



2007

Shelter competition between native signal crayfish and non-native red swamp crayfish in Pine Lake, Sammamish, Washington: the role of size and sex

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**SHELTER COMPETITION BETWEEN NATIVE SIGNAL CRAYFISH AND
NON-NATIVE RED SWAMP CRAYFISH IN PINE LAKE, SAMMAMISH,
WASHINGTON: THE ROLE OF SIZE AND SEX**

By

Karl W. Mueller

Accepted in Partial Completion
of the Requirements for the Degree
Master of Science

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MASTER'S THESIS

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NON-NATIVE RED SWAMP CRAYFISH IN PINE LAKE, SAMMAMISH,
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A Thesis

Presented to

The Faculty of

Western Washington University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

By

Karl W. Mueller

May 2007

ABSTRACT

Freshwater crayfish (Decapoda) communities worldwide are becoming increasingly similar from location to location by the intentional or accidental introduction of North American crayfishes. The red swamp crayfish (*Procambarus clarkii*, Cambaridae), which is native to the south-central United States and northeastern Mexico, is the most widely introduced crayfish in the world. It was first discovered in Pine Lake, Sammamish, Washington in 2000.

The results of a 2005 baseline survey of the crayfish in Pine Lake suggested that the red swamp crayfish was displacing the native signal crayfish (*Pacifastacus leniusculus*, Astacidae). One mechanism through which non-native crayfishes displace native species is competitive interaction over shelter that influences susceptibility to predation. Field experiments were designed to explore how crayfish size and sex influence shelter occupancy in mixed-species pairs of signal crayfish and red swamp crayfish competing for limited shelter inside enclosures placed on the bottom of Pine Lake. In addition, the relative survivorship of signal crayfish and red swamp crayfish was quantified in experiments where mixed-species pairs were tethered outside of single shelters.

Irrespective of species and sex, when paired with smaller heterospecifics, large crayfish readily monopolized the shelters inside the enclosures. When contestants were size-matched, the dominant crayfish or 'winner' was typically the one with longer chelae; frequently, this was the signal crayfish. Female crayfishes also were adept at monopolizing the shelter. The tether experiments revealed no significant differences in

survivorship between species. These results suggest that additional mechanisms besides shelter competition are contributing to the possible displacement of signal crayfish at Pine Lake.

ACKNOWLEDGMENTS

I thank Chuck and Gloria Watson for their hospitality and technical assistance, my advisory committee, Leo Bodensteiner (chair), Brian Bingham, and Al Stoner, for guidance, Robin Matthews and Ben Miner for helpful statistical advice, Carl Denooyer for use of his digital camera and underwater housing, Pete Butkus and Kevin Husemann for providing easy access to Pine Lake, Geoff Landis for constructing the crayfish holding tables, and Jim Mullen for preparing the PVC shelters. Crayfishes were collected under Permit # 06-325 from the Washington Department of Fish and Wildlife. This research was funded by Chimaera Endeavors, a small grant from Huxley College, Western Washington University, and scholarships from the American Academy of Underwater Sciences and the Washington State Lake Protection Association.

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INTRODUCTION

Freshwater biota is becoming increasingly similar on a global scale by the accidental or intentional introduction of non-native aquatic species. For example, in North America, introduced centrarchids, e.g., the black basses (*Micropterus* spp.) and other sunfishes (*Lepomis* spp.), dominate freshwater fish communities throughout the United States (Fuller et al. 1999). Likewise, in Africa, diverse native fish assemblages that contributed to the bulk of the catch of traditional freshwater fisheries have been replaced by ones composed of a few prolific non-native species (Kudhongania and Chitamwebwa 1995). Many freshwater plant species are now cosmopolitan, occurring around the world as a result of human-mediated range expansions (Groombridge and Jenkins 2002). Moreover, freshwater crayfish communities worldwide are becoming increasingly homogenized by the introduction of the same three or four species of North American crayfish (Taylor 2002). One of these, the red swamp crayfish (*Procambarus clarkii*, Cambaridae; Figure 1), which is native to the south-central United States and northeastern Mexico, is the most widely introduced crayfish in the world (Hobbs et al. 1989). In Europe, the red swamp crayfish is rapidly spreading from Spain and Portugal on the Iberian Peninsula to neighboring countries (Gherardi 2006).

Why should we be concerned about the spread of red swamp crayfish? Quite simply, wherever red swamp crayfish are introduced, the potential for profound, negative ecological impacts is great (Hobbs et al. 1989; Barbaresi and Gherardi 2000; Gherardi 2006). Crayfish are keystone organisms that structure food webs and aquatic communities, acting as predators and grazers, and as prey for fishes, birds, mammals, and

even other crayfish (Nyström 2002). Furthermore, crayfish influence energy flow and nutrient cycling of freshwater ecosystems through feeding and burrowing (Covich et al. 1999). Consequently, once established, a non-native crayfish can alter, directly or indirectly, whatever lake, pond, or stream it was introduced into (Gherardi 2006). Additionally, the red swamp crayfish is capable of displacing or extirpating native species of crayfish, changing local biodiversity (Gil-Sánchez and Alba-Tercedor 2002, 2006).

The red swamp crayfish was first discovered in Washington state in 2000 (Mueller 2001). Several live specimens were collected by Washington Department of Fish and Wildlife personnel during a routine survey of the littoral zone fish community of Pine Lake, located on the Sammamish Plateau in King County, approximately 12 km east of Seattle (Figure 2). Of concern were the facts that 1) Pine Lake was home to a population of native signal crayfish (*Pacifastacus leniusculus*, Astacidae; Figure 3), which ranges naturally from southern British Columbia to northern California and east to Idaho and western Nevada (Lewis 2002), and 2) most of the red swamp crayfish sampled were sexually mature adults (Mueller 2001). In 2005, a baseline survey of the crayfishes in Pine Lake revealed that the size distributions of both populations were similar and that carapace lengths were modal at ~ 35 – 45 mm. Furthermore, the non-native red swamp crayfish outnumbered the native signal crayfish by more than two to one during most sampling periods. One explanation for these data is that the red swamp crayfish is displacing the signal crayfish in Pine Lake (Mueller 2007).

Non-native crayfishes displace native species in a number of ways. Some possible mechanisms of displacement include competition for food (Hill and Lodge 1999;

Gherardi and Cioni 2004), reproductive interference (Butler and Stein 1985), increased vulnerability to predation based on differences in crayfish size and escape behavior (Söderbäck 1991; DiDonato and Lodge 1993; Garvey et al. 1994; Hazlett et al. 2003), and competitive interactions over shelter that influence susceptibility to predation (Antonelli et al. 1999; Usio et al. 2001; Gherardi and Cioni 2004).

Shelter is critical for crayfish survival, serving as a refuge against predation or environmental extremes and providing habitat suitable for reproductive purposes (Gherardi 2002). During the day, nocturnally active crayfish seek shelter in crevices, under rocks and woody debris, or in burrows intentionally excavated by the crayfish. During summer, the peak reproductive period for red swamp crayfish, sexually mature adults (carapace length ≥ 40 mm) actively seek shelter (Penn 1943; Gherardi et al. 1999; Ilhéu et al. 2003). Shelter is often limited in natural settings; thus, the potential for intraspecific or interspecific competitive interactions over shelter is high. In fact, disputes over shelter are one of the main reasons crayfish fight one another in the wild (Gherardi 2002; Gherardi and Cioni 2004). The outcome of these disputes is often dictated by size, sex, and reproductive status of the contestants (Nakata and Goshima 2003; Figler et al. 2005).

Non-native red swamp crayfish exert dominance over native species when competing for limited shelter under controlled laboratory conditions; however, field trials are lacking (Gherardi and Cioni 2004; Gherardi and Daniels 2004). Hence, the goal of the present study was to evaluate, through a series of field experiments, if sheltering ability might confer an advantage to the non-native red swamp crayfish at Pine Lake. Observations of behavioral interactions among crayfishes in a natural setting such as Pine

Lake can validate laboratory studies (Bergman and Moore 2003) and provide useful insights into species replacements and the invasion process (Sutherland 1998; Holway and Suarez 1999). Since competition and predation frequently interact with one another to structure communities (Gurevitch et al. 2000; Mills et al. 2004) elements of both were incorporated into the field experiments. The main objective of this study was to explore how size and sex influenced shelter occupancy in mixed-species pairs of signal crayfish and red swamp crayfish competing for limited shelter inside enclosures placed on the bottom of Pine Lake. A second objective was to quantify the relative survivorship of the two crayfishes in experiments where mixed-species pairs were tethered outside of single shelters. This study was restricted to the interactions of heterospecifics only, because intraspecific sheltering and aggression in signal crayfish and red swamp crayfish have been studied elsewhere (e.g., Ranta and Lindström 1993 and Figler et al. 2005).



Figure 1. Red swamp crayfish (*Procambarus clarkii*, Cambaridae).

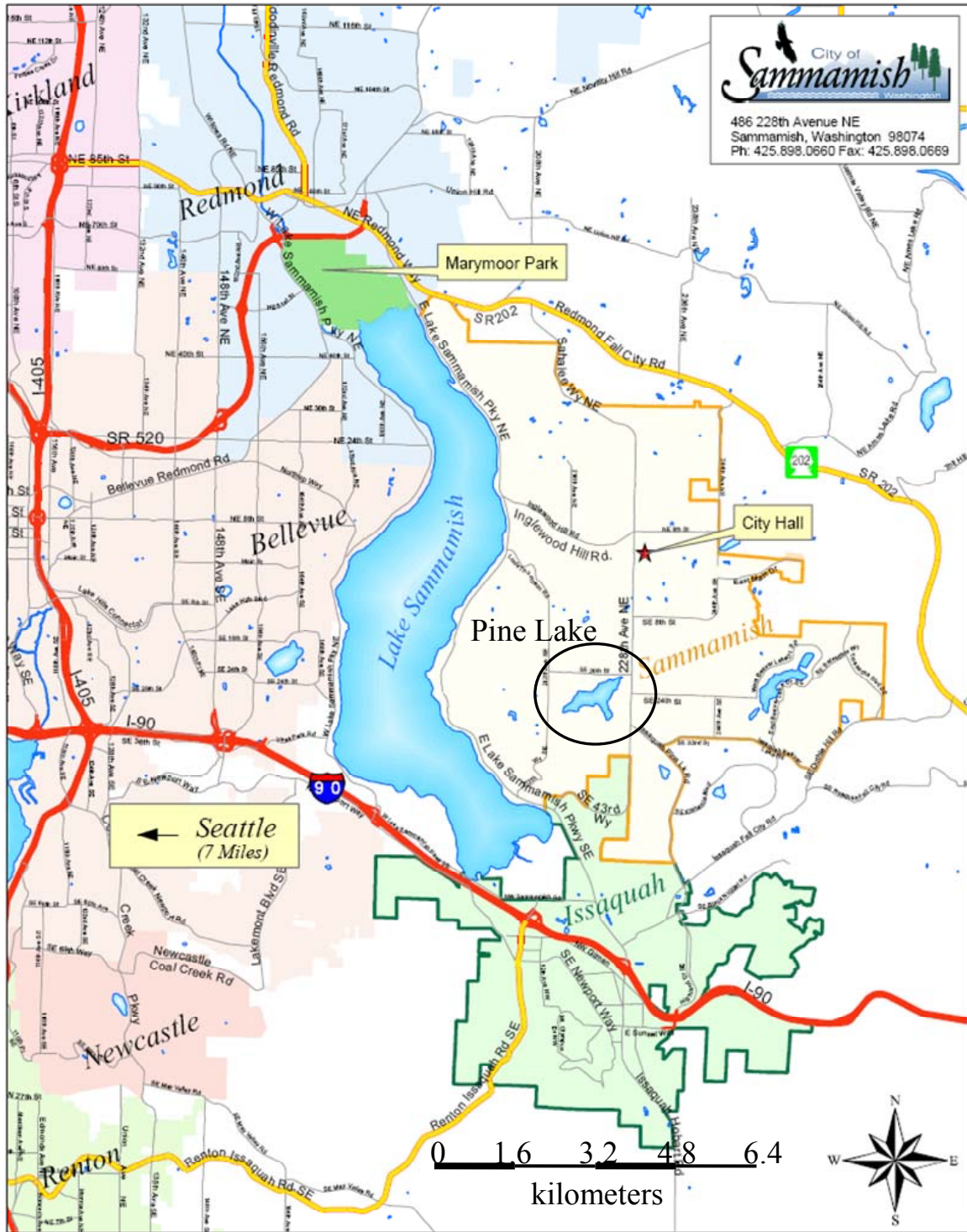


Figure 2. Vicinity map showing location of Pine Lake, Sammamish, Washington. Pine Lake, the small, T-shaped lake located east of Lake Sammamish and west of 228th Avenue NE, is circled (source: City of Sammamish, Washington).



Figure 3. Signal crayfish (*Pacifastacus leniusculus*, Astacidae).

MATERIALS AND METHODS

Study area

Pine Lake is a small (34.8 ha), shallow (mean depth = 6.1 m, maximum depth = 11.9 m), T-shaped lake (shoreline length ~ 3.9 km) with a history of eutrophication; however, in recent years, it has exhibited oligotrophic conditions. Secchi depth transparencies range from approximately 2 m to 7 m annually, and water temperatures range from approximately 5° to 24° C annually (Abella et al. 2005). The lake thermally stratifies for several months each year, beginning in April and ending with the fall turnover in November. During the stratification period, the dissolved oxygen level decreases rapidly in the metalimnion (depth ~ 5 – 8 m), from approximately 8 mg/L to 2 mg/L, and is depleted in the hypolimnion (depth > 8 m). The substrates of Pine Lake are glacial till, clay, and gravelly, sandy loam (Jacoby et al. 1997). Aquatic plant coverage is < 25% of the lake surface area and primarily consists of water weed (*Elodea canadensis*), bushy pondweed (*Najas flexilis*), and small pondweed (*Potamogeton pusillus*) (Walton 1996). The littoral zone fish community is composed mostly of introduced species and includes largemouth bass (*Micropterus salmoides*), smallmouth bass (*M. dolomieu*), pumpkinseed (*Lepomis gibbosus*), yellow perch (*Perca flavescens*), rainbow trout (*Oncorhynchus mykiss*), cutthroat trout (*O. clarki*), brown trout (*Salmo trutta*), and brown bullhead (*Ameiurus nebulosus*). Like many lowland lakes in western Washington, over 90% of Pine Lake's shoreline has been developed for residential purposes; there are approximately three docks for every 100 m of shoreline (Verhey and Mueller 2001).

