habitats. Furthermore, more energy must be expended to move about or simply hold position in the high water velocities of riffles and thus, it is likely less optimal habitat for crayfish than low flow habitats.
Along with current velocity preferences, small and medium crayfish tend to prefer shallow water depths whereas large crayfish are associated with deep water habitats. In fact, *O. obscurus* appeared to match up well with the deep water-big fish and shallow water-small fish hypothesis (primarily driven by terrestrial predators on fish and larger crayfish) frequently observed in stream fish (Schlosser 1982, Power 1984, Harvey and Stewart 1991) and crayfish distributions (Rabeni 1985, Creed 1994, Englund and Krupa 2000, Usio and Townsend 2000, DiStefano *et al*. 2003, Flinders and Magoulick 2003, 2007b). This trend matches distribution patterns observed in my study system as well, with small crayfish avoiding deep water habitats most likely due to high predation risk associated with large fish predators in these habitats. Ultimately though, it appears that water depth will only be an important structuring mechanism for small crayfish when predaceous fish are present (Mather and Stein 1993a,b, Englund and Krupa 2000, Usio and Townsend 2000). However, survival of small crayfish was similarly low in shallow pool habitats where larger fish predators were less common. In order to gain insight into factors driving this pattern, studies assessing diel (and particularly) nocturnal patterns of predatory fish movement and size-selective feeding of terrestrial predators are necessary to tease apart predator-prey interactions in shallow pool habitats.

In general, large rocky substrate appears to be an important structuring mechanism for small and medium crayfish, as these two size classes are positively correlated with increased grain sizes. However, large crayfish do not display this association with large grain sizes, commonly occupying habitats composed primarily of small grain sizes (mostly sand and clay). These substrate use patterns for large crayfish
likely result from reduced predation risk (due to their size) and thus, a corresponding
decreased need for refugia use. Large crayfish, however, were commonly observed
hiding under large cobbles (even in deep pools), possibly defending territories. However,
this pattern is likely not linked to predation risk, rather this pattern may be driven by
mating, reproduction, and/or competition with conspecifics for optimum territories.

Predation is often an important source of mortality in early life stages of
organisms and can restrict juvenile crayfish to refugia while adults may range freely
(Stein and Magnuson 1976, Werner et al. 1983a,b, Schlosser 1987). Grain manipulation
tethering experiments showed that survival was higher at plots composed cobble than
sand. Overall, it appears that large rocky substrate may provide necessary refugia from
the suite of predators, allowing for survival of small crayfish in high risk habitats.

Although crayfish densities seemed to correlate strongly with some abiotic
variables in field surveys, interactions between abiotic variables and predation risk were
not consistent in the tethering assays, implying that most abiotic variables, do not mediate
predation events in this population of crayfish. Although all crayfish sizes were
vulnerable to predation, only small crayfish showed consistent differences in survival
across habitats whereas medium and large crayfish generally had similar survival across
all three habitat types. In summary, small crayfish distributions appear to be structured
primarily by predation and substrate availability whereas larger crayfish appear to be
structured by a combination of substrate availability, current velocity, and competition
for food resources. Other factors, however, could be playing a role in overall distribution
patterns, including competition for refugia (Bovbjerg 1970, Fero and Moore 2008) and
optimal food resources and food availability (Nyström et al. 2006, Flinders and Magoulick 2007a).

Lotic systems, in particular, are extremely dynamic and are prone to disturbance (i.e., flooding, seasonal drying) causing a frequently changing landscape for biota. Since small, headwater streams are often intermittent, abiotic factors most likely have the greatest effects on community structure (Creed 2006) and organismal distribution. However, in larger, permanent streams (e.g., West Branch of the Mahoning River) abiotic conditions tend to be less severe and there tends to be an increase in trophic complexity allowing biotic interactions to also play a role (Creed 2006). Disturbance events (e.g., flooding), however, do occur in larger, permanent streams and can have dramatic effects on crayfish population structure often overriding biotic interactions.

**Importance of Study**

Interactions among biotic and abiotic variables drive a suite of ecosystem functions and processes. Since crayfish can be characterized as ‘keystone species’ and ‘ecosystem engineers’, it is not surprising that they are often an important component of community structure, trophic cascades, organic matter processing, and can influence species replacement with respect to invasive species. Unfortunately, with increases in habitat loss, the introduction of invasive species, and watershed degradation, native species, including crayfish, are suffering tremendously. While the importance of managing this problem has gotten substantive attention, many restoration and management efforts are often hampered by the lack of background data associated with
the ecology, life history, and behavior of native species. Behavioral studies are particularly important in conservation biology as they can contribute greatly to a stronger, more complete understanding of species-specific resource utilization patterns and habitat requirements (Sutherland 1998). Similarly, understanding an organism’s ecology and life history can be critically important in predicting their importance in ecosystem function and community dynamics.

In streams, the influence of predators on community structure is not well understood (Mather and Stein 1993a). Although little is known about the risk caused by terrestrial predators on stream prey or the cannibalistic behavior of crayfish, some studies have shown that terrestrial predators can, in fact, affect stream prey (Power et al. 1989), and cannibalism among crayfish can occur in some species when they constrained to high density situations (Alcorlo et al. 2004). Secondly, correlation between predation risk and abiotic variables, including current velocity and substrate heterogeneity, is an important interaction that has not been well tested. Understanding the interplay of biotic and abiotic factors in determining habitat requirements (e.g., current velocity and refugia) is necessary for management and restoration practices. Furthermore, interactions between crayfish and non-predatory fishes are practically unknown (Dorn and Mittlebach 1999). While this may not appear to be a critical food web interaction, non-predatory fish may act as competitors for resources (shelter and/or food) or may have effects similar to predatory fishes, where their very presence can cause behavioral changes and avoidance.

Further, understanding the role of disturbance in aquatic systems is a research area in ecology that continues to develop. In fact, little is known about effects of
flooding on crayfish and maybe more importantly, little is also known about how
disturbance intensity and frequency interact to actually constitute a disturbance for a
given species. Contributing to this area of research may prove important for ecological
management purposes and reintroduction practices in the future. With increases in the
building of dams, increased runoff due to urbanization, and potential increases of
precipitation due to global climate change, understanding the effects of water velocity on
stream organisms will be important for managing and preserving these ecosystems.

Ultimately, studying interactions between biotic and abiotic interactions will aid
in predicting the effects of environmental and global change on native species.
Reintroductions, restocking, management, and restoration efforts will prove more
effective with knowledge of the organism’s ecology. Contributions to understanding
predation and disturbance will be important for management and conservation practices.
References