This study was conducted over an 8-week period in summer from 19 June 2006 to 11 August 2006. The field experiments took place on a small, gradually sloping shoal (47° 35.191 N, 122° 02.924 W) at the west end of Pine Lake along its southern shore (Figure 4). The shoal was comprised of hardpan clay substrate, and was free of aquatic vegetation. Here, depths ranged to 4 m, and considerable crayfish burrowing activity was evident to a depth of about 4 m. Below 4 m, the substrate changed to soft fine sediments and gave way to a band of dense aquatic vegetation that extended to a depth of about 6 m. This site was selected for both logistical and biological reasons. It was conveniently located near an access point and its shallow, homogenous topography facilitated setting up and running the experiments. In addition, Mueller (2007) found that signal crayfish and red swamp crayfish were relatively abundant at this location, underscoring its importance to both species.

Experimental animals

Sixty-one signal crayfish and 79 red swamp crayfish were collected from Pine Lake with baited funnel traps and by hand using scuba during the first week of the study (19–23 June) and on four subsequent dates (28 June, 13 and 14 July, and 4 August). Upon capture, the crayfishes were identified to species and their morphometric data [total, carapace, and chelae lengths (mm), chelae width (mm), and wet weight (g)] were recorded. In addition, the sex and reproductive status of each crayfish were noted. The size distributions of the crayfishes used in the study were significantly different (Kolmogorov-Smirnov test, $D = 0.46$, $P < 0.0001$). The carapace length of signal

crayfish was modal at 35 mm, whereas the carapace length of red swamp crayfish was modal at 50 mm (Figure 5).

When not being used in field trials, the experimental animals were held individually in clear, uniquely-labeled, vented plastic boxes (Sterilite® Flip-Tops) that were placed in two 300-L plywood holding tables (1.2 x 1.5 x 0.2 m each) located on a dock near the study site (Figure 6). The holding tables were supplied with continuous flow-through water drawn directly from the epilimnion of Pine Lake using a small, submerged magnetic-drive pump. Since crayfish are able to chemically recognize conspecifics (Rose 1986; Oh and Dunham 1991), sexes (Dunham and Oh 1992), and social status (Zulandt Schneider et al. 1999), and since chemical signals can influence the outcome of physical encounters between individuals (Zulandt Schneider et al. 2001; Moore and Bergman 2005), it was considered critical that experimental animals be held singly in this continuous flow-through system to minimize individual recognition before conducting the pair-wise field trials. Furthermore, crayfish held singly survive better in captivity compared to those held communally (Sáez-Royuela et al. 1995).

Throughout the study, the holding tables were covered with a durable shade tarp composed of a breathable fabric which prevented damage to the light-sensitive eyes of the crayfish during daylight hours (Kong and Goldsmith 1977; Hafner et al. 1982) and reduced any 'greenhouse' effects. Water temperature varied from the beginning (19° C) to the end (23° C) of the 8-week study period. The tarp also deterred any mammalian or avian crayfish predators, such as river otter (*Lutra canadensis*) and great blue heron (*Ardea herodias*), that reside at the lake. Finally, while in the holding tables, the

crayfishes were fed a commercially prepared, pelleted diet (cat food or shrimp food) *ad libitum* once daily (Figure 7).

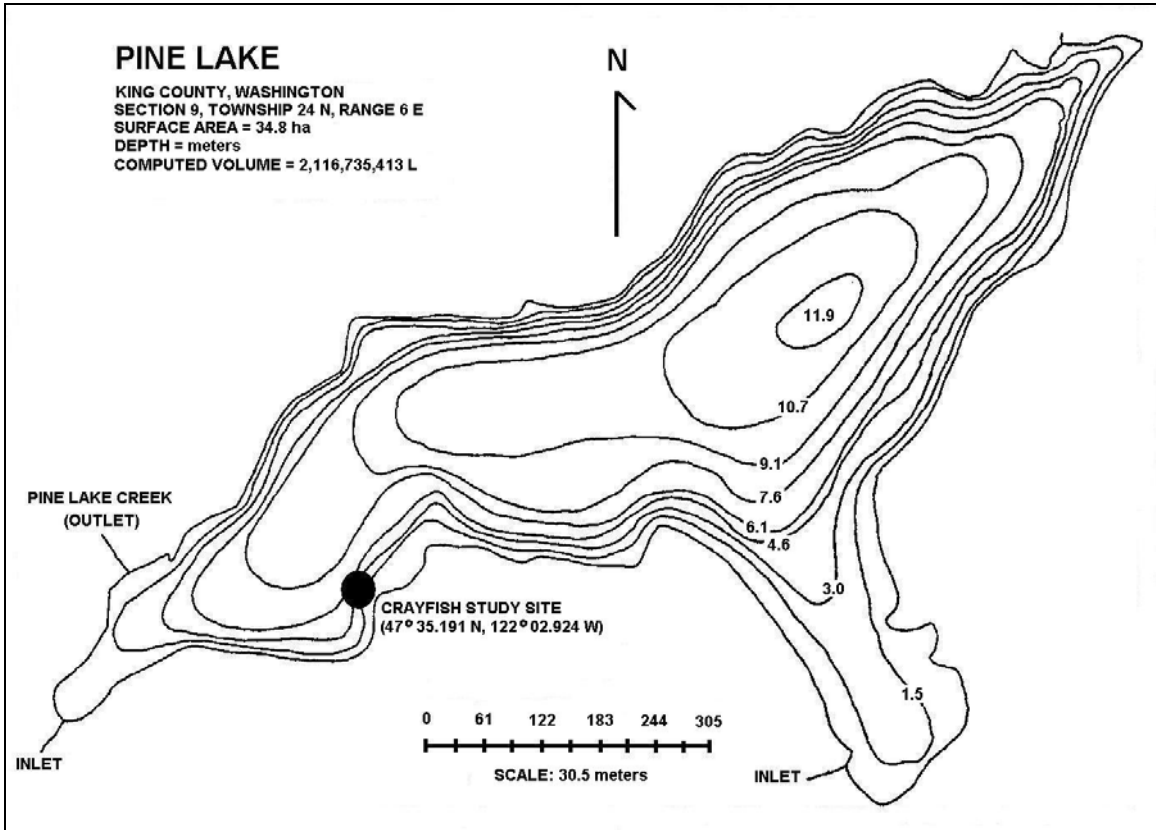


Figure 4. Bathymetric map of Pine Lake, Sammamish, Washington showing location of crayfish study site (redrawn from map provided by the Washington Department of Fish and Wildlife).

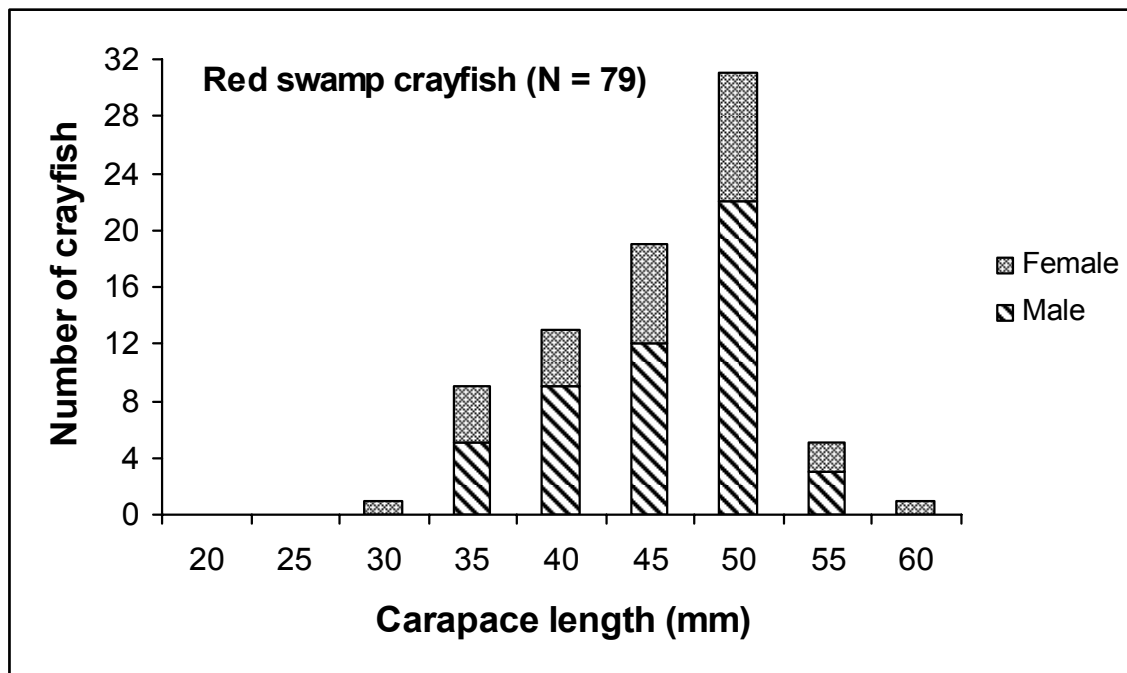
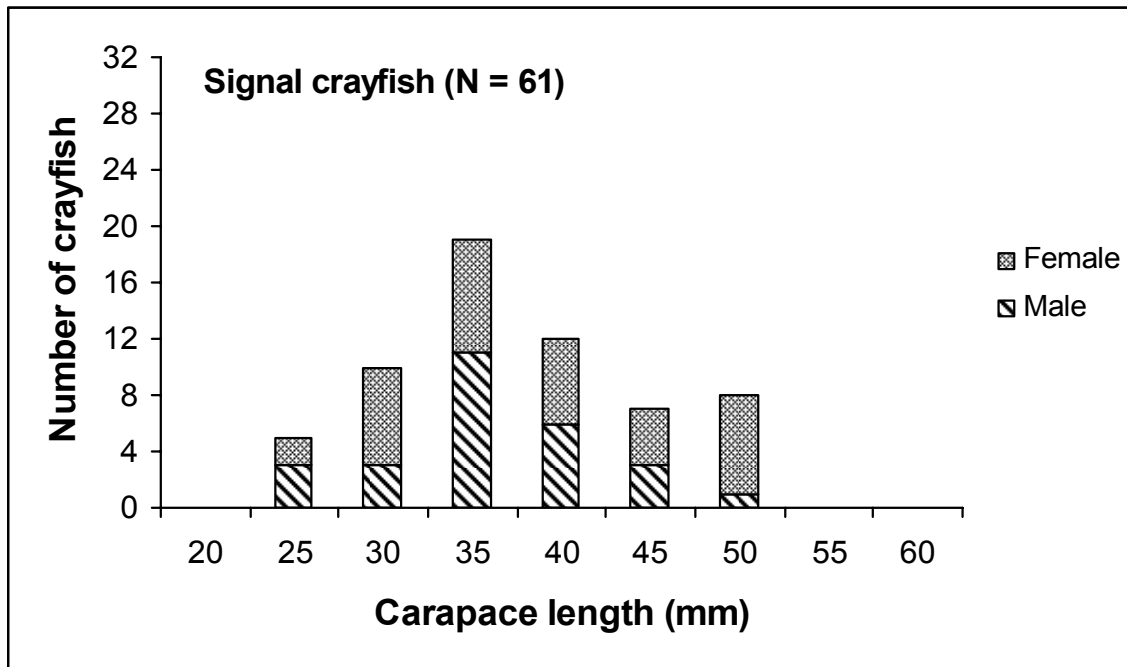


Figure 5. Length frequency of signal crayfish (*Pacifastacus leniusculus*; top) and red swamp crayfish (*Procambarus clarkii*; bottom) used in experiments at Pine Lake, Sammamish, Washington during summer 2006.



Figure 6. Continuous flow-through water tables (1.2 x 1.5 x 0.2 m each) used to hold crayfishes when not being used in field experiments at Pine Lake, Sammamish, Washington.



Figure 7. Feeding captive red swamp crayfish (*Procambarus clarkii*). Note the unique labels and vents in plastic boxes. The labels were used to identify individual crayfish. The vents allowed water to continuously flow through the plastic boxes.

Enclosure experiments

In these experiments, mixed-species pairs of crayfish competed for single artificial shelters inside enclosures placed on the bottom of the lake to test the null hypothesis of equal use of shelter by signal crayfish and red swamp crayfish. The mixed-species pairs of crayfish in the first four experiments were composed of same-sex or mixed-sex dyads with individuals of similar size [the difference in carapace length between contestants was $\leq 10\%$] to examine the effect of sex differences on shelter competition. The mixed-species pairs in the remaining four experiments were composed of same-sex dyads with individuals of different sizes (the difference in carapace length between contestants was $> 10\%$) to examine the effect of size differences on shelter competition. The percent difference in carapace length (CL) was calculated as $[(CL \text{ large} - CL \text{ small})/CL \text{ large}] \times 100$.

The enclosures were constructed of 0.5 x 0.5 x 0.3 m wire baskets lined with 1.3 cm plastic mesh on all sides. Previous studies demonstrated that crayfish prefer black or dark shelters with rough or uneven interiors over light shelters with smooth interiors (Blank and Figler 1996; Antonelli et al. 1999); therefore, a piece of black PVC pipe (5 cm diameter) was roughened inside with a file then centered obliquely and secured to the bottom of each enclosure with cable ties (Figure 8). Each piece of PVC pipe measured 25 cm in length, was capped on one end, and provided refuge for one crayfish. The length of the artificial shelter was based on the work of Nakata and Goshima (2003) who found that signal crayfish preferred shelters at least twice their own total length (at Pine Lake, the typical total length of an adult signal crayfish is ~ 12.5 cm).

On the first day of a trial, the experimental animals were transferred from the holding tables to water-filled tubs and buckets onboard a small research boat and ferried to the study site. Mixed-species pairs of crayfish were placed into enclosures (one pair of crayfish per enclosure), which were then closed with cable ties to prevent their escape and to prevent intrusion by free-ranging crayfishes or potential predators. A snorkeler (Figure 9) attached the enclosures 4 m apart to three 20-m polypropylene ground lines at the study site (Figure 10).

Up to 16 replicate enclosures were used for each experiment (Table 1). For some experiments, too few crayfishes were collected at the beginning of the study to simultaneously run all 16 replicate contests; therefore, some individuals were reused during an experiment to increase the number of replicates. After their first deployment, the crayfishes intended for reuse were returned to the holding tables for at least one day before being systematically reassigned to different animals in a subsequent deployment; this insured independence between experimental runs and minimized pseudoreplication (Hurlbert 1984). The enclosures remained on the bottom of the lake for the duration of the study except during the changeover between experiments. No fouling of enclosures was evident between trials.



Figure 8. Example of enclosure used in study of shelter competition between native and non-native species of crayfish.

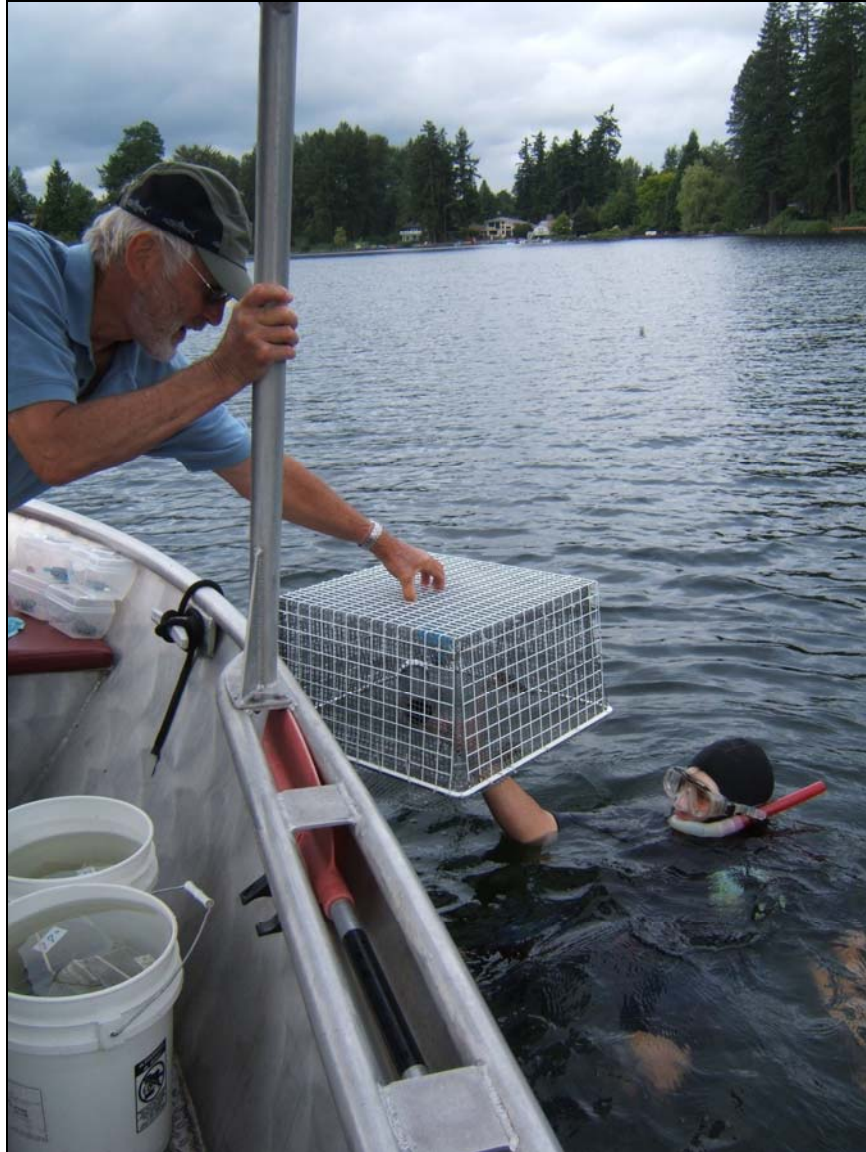


Figure 9. Snorkeler preparing to deploy enclosure containing mixed-species pair of crayfish during study of shelter competition at Pine Lake, Sammamish, Washington (photo by Karen E. Mueller).

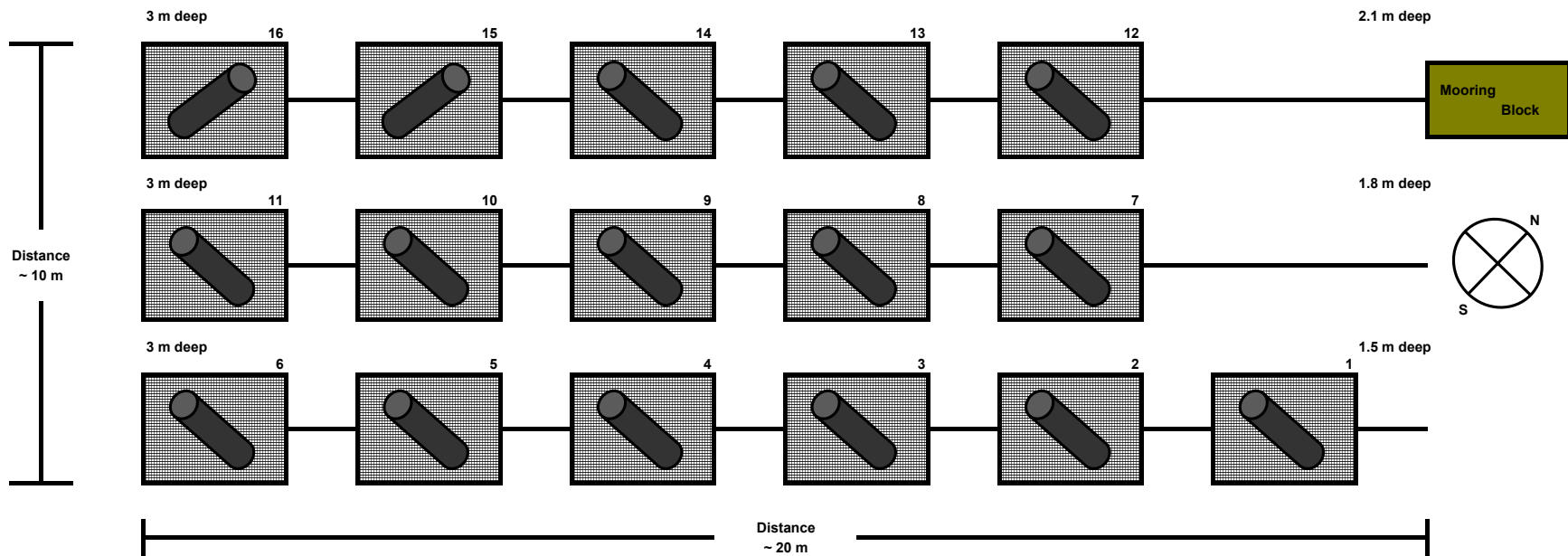


Figure 10. Layout of enclosure experiments at Pine Lake, Sammamish, Washington. Enclosures 15 and 16 were constructed first and served as prototypes for the remaining enclosures. The PVC shelters within the enclosures were reversed by chance.

Table 1. Mixed-species pairs of crayfish used in enclosure experiments to examine shelter competition between native signal crayfish (*Pacifastacus leniusculus*) and non-native red swamp crayfish (*Procambarus clarkii*) at Pine Lake, Sammamish, Washington in 2006.

Date	Enclosure experiment	Number of pairs or replicates	Contestants	Carapace length range (mm)
Similar size contestants				
26–28 June	1 ^a	15	<i>Pacifastacus</i> ♀ <i>Procambarus</i> ♀	31–52 33–52
29 June–1 July	2 ^a	15	<i>Pacifastacus</i> ♂ <i>Procambarus</i> ♂	33–51 35–51
3–5 July	3 ^a	15	<i>Pacifastacus</i> ♀ <i>Procambarus</i> ♂	35–51 35–50
6–8 July, 10–12 July	4 ^a	16	<i>Pacifastacus</i> ♂ <i>Procambarus</i> ♀	33–51 33–51
Different size contestants				
29 June–1 July, 6–8 July, 10–12 July	5 ^b	14	<i>Pacifastacus</i> ♂ <i>Procambarus</i> ♂	29–38 43–58
26–28 June, 13–15 July	6 ^b	13	<i>Pacifastacus</i> ♀ <i>Procambarus</i> ♀	26–41 37–60
17–19 July, 20–22 July	7 ^b	16	<i>Pacifastacus</i> ♀ <i>Procambarus</i> ♀	48–52 33–45
17–19 July, 20–22 July	8 ^b	16	<i>Pacifastacus</i> ♂ <i>Procambarus</i> ♂	40–51 35–40

^a The difference between carapace length of contestants was $\leq 10\%$.

^b The difference between carapace length of contestants was $> 10\%$.

Two experiments, whole or in part, were run each week (Table 1). All replicate enclosures were submerged for three days. Although dominance in crayfish is often established within one day and sometimes within minutes (Figler et al. 1995a; Goessmann et al. 2000; Herberholz et al. 2003), extra time was allowed for the crayfishes to acclimate to their enclosures and to recover from handling stress. On two occasions, a replicate from one experiment was mistakenly run with the replicates from another experiment. Furthermore, the reuse of crayfish in four of the experiments precluded running the replicates simultaneously during a three-day period; therefore, these experiments were completed over multiple days (Table 1).

During an experimental run, a scuba diver performed nine observation bouts on each enclosure during daylight hours (Figure 11) when crayfish were most likely sheltering or seeking shelter. Each observation bout lasted up to five minutes, which allowed the scuba diver time to discern shelter occupancy by the crayfishes inside an enclosure (Figure 12). A score of 1 was given to any crayfish occupying the shelter during an observation bout. Besides recording shelter occupancy, the scuba diver collected information on the position of the subordinate crayfish (i.e., the one not occupying the shelter; Figure 13), movements of or behavioral interactions between the crayfishes, and the presence of potential predators (Figure 14). During enclosure experiments 5 and 6, large red swamp crayfish killed and consumed smaller signal crayfish in a few replicates before the end of the 3-d experimental run. These replicates were removed from the statistical analysis.



Figure 11. Scuba diver performing observation bout on enclosure during study of shelter competition between crayfishes at Pine Lake, Sammamish, Washington.



Figure 12. Signal crayfish (*Pacifastacus leniusculus*) occupying artificial shelter inside enclosure on the bottom of Pine Lake, Sammamish, Washington.



Figure 13. Red swamp crayfish (*Procambarus clarkii*) crawling along top edge of enclosure used in study of shelter competition at Pine Lake, Sammamish, Washington.



Figure 14. Smallmouth bass (*Micropterus dolomieu*) inspecting crayfishes held in enclosure on bottom of Pine Lake, Sammamish, Washington. Note the free-ranging red swamp crayfish (*Procambarus clarkii*) emerging from its burrow at center.

Tether experiments

The primary goal of tethering crayfishes was to examine differences in relative survivorship among contestants; in particular, how size and sex might influence survival. Although tethering has its detractors (Knieb and Scheele 2000), it has been used successfully on other large decapod crustaceans (Eggleston et al. 1992; DiDonato and Lodge 1993; Childress and Herrnkind 1994) and remains a useful method for exploring relative predation and sheltering mechanisms in benthic ecology (Aronson et al. 2001).

The mixed-species pairs of crayfish in the first two tether experiments (Table 2) were composed of same-sex or mixed-sex dyads to test the null hypothesis of no difference in survivorship among crayfishes. In these experiments, the individuals were of similar size (the difference in carapace length between contestants was $\leq 10\%$). The mixed-species pairs in the final tether experiments (Table 2) were composed of same-sex dyads with individuals of different size (the difference in carapace length between contestants was $> 10\%$). The final tether experiments also tested the hypothesis of no difference in survivorship among crayfishes.

Table 2. Mixed-species pairs of crayfish used in tether experiments to examine survivorship among native signal crayfish (*Pacifastacus leniusculus*) and non-native red swamp crayfish (*Procambarus clarkii*) at Pine Lake, Sammamish, Washington in 2006.

Date	Tether experiment	Number of pairs or replicates	Contestants	Carapace length range (mm)
Similar size contestants				
1–5 August	1 ^a	16	<i>Pacifastacus</i> ♀ <i>Procambarus</i> ♂	40–53 41–53
1–5 August	2 ^a	16	<i>Pacifastacus</i> ♂ <i>Procambarus</i> ♂	38–53 36–53
Different size contestants				
7–11 August	3 ^b	15	<i>Pacifastacus</i> ♀ <i>Procambarus</i> ♀	38–53 50–58
7–11 August	4 ^b	10	<i>Pacifastacus</i> ♂ <i>Procambarus</i> ♂	30–44 52–59

^a The difference between carapace length of contestants was $\leq 10\%$.

^b The difference between carapace length of contestants was $> 10\%$.

The protocols for tethering crayfishes were adapted from Eggleston et al. (1992), DiDonato and Lodge (1993), and Childress and Herrnkind (1994). The carapaces of crayfishes used in these experiments were wiped clean with alcohol and brass barrel swivels were attached to the dorsal sides of the carapaces with cyanoacrylate, or ‘super glue’ (Figure 15). The tethers were composed of short pieces of monofilament fishing line with loops and brass snap swivels tied at opposite ends of the tethers. The PVC shelters were 25 cm long (5 cm diameter), roughened inside with a file, and open at both ends. The shelters were staked to the bottom of the lake, approximately 2 m apart, and marked with surveyor flags (Figure 16). Prior to the experiments, a scuba diver threaded the loop-ends of the tethers through 25-cm wire stakes positioned 25 cm away from either end of 32 PVC shelters. The tether stakes were then pounded in flush with the substrate using a rubber mallet. In this way, two experiments could be run concurrently with up to 16 replicates each, and the experimental animals could not entangle themselves on their tether stakes. The treatments were randomly dispersed among the PVC shelters (Figure 17).

On the first day of a trial, the experimental animals were ferried to the study site. A scuba diver then descended with mixed-species pairs of crayfish and attached the snap swivels of the tethers to the barrel swivels on the experimental animals. After securing the experimental animals to the tethers, the scuba diver briefly (< 2 min) observed each mixed-species pair to insure that the tethers held fast even when the crayfishes tail-flipped away. About 7% of the barrel swivels broke off the carapaces (mostly the smooth-shelled signal crayfish) when the crayfishes were being deployed. These were immediately replaced in the field with small cable ties that were locked around the

crayfish's cephalothorax between the first and second or second and third walking legs. The snap swivels on the tethers were then re-attached to the cable ties. The mixed-species pairs of crayfish were tethered at opposite ends of the PVC shelters to minimize entanglement of the contestants.

In the first two experiments, the tethers were composed of 1.8 kg test line and were approximately 43 cm long. This length allowed contestants to physically interact because their tethers overlapped. In one replicate, the mixed-species pair repeatedly became entangled and had to be untangled by the scuba diver during subsequent observation bouts. Tangling was not observed in the other replicates; however, many tethers broke at their swivel points when the crayfishes were removed by predators. Consequently, in the last two experiments, a heavier monofilament fishing line (11.3 kg test) was used for the tethers to prevent line breakage. Moreover, the tethers were shortened to a length of approximately 33 cm to further minimize entanglement between contestants. In all experiments, each contestant had access to the PVC shelter but only at the end where it was tethered (Figures 18, 19).

The duration of each tether experiment was five days. During an experimental run, a scuba diver performed 12 observation bouts on each shelter during daylight hours. Each observation bout lasted up to five minutes, which allowed the scuba diver time to discern which crayfish, if any, had been killed. A kill was indicated by obvious crayfish remains (Figure 20), a piece of carapace stuck to the snap swivel (Figure 21), or a broken tether. During the first two experiments, about 10% of the crayfishes escaped, but all of these were found or recaptured on-site by the end of the study. If a crayfish was killed, the time was noted and that crayfish was given a score of 1; if a crayfish survived or

escaped, it was given a score of 0. Besides recording mortalities, the scuba diver collected information on the position, including shelter occupancy, of all crayfishes (Figure 22), movements of or behavioral interactions between crayfishes, and the presence of free-ranging crayfish (Figure 23) and potential predators (Figure 24).



Figure 15. Red swamp crayfish (*Procambarus clarkii*) with brass barrel swivel attached to its carapace.



Figure 16. Example of shelter, stakes, and flag used in tether experiments at Pine Lake, Sammamish, Washington.

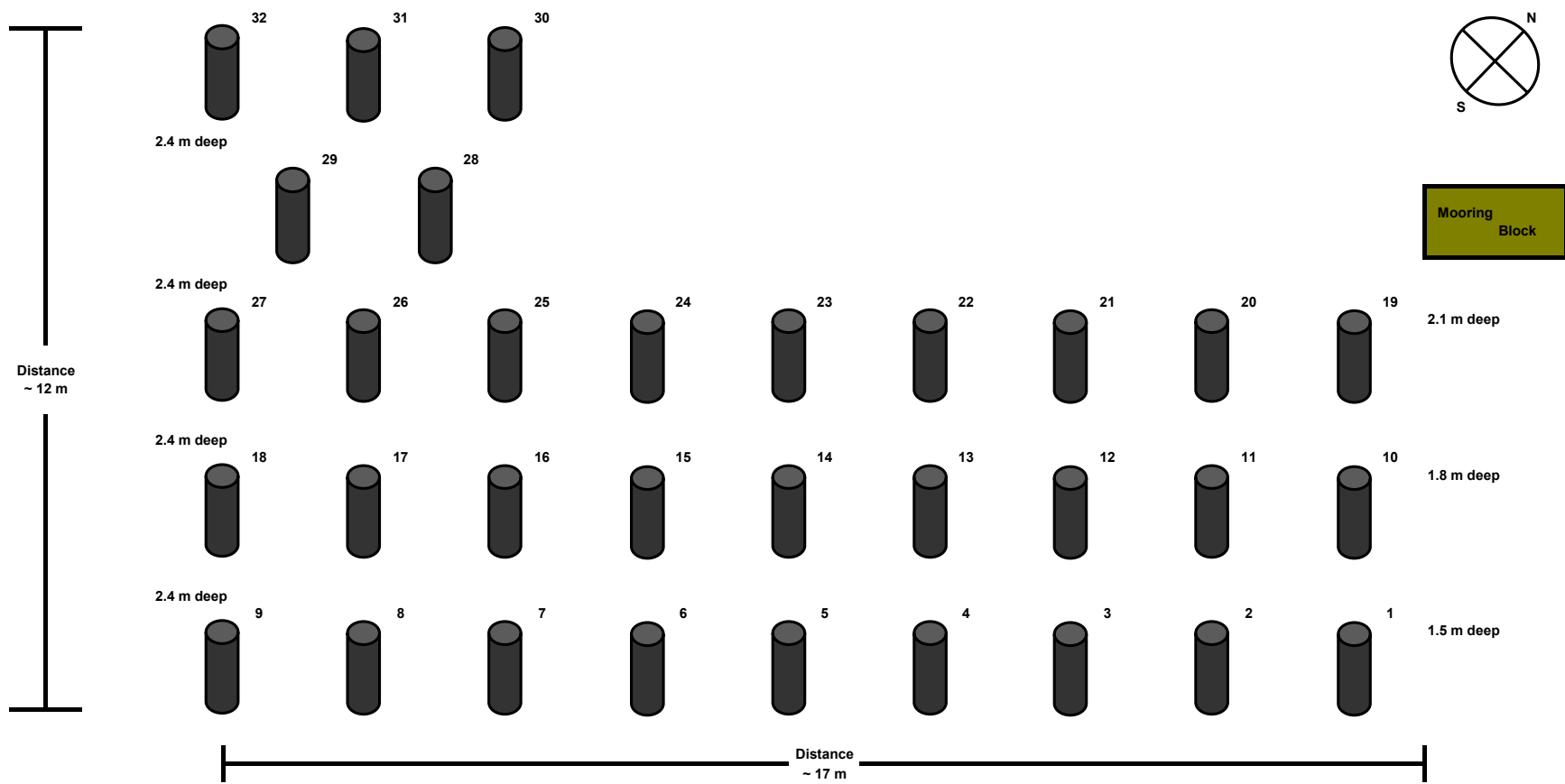


Figure 17. Layout of tether experiments at Pine Lake, Sammamish, Washington.

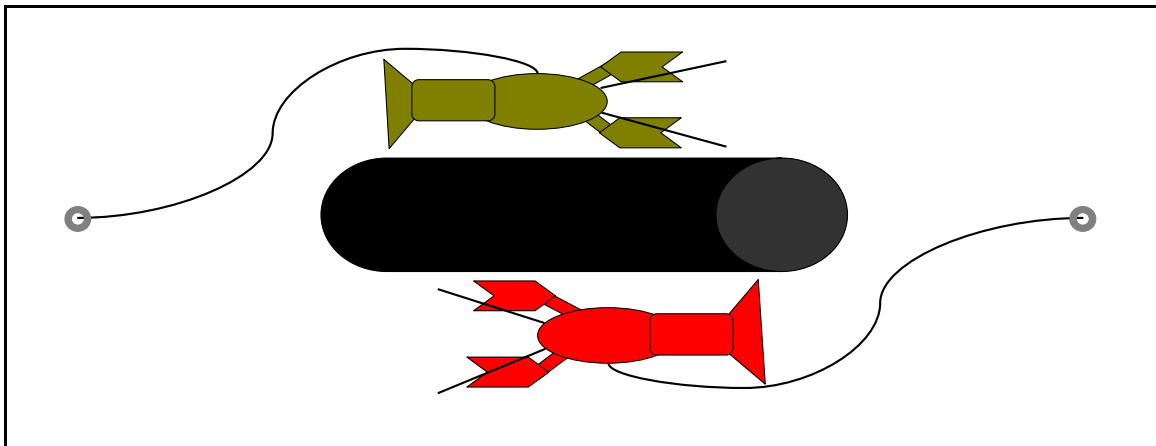


Figure 18. Schematic of mixed-species pair of crayfish showing orientation of tethers to the PVC shelter. The contestants had access to the shelter from the end closest to their tether stakes. In this example, the tethers overlap; hence, the crayfishes would be able to physically interact.



Figure 19. Red swamp crayfish (*Procambarus clarkii*) (top) and signal crayfish (*Pacifastacus leniusculus*) (bottom) tethered on either side of 25-cm long PVC shelter during survivorship experiment at Pine Lake, Sammamish, Washington.

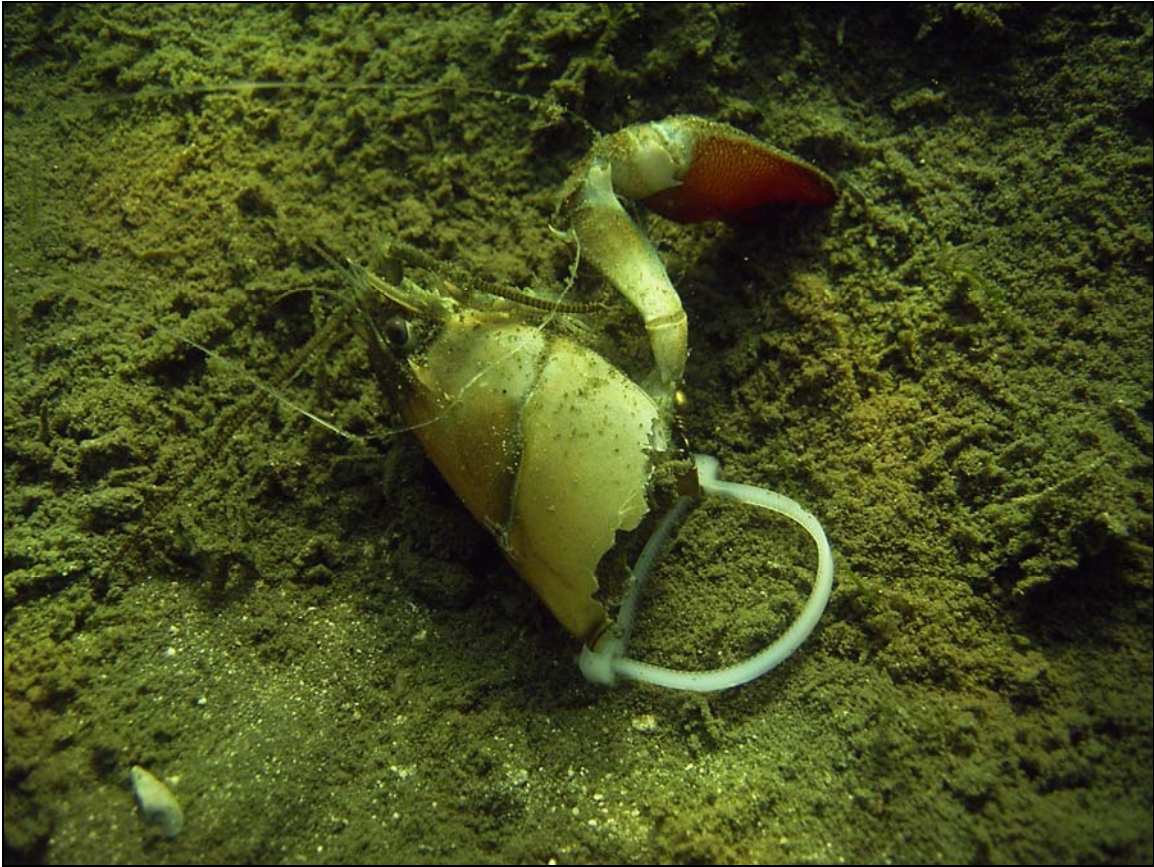


Figure 20. Remains of tethered signal crayfish (*Pacifastacus leniusculus*) after being killed and partially eaten by unknown predator at Pine Lake, Sammamish, Washington. Note the cable tie. These were used occasionally to replace brass barrel swivels that had come unglued from the carapace.



Figure 21. Carapace remains of tethered red swamp crayfish (*Procambarus clarkii*) after being killed and eaten by unknown predator at Pine Lake, Sammamish, Washington.



Figure 22. Signal crayfish (*Pacifastacus leniusculus*) occupying PVC shelter during tether experiment at Pine Lake, Sammamish, Washington.



Figure 23. Free-ranging male red swamp crayfish (*Procambarus clarkii*) interacting with tethered female red swamp crayfish outside PVC shelter during experiment at Pine Lake, Sammamish, Washington.



Figure 24. Trophy-size largemouth bass (*Micropterus salmoides*) inspecting PVC shelter during tether experiment at Pine Lake, Sammamish, Washington (note the PVC shelter in upper right-hand corner). The largemouth bass was one of several potential crayfish predators observed on-site throughout the study.

Data analysis

The enclosure experiment data (= the total count or score for each crayfish during the 3-d experimental run) were analyzed with replicated tests of goodness of fit (G test) followed by unplanned tests of differences among replicates using the simultaneous test procedure (STP) described by Sokal and Rohlf (1981). First, for each experiment, individual values of G were computed for each replicate to test the null hypothesis of equal use of the shelter by the crayfishes. The expected values were based on the assumption of 50% occupancy by each crayfish. Next, the total G was obtained by summing the individual G values. Pooling the replicates together from an experiment and testing the hypothesis of equal use of the shelter by the crayfishes resulted in a pooled G value. The interaction or heterogeneity G for each experiment was obtained by subtracting the pooled G from the total G . Finally, where there was significant heterogeneity among replicates, the STP was used to locate the source(s) of heterogeneity [i.e., according to Sokal and Rohlf (1981), to test whether all replicates differed from one another or whether there were homogenous sets of replicates differing from other such sets or single replicates].

Since body size and chelae length often influence the outcome of shelter disputes among crayfish (Garvey and Stein 1993; Figler et al. 1999; Nakata and Goshima 2003), biological explanations for heterogeneity among replicates were explored by plotting the carapace length against the chelae length of all contestants involved in an experiment. Proportional occupancy by species was calculated for each replicate of an enclosure experiment by dividing the total occupancy count for each crayfish during the 3-d

experimental run by the number of observation bouts ($n = 9$). Median proportional occupancy by species and the upper and lower quartiles were calculated for each enclosure experiment. Differences in these distributions were examined using the Wilcoxon rank sum (WRS) test. All G tests were performed using a program by John McDonald of the University of Delaware (<http://udel.edu/~mcdonald/statgtestgof.html>). The WRS tests were performed using Statistix® 8 software (Analytical Software, Tallahassee, Florida).

The tether experiment data (= number of mortalities by species occurring at observation bout time, t) were analyzed using survival analysis. This statistical approach concerns the time between entry to a study and a subsequent event (Khan and Prescott 2004). Predation or death of a crayfish was the event of interest in the tether experiments. The probability or proportion of subjects surviving to at least time t (here, the time of individual observation bouts) is termed the survival function, $S(t)$, which is commonly estimated using non-parametric methods developed in the 1950s by E. L. Kaplan and P. Meier (Oakes 2001). At the start of an observation period, $S(t) = 1$. Survivorship then decreases from 1 to 0, in step-wise fashion, with increasing time. The observation period generally ends before $S(t) = 0$. Of particular interest is the median survival time or the time at which the cumulative $S(t) = 0.5$ (Khan and Prescott 2004). The advantages of using Kaplan-Meier methods are that no assumptions about the form of $S(t)$ are required, the techniques are suitable for small data sets, and the methods can handle censored data. Censoring occurs when 1) the subject is removed before the event of interest (death) can be observed or the event of interest occurs after the end of data collection, 2) the event of interest occurs before data collection begins, or 3) the event of

interest occurs during a break in data collection (Kachman 1999). Examples of censored data from the tether experiments included the survival times of escaped crayfishes and those of crayfishes surviving until the end of data collection. The Kaplan-Meier product-limit estimate of $S(t)$, including standard error and, when possible, the median survival time, were calculated by species using Statistix® 8 software. Survivorship curves [i.e., $S(t)$ over time] were plotted according to Atkinson (1995) and Motulsky (1999). Differences in survivorship curves were evaluated with logrank tests recommended by Jones and Crowley (1990) and Khan and Prescott (2004) and were performed using Statistix® 8 software.

RESULTS

Enclosure experiments

Shelter occupancy during the enclosure experiments was rarely equal between contestants. Typically, one crayfish would establish dominance and monopolize or hold the shelter for the entire experimental run. The dominant crayfish was usually observed inside the shelter, facing out, occupying a position near the opening. The subordinate crayfish was observed resting or crawling outside the shelter, usually > 25 cm away from the opening in any direction. If a subordinate crayfish passed by the opening of the shelter, the dominant crayfish threatened (e.g., lifted and opened one or both chelae) or attacked the intruder. If both crayfishes were outside the shelter and the subordinate crayfish moved toward the shelter or the opening, the dominant crayfish would quickly

resume its position at the front end of the shelter. Likewise, if both crayfishes were outside the shelter when predatory fishes approached, the dominant crayfish returned to the shelter while the subordinate crayfish remained still or moved elsewhere within the enclosure. Occasionally, the crayfishes within an enclosure alternated use of the shelter with no clear hierarchy.

In each experiment, the null hypothesis of equal use of the shelter by signal crayfish and red swamp crayfish was rejected (G tests, $P < 0.05$; Table 3). Furthermore, the replicated G tests indicated significant heterogeneity among replicates ($P < 0.0001$) and the STP revealed two distinct homologous groups of winners, one dominated by signal crayfish, the other, red swamp crayfish (Tables A.1 – A.8).

When contestants were size-matched, signal crayfish occupied the shelter proportionally more often than red swamp crayfish in three out of four experiments; this was significant in two of the experiments (WRS test, $P < 0.05$; Table 3). Female red swamp crayfish occupied the shelter proportionally more often than similarly-sized male signal crayfish, but this difference was not significant (WRS test, $P > 0.05$; Table 3). When contestants differed in size, the larger crayfish, irrespective of species and sex, occupied the shelter proportionally more often than its smaller counterpart. This was significant in three out of four experiments (WRS test, $P < 0.05$; Table 3).

By plotting the carapace lengths against the chelae lengths of all contestants involved in the experiments, some biological differences between the winners and losers were revealed. For example, in the experiments where contestants were size-matched, the groups of winners dominated by signal crayfish were composed mostly of individuals with $CL < 45$ mm (Figures 25 – 28). The signal crayfish in these groups had longer

chelae than their red swamp crayfish counterparts. In contrast, the groups of winners dominated by red swamp crayfish were composed mostly of individuals with CL > 40 mm (Figures 25 – 28). The red swamp crayfish in the latter groups generally had similar-sized or shorter chelae than their signal crayfish counterparts. Finally, in the experiments where contestants differed in size, with few exceptions, the winners clearly had distinct size advantages over the losers (Figures 29 – 32).

Table 3. Results of replicated *G* tests and Wilcoxon rank sum tests of crayfish occupancy data from the 2006 shelter competition study at Pine Lake, Sammamish, Washington. *Pacifastacus* = *Pacifastacus leniusculus*, the native signal crayfish; *Procambarus* = *Procambarus clarkii*, the non-native red swamp crayfish.

Enclosure experiment	Contestants	Pooled occupancy (#)	Pooled <i>G</i>	Heterogeneity <i>G</i>	Total <i>G</i>	Median proportional occupancy (lower quartile, upper quartile)	Wilcoxon rank sum test
1	<i>Pacifastacus</i> ♀	77	*	****	****	0.8 (0.1, 1.0)	NS
	<i>Procambarus</i> ♀	50				0.0 (0.0, 0.9)	
2	<i>Pacifastacus</i> ♂	82	***	****	****	0.9 (0.0, 1.0)	*
	<i>Procambarus</i> ♂	36				0.0 (0.0, 0.8)	
3	<i>Pacifastacus</i> ♀	89	****	****	****	0.8 (0.3, 1.0)	**
	<i>Procambarus</i> ♂	27				0.0 (0.0, 0.3)	
4	<i>Pacifastacus</i> ♂	53	**	****	****	0.1 (0.0, 0.9)	NS
	<i>Procambarus</i> ♀	84				0.9 (0.0, 1.0)	
5	Small <i>Pacifastacus</i> ♂	18	****	****	****	0.0 (0.0, 0.1)	***
	Large <i>Procambarus</i> ♂	97				0.9 (0.7, 1.0)	
6	Small <i>Pacifastacus</i> ♀	8	****	****	****	0.0 (0.0, 0.0)	****
	Large <i>Procambarus</i> ♀	105				1.0 (0.9, 1.0)	
7	Large <i>Pacifastacus</i> ♀	86	**	****	****	0.7 (0.1, 1.0)	NS
	Small <i>Procambarus</i> ♀	49				0.1 (0.0, 0.9)	
8	Large <i>Pacifastacus</i> ♂	96	****	****	****	0.8 (0.5, 1.0)	*
	Small <i>Procambarus</i> ♂	32				0.0 (0.0, 0.2)	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, NS – not significant.

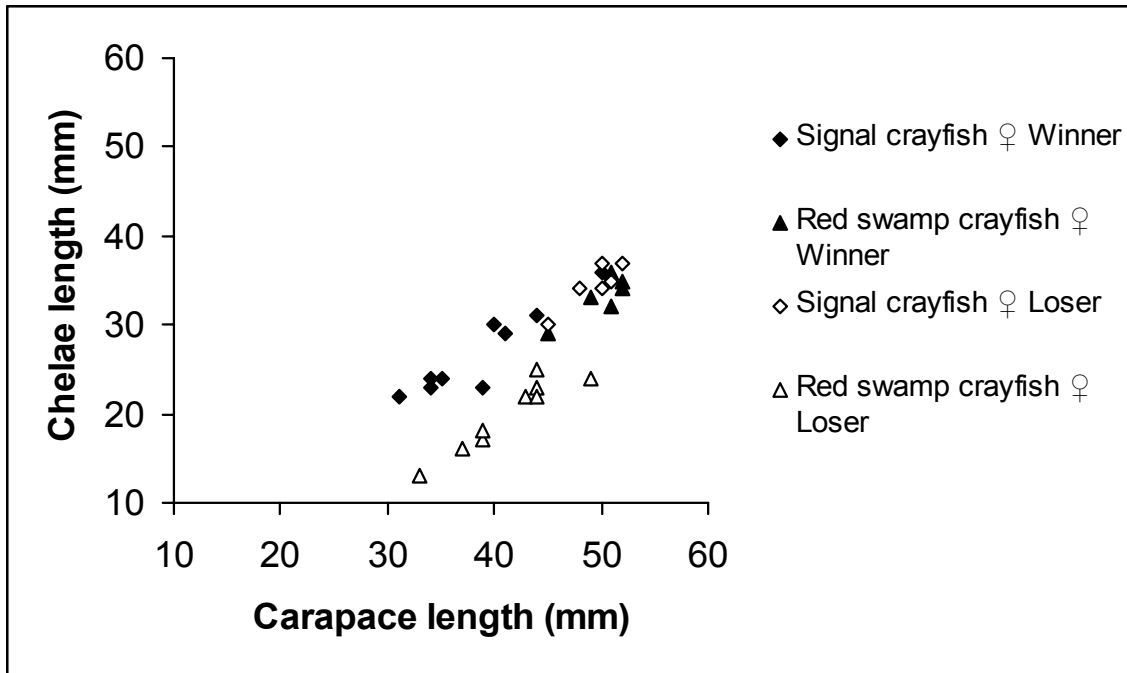


Figure 25. Relationship between carapace length and chelae length of female signal crayfish (*Pacifastacus leniusculus*) and similarly-sized female red swamp crayfish (*Procambarus clarkii*) from enclosure experiment 1.

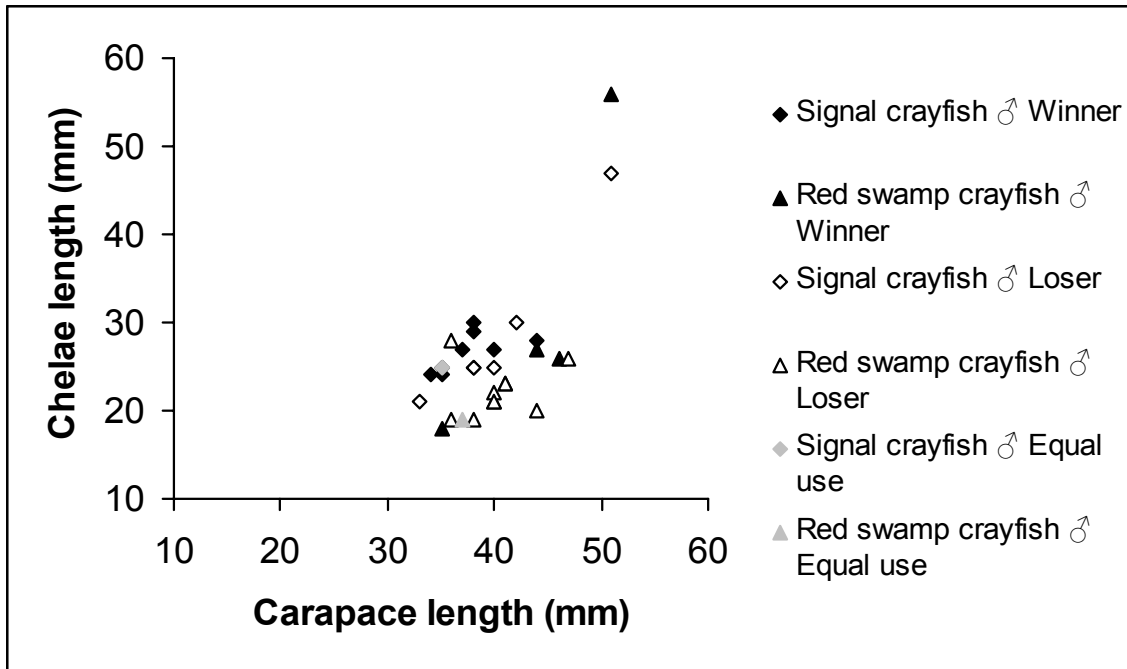


Figure 26. Relationship between carapace length and chelae length of male signal crayfish (*Pacifastacus leniusculus*) and similarly-sized male red swamp crayfish (*Procambarus clarkii*) from enclosure experiment 2.

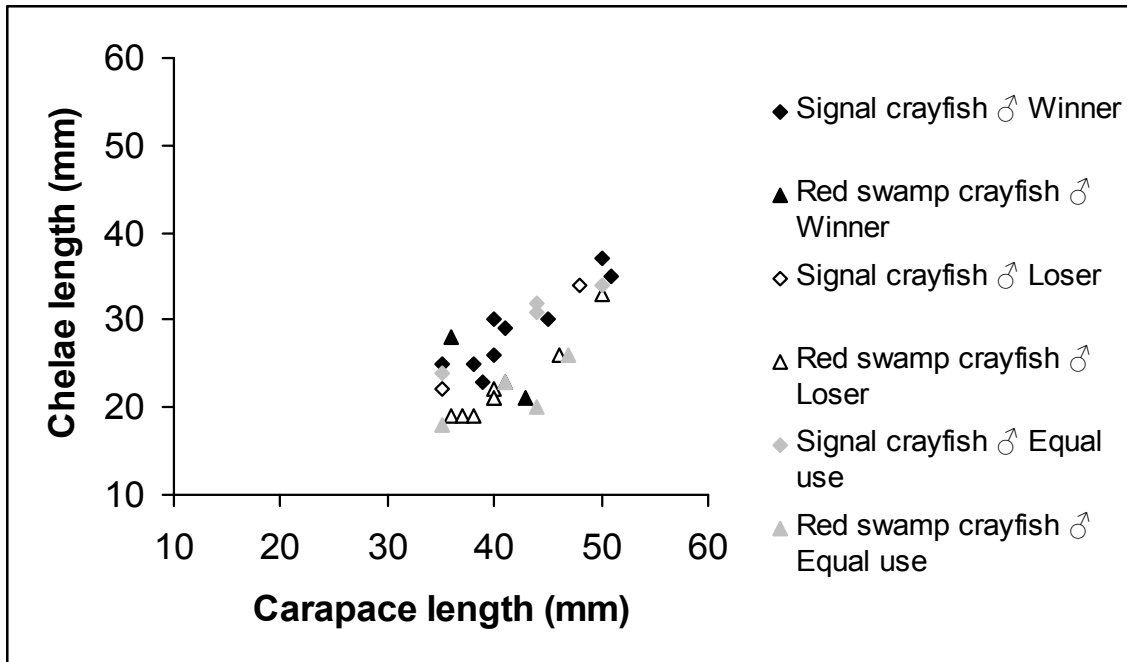


Figure 27. Relationship between carapace length and chelae length of female signal crayfish (*Pacifastacus leniusculus*) and similarly-sized male red swamp crayfish (*Procambarus clarkii*) from enclosure experiment 3.

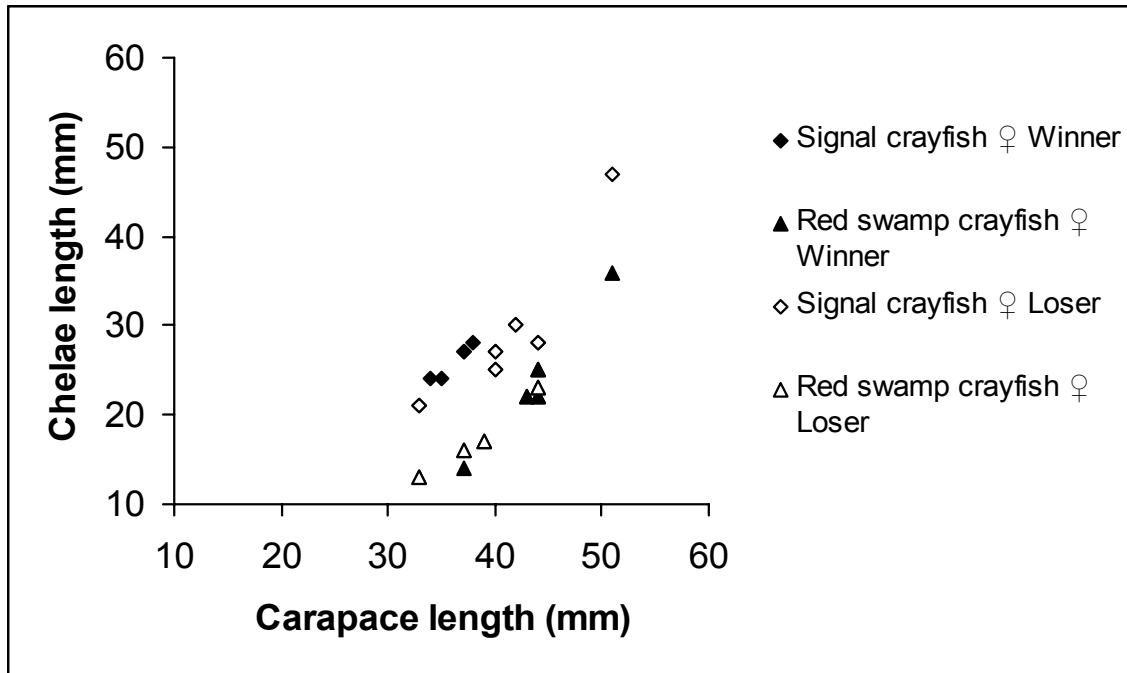


Figure 28. Relationship between carapace length and chelae length of male signal crayfish (*Pacifastacus leniusculus*) and similarly-sized female red swamp crayfish (*Procambarus clarkii*) from enclosure experiment 4.

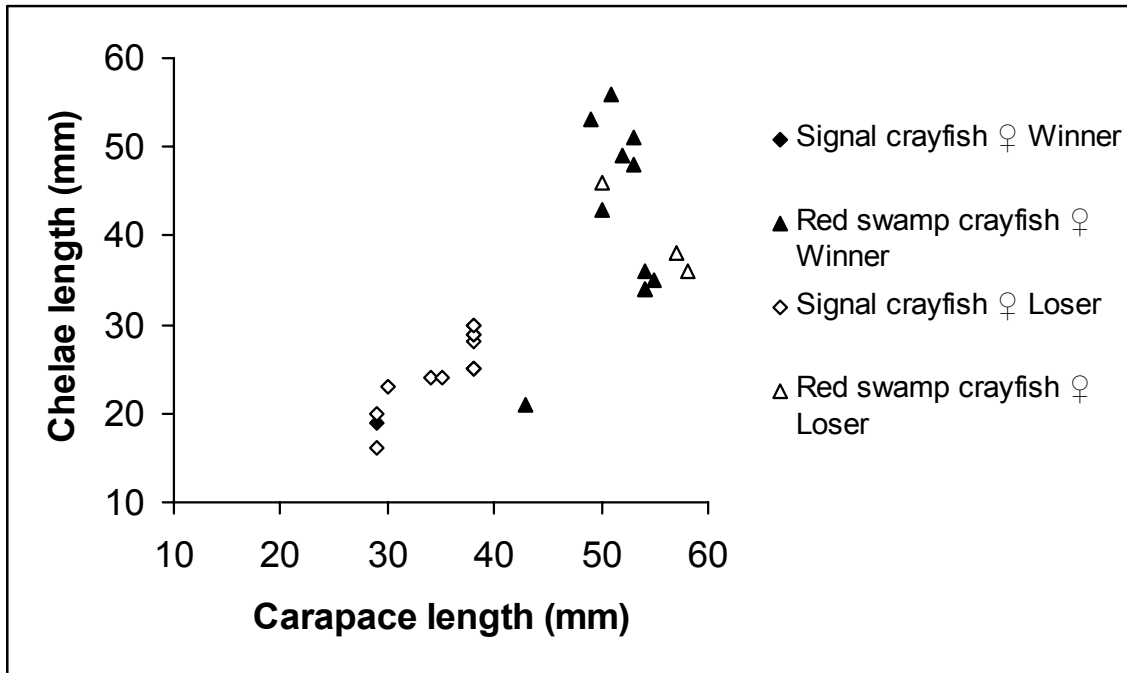


Figure 29. Relationship between carapace length and chelae length of small male signal crayfish (*Pacifastacus leniusculus*) and large male red swamp crayfish (*Procambarus clarkii*) from enclosure experiment 5.

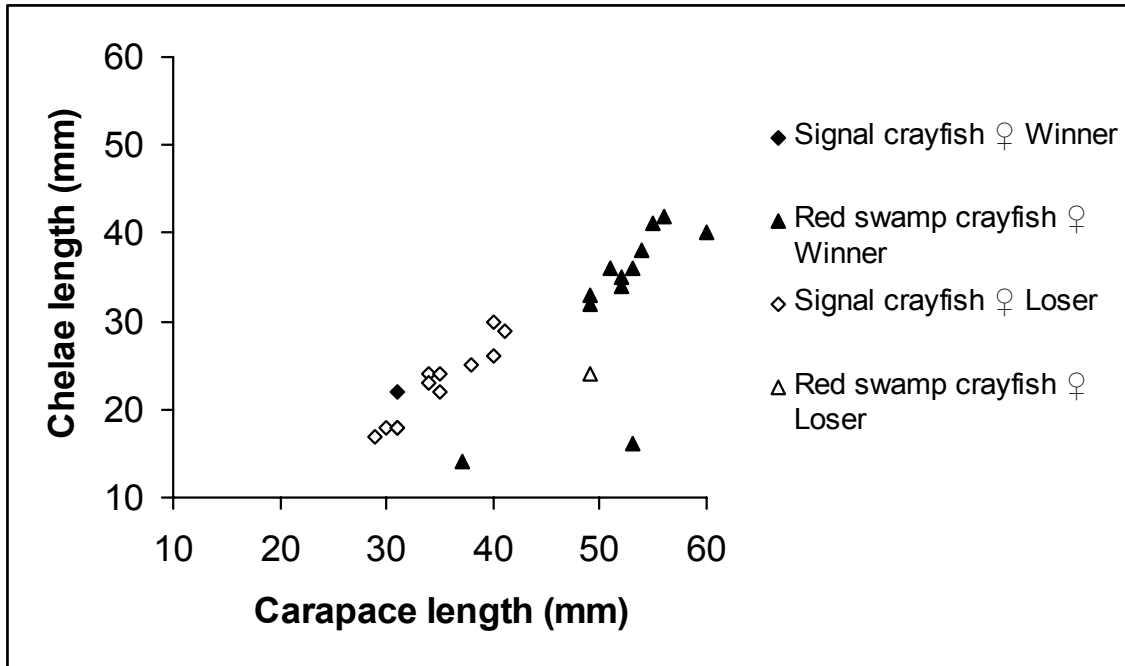
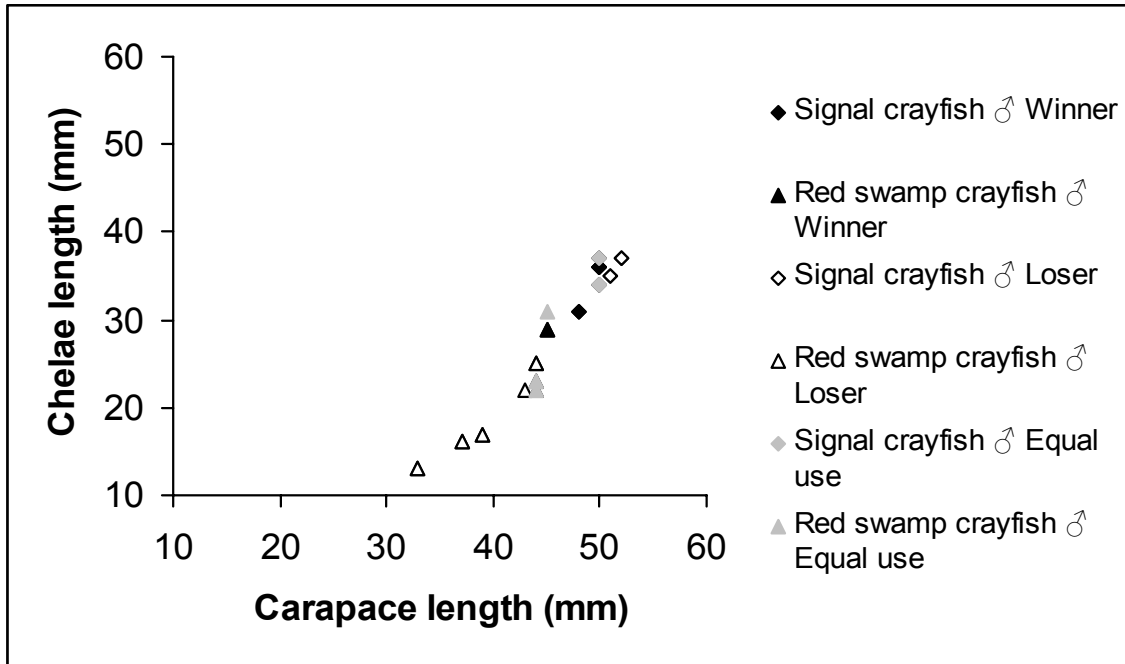


Figure 30. Relationship between carapace length and chelae length of small female signal crayfish (*Pacifastacus leniusculus*) and large female red swamp crayfish (*Procambarus clarkii*) from enclosure experiment 6.



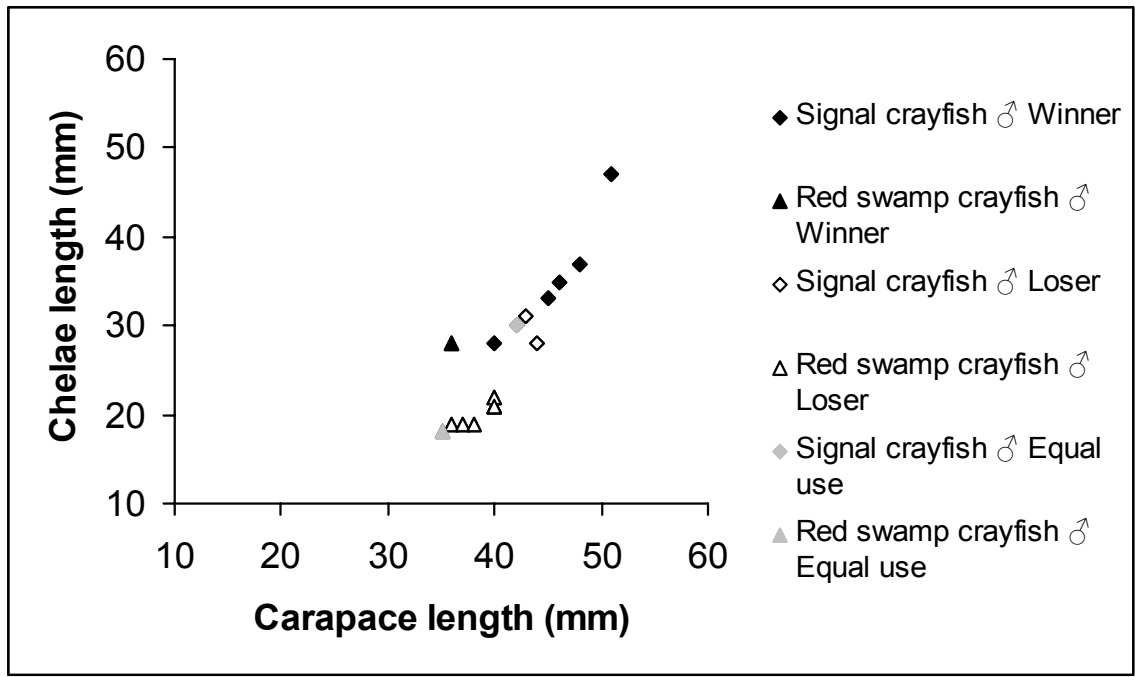


Figure 32. Relationship between carapace length and chelae length of large male signal crayfish (*Pacifastacus leniusculus*) and small male red swamp crayfish (*Procambarus clarkii*) from enclosure experiment 8.

Tether experiments

Sheltering was not as clearly defined during these trials as it was during the enclosure experiments. Without the enclosures, alternative refuges were available to contestants that were excluded from the PVC shelters. For example, if one crayfish was monopolizing the PVC shelter, its counterpart might be buried outside the shelter (Figure 33), lying alongside the shelter (Figure 34), or seeking refuge on or near coarse substrates within the radius of its tether (Figure 35). With both ends of the PVC shelter open, ‘double occupancy’ was possible. Indeed, on two occasions during tether experiments 1 and 2, the crayfish pairs shared the refuge, the contestants occupying opposite ends of the PVC shelter (Figure 36). Because of the alternative refuges available to contestants, the influence of the PVC shelter on survival became somewhat equivocal; thus, shelter use during the tether trials was not analyzed.

In the first experiment, where female signal crayfish were tethered with male red swamp crayfish of the same size, the overall survivorship for both species was low (Figure 37). The median survival time of female signal crayfish was 32 hours, whereas the median survival time of male red swamp crayfish was 20 hours. There was no significant difference in overall survivorship (range = 23 – 25%) among the crayfishes after five days (logrank test, $P = 0.80$). In the second experiment, where male signal crayfish were tethered with male red swamp crayfish of the same size, the overall survivorship for both species also was low (Figure 38). The median survival time of male signal crayfish was 43 hours, whereas the median survival time of male red swamp

crayfish was 26 hours. Again, there was no significant difference in overall survivorship (range = 16 – 17%) among the crayfishes after five days (logrank test, $P = 0.76$).

In the third experiment, where small female signal crayfish were tethered with larger female red swamp crayfish, the overall survivorship for both species was higher than the previous experiments (Figure 39). There was no significant difference in overall survivorship (range = 73 – 87%) among the crayfishes after five days (logrank test, $P = 0.38$). And in the last experiment, where small male signal crayfish were tethered with larger male red swamp crayfish, the overall survivorship for both species also was high (Figure 40). Here, too, there was no significant difference in overall survivorship (range = 70 – 80%) among the crayfishes after five days (logrank test, $P = 0.58$). It should be noted that in the final experiments, most (> 80%) surviving crayfish – paired or otherwise – were observed sharing or occupying the PVC shelter by the end of the study.



Figure 33. Tethered red swamp crayfish (*Procambarus clarkii*) buried outside PVC shelter during survivorship experiment at Pine Lake, Sammamish, Washington.



Figure 34. Tethered signal crayfish (*Pacifastacus leniusculus*) seeking refuge alongside PVC shelter occupied by tethered red swamp crayfish (*Procambarus clarkii*) during survivorship experiment at Pine Lake, Sammamish, Washington.

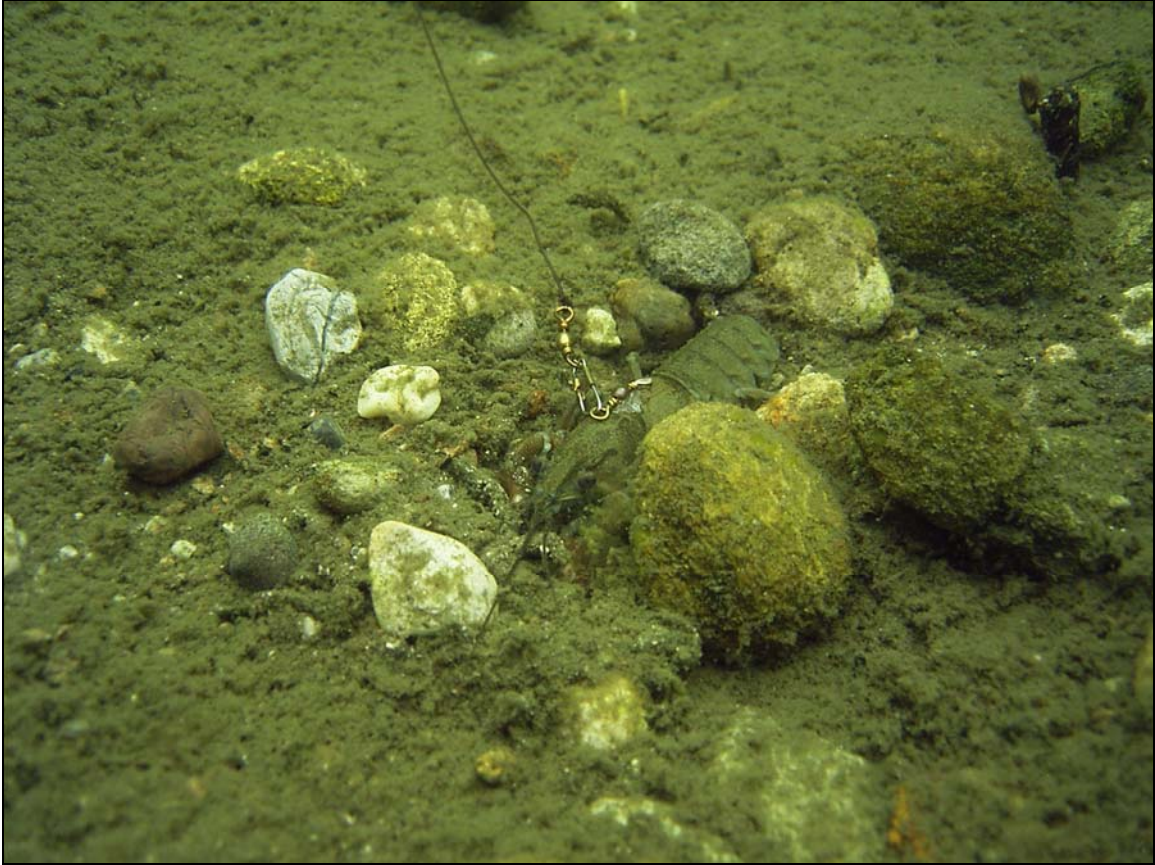


Figure 35. Tethered signal crayfish (*Pacifastacus leniusculus*) hiding amongst gravel and pebbles during survivorship experiment at Pine Lake, Sammamish, Washington.



Figure 36. Tethered signal crayfish (*Pacifastacus leniusculus*) (left) and tethered red swamp crayfish (*Procambarus clarkii*) (right) sharing PVC shelter during survivorship experiment at Pine Lake, Sammamish, Washington.

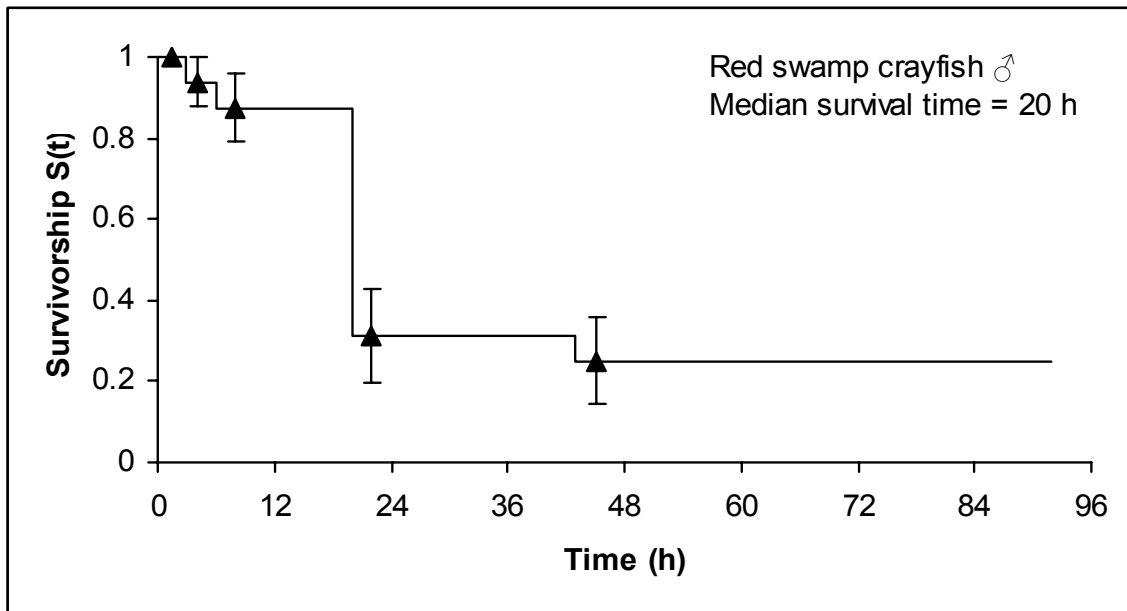
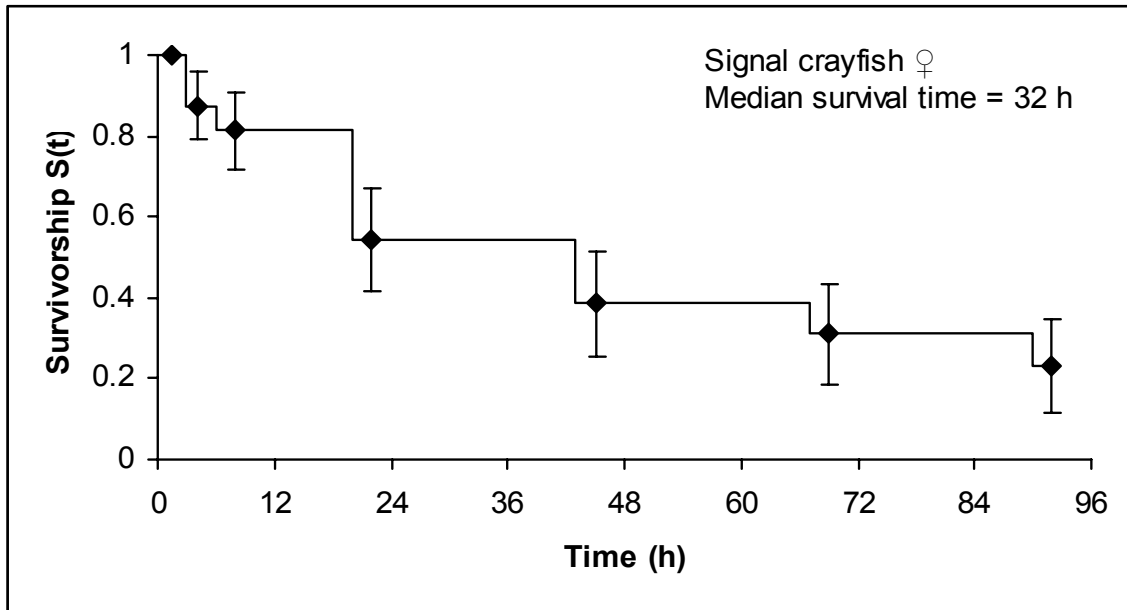


Figure 37. Survivorship curves for similar-sized female signal crayfish (*Pacifastacus leniusculus*) (top) and male red swamp crayfish (*Procambarus clarkii*) (bottom) tethered in mixed-species pairs outside single PVC shelters placed on the bottom of Pine Lake, Sammamish, Washington. Values are Kaplan-Meier estimates of the survival function $S(t)$, the proportion of subjects surviving to at least time t , and their standard errors.

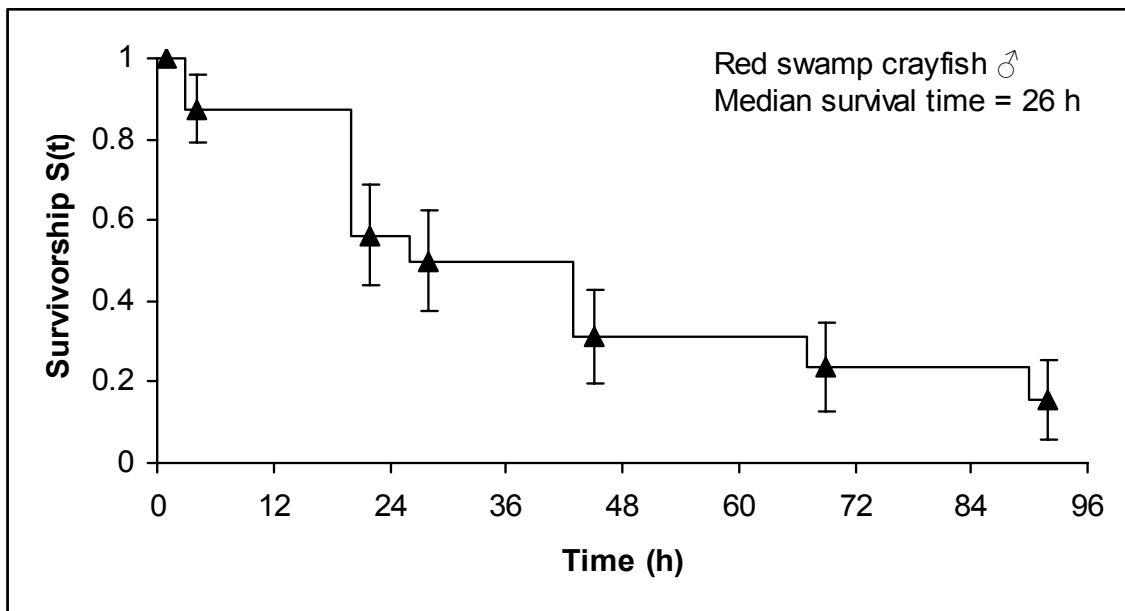
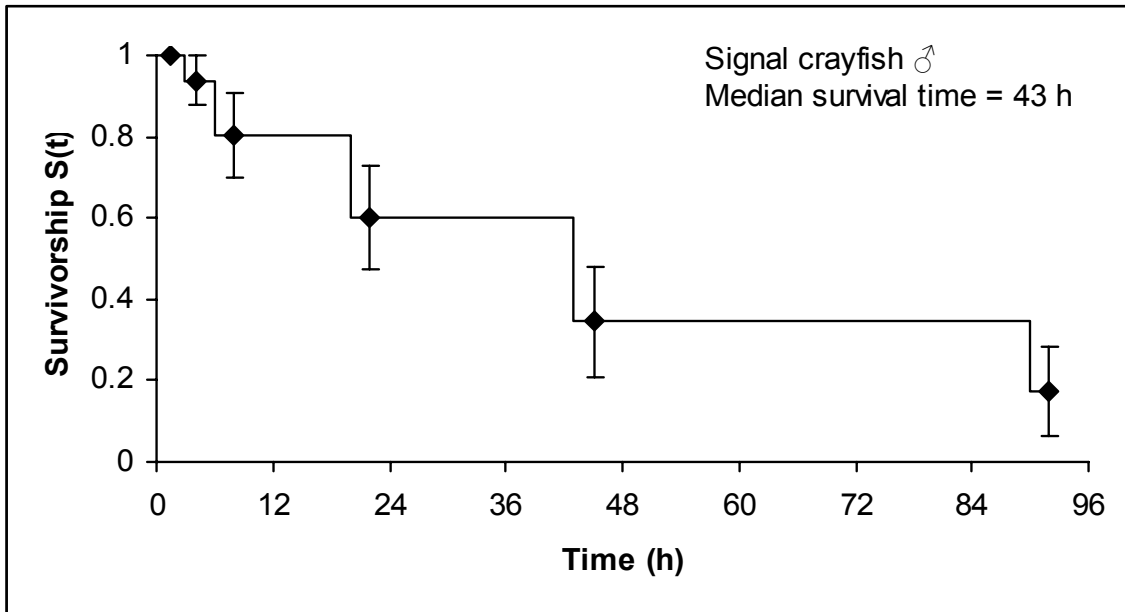


Figure 38. Survivorship curves for similar-sized male signal crayfish (*Pacifastacus leniusculus*) (top) and male red swamp crayfish (*Procambarus clarkii*) (bottom) tethered in mixed-species pairs outside single PVC shelters placed on the bottom of Pine Lake, Sammamish, Washington. Values are Kaplan-Meier estimates of the survival function $S(t)$, the proportion of subjects surviving to at least time t , and their standard errors.

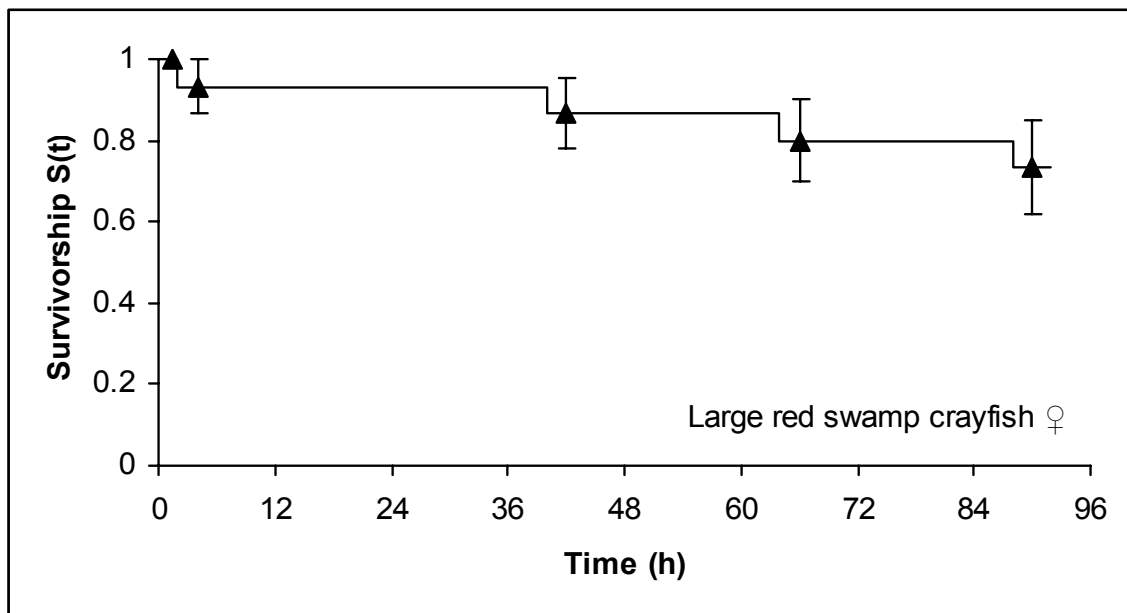
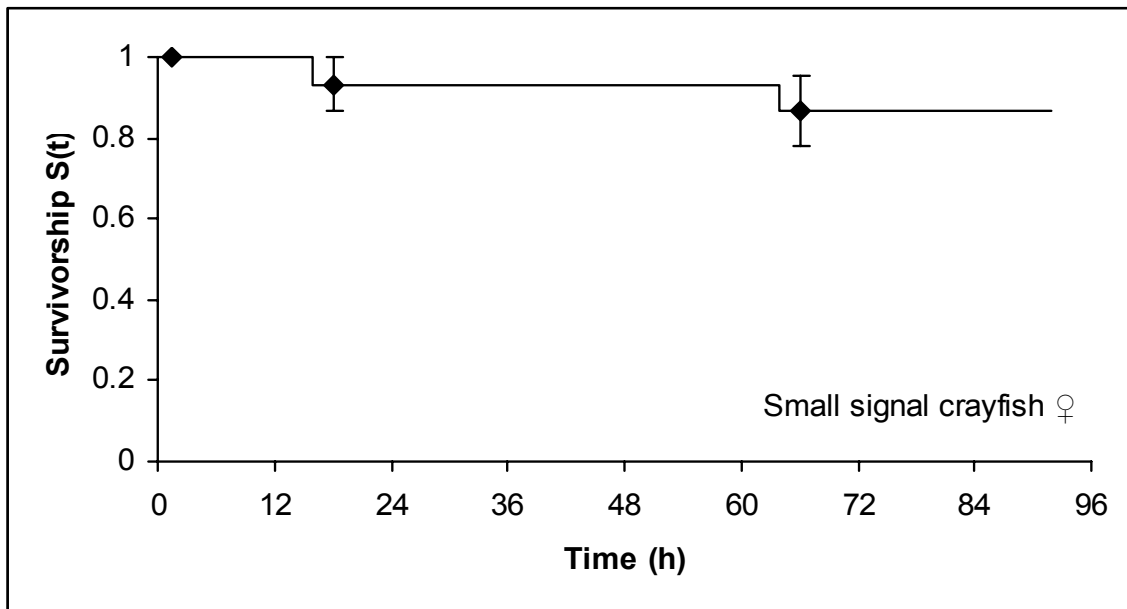


Figure 39. Survivorship curves for small female signal crayfish (*Pacifastacus leniusculus*) (top) and large female red swamp crayfish (*Procambarus clarkii*) (bottom) tethered in mixed-species pairs outside single PVC shelters placed on the bottom of Pine Lake, Sammamish, Washington. Values are Kaplan-Meier estimates of the survival function $S(t)$, the proportion of subjects surviving to at least time t , and their standard errors.

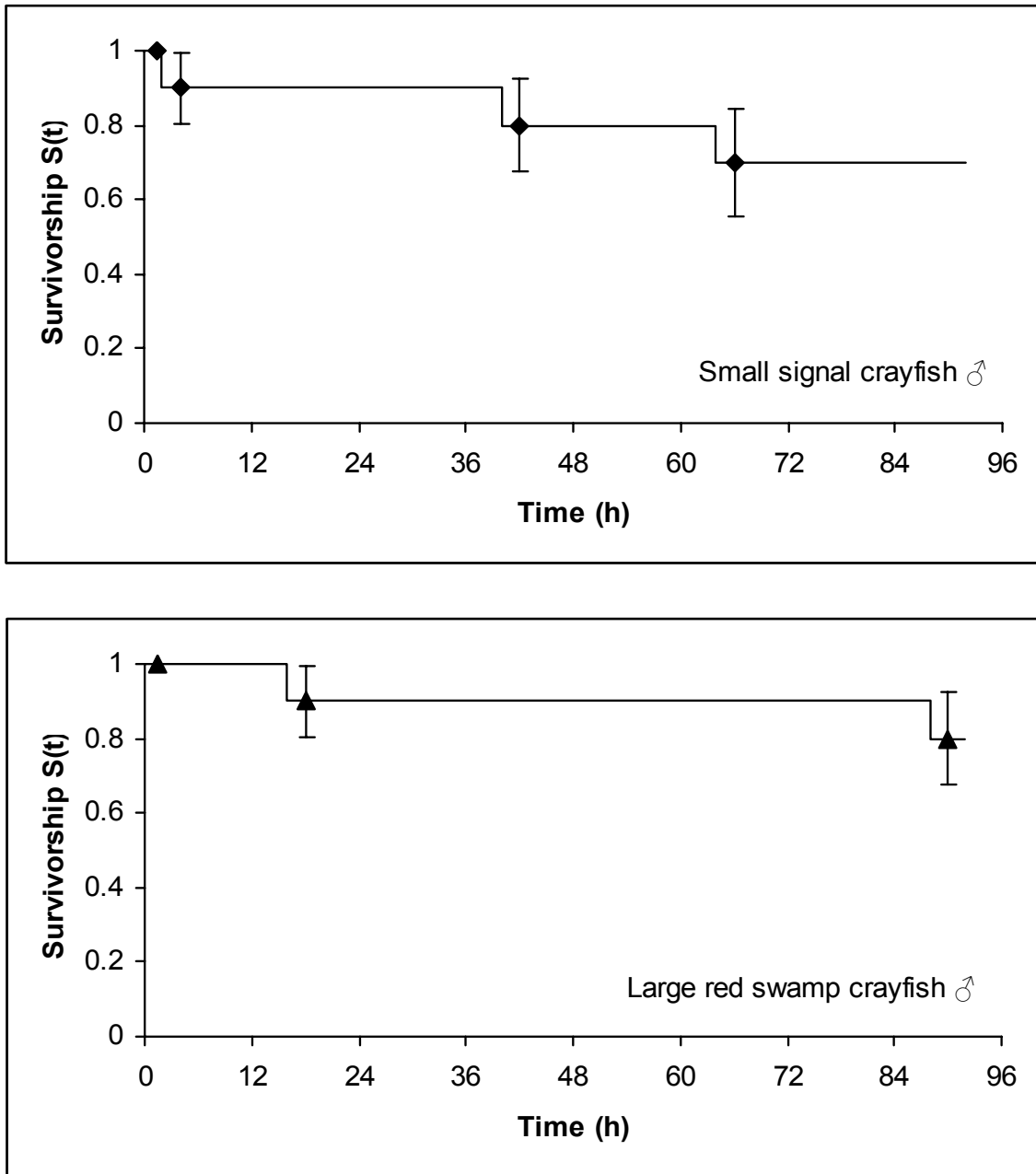


Figure 40. Survivorship curves for small male signal crayfish (*Pacifastacus leniusculus*) (top) and large male red swamp crayfish (*Procambarus clarkii*) (bottom) tethered in mixed-species pairs outside single PVC shelters placed on the bottom of Pine Lake, Sammamish, Washington. Values are Kaplan-Meier estimates of the survival function $S(t)$, the proportion of subjects surviving to at least time t , and their standard errors.

DISCUSSION

The results of the enclosure experiments at Pine Lake demonstrated that, irrespective of species and sex, when paired with smaller heterospecifics, larger crayfish readily monopolized the shelters inside the enclosures. Furthermore, larger red swamp crayfish could be extremely aggressive as indicated by the predation on smaller signal crayfish in about 20% of the replicates. These findings are consistent with previous studies of sheltering behavior and aggression in crayfish. For example, Rabeni (1985) demonstrated that dominance in sheltering cambarid crayfishes (*Orconectes* spp.) was a function of carapace length. Likewise, Figler et al. (1999) found that, in juvenile red swamp crayfish, carapace length was the most important factor in determining the winner of disputes over shelter. Ranta and Lindström (1992) reported that large juvenile signal crayfish evicted smaller conspecifics from shelter and successfully defended the same resource from intruding conspecifics. The authors demonstrated that the likelihood of an adult signal crayfish winning a shelter increased with increasing difference in body weight between the contestants (Ranta and Lindström 1993). In addition, Issa et al. (1999) found that large juvenile red swamp crayfish aggressively subordinated smaller conspecifics, a pattern that was also observed by Pavey and Fielder (1996) in fighting pairs of juvenile Australian crayfish (*Cherax cuspidatus*).

When contestants were size-matched, the dominant crayfish or 'winner' was typically the one with longer chelae; frequently, this was the signal crayfish. This was consistent with previous studies that found chelae length to be a reliable predictor of fighting ability in crayfish. For example, male crayfish with long chelae were more

likely to be the winners during conflicts with same-sex conspecifics having shorter chelae (Rutherford et al. 1995; Edsman and Jonsson 1996).

The findings at Pine Lake indicated that female crayfishes were adept at monopolizing shelter. In the case of female signal crayfish, superior chelae length likely contributed to their success; however, the same could not be said of female red swamp crayfish, since they had similar-size or shorter chelae than their signal crayfish counterparts. The success of female and male red swamp crayfish paired with similar-sized signal crayfish can be explained, in part, by the reproductive status of the non-native species. Red swamp crayfish typically reach sexual maturity at ~ 40 mm CL and mate during summer, a period when reproductive adults, especially females, often seek shelter in burrows (Penn 1943; Gherardi et al. 1999; Ilhéu et al. 2003). During the enclosure experiments, the size of 'winning' red swamp crayfish was typically ≥ 40 mm CL. Given the time of this study (summer), these were probably reproductively active adults that were highly motivated to acquire shelter. Indeed, brooding female red swamp crayfish (i.e., carrying eggs or hatched young) are very aggressive toward intruding males and non-maternal female red swamp crayfish (Figler et al. 1995b), and are the winners in most territorial disputes with conspecifics (Figler et al. 2001). Similarly, large, sexually mature male red swamp crayfish have little trouble exerting dominance over established, mixed-sex communities of conspecifics (Figler et al. 1995a). Sexually mature male red swamp crayfish also easily out-compete non-maternal female red swamp crayfish for shelter (Figler et al. 2005), whereas adult female signal crayfish subordinate adult male signal crayfish when competing for shelter (Peeke et al. 1995).

Social experience also influences the outcome of agonistic encounters among crayfish. For example, the original occupier of a shelter may exhibit a prior residence advantage over intruders, a social benefit that transcends size or sex differences between contestants (Peeke et al. 1995; Blank and Figler 1996; Edsman and Jonsson 1996; Figler et al. 2005). Daws et al. (2002) found that if an individual red swamp crayfish won or lost a series of fights, the outcome of those encounters would dictate whether the individual would win subsequent bouts with conspecifics, even if there was a size disparity between the contestants, e.g., a small ‘winner’ was likely to win against a large ‘loser’. Likewise, Goessmann et al. (2000) reported that winning enhanced further success in juvenile noble crayfish (*Astacus astacus*) and losing decreased an individual’s subsequent chances for dominance. The latter is referred to as the loser effect. According to Hock and Huber (2006), loser effects are the most important in determining the social rank of individual crayfish. It is possible that ‘winner’ and ‘loser’ effects played a role in the outcome of contests where crayfishes were reused at Pine Lake; however, because the field experiments were not designed to isolate these effects, the social experiences of the crayfishes were not evaluated.

Several studies of native and non-native species of crayfish have shown that agonistic encounters and competitive interactions over shelter often favor the non-native species (Vorburger and Ribi 1999; Usio et al. 2001; Gherardi and Cioni 2004; Gherardi and Daniels 2004). This was not the case at Pine Lake. The results of the enclosure experiments showed that the native signal crayfish was capable of ‘holding its own’ against similarly-sized non-native red swamp crayfish, even during the peak reproductive period of the red swamp crayfish when individuals are highly motivated to obtain shelter.

Conversely, in Sweden, Söderbäck (1991, 1994) reported that non-native signal crayfish were very aggressive toward similar-sized native noble crayfish and displaced them from shelter, substantially increasing the noble crayfish's exposure to predation risk. In Wisconsin, DiDonato and Lodge (1993) found that the native virile crayfish (*Orconectes virilis*) was removed by fish at significantly higher rates than similarly-sized non-native rusty crayfish (*Orconectes rusticus*) and northern clearwater crayfish (*Orconectes propinquus*). Garvey and Stein (1993) hypothesized that non-native rusty crayfish used their superior body size and chelae to dominate a smaller, native species of crayfish. Indeed, when shelter was limiting and predaceous fish present, the native species was excluded from shelter and consumed at a higher rate than the invader (Garvey et al. 1994). Similarly, Hill and Lodge (1994) demonstrated that non-native rusty crayfish inhibited native congeners (*Orconectes* spp.) from using habitats that provided refuge from predation. Since the native congeners were the preferred prey of resident fish, the authors concluded that competitive displacement from shelter by rusty crayfish contributed to the decline of the native species by indirectly increasing predation pressure on them.

The results of the tether experiments demonstrated that selective predation did not occur, i.e., the signal crayfish and the red swamp crayfish appeared to suffer equally from predation. The low survivorship ($\leq 25\%$) of similar-sized contestants in the first two experiments was attributed to the longer tethers which allowed physical interaction between the crayfishes. It was surmised that aggressive interaction among contestants increased their exposure to predation, a mechanism that has been demonstrated elsewhere (e.g., Garvey et al. 1994). The high survivorship ($\geq 70\%$) of disparate-sized contestants

in the final experiments was attributed to the shorter tethers which greatly reduced the physical interaction between crayfishes. Ostensibly, rather than fighting each other, for shelter or otherwise, the contestants reaped the reward of increased survival by sharing the resource between them.

Besides shelter competition, non-native species can alter crayfish community structure by competing with native species for food (Hill and Lodge 1999). If the non-native crayfish is successful, then competition for food will lead to reduced growth in the native species. Reduced growth translates to smaller body sizes at given ages which can negatively affect the native population in a couple of ways. First, if individuals remained smaller for a longer period of time, they would be at greater risk of size-selective predation (Stein 1977). Second, having smaller females in the native population might lead to decreased fecundity with a subsequent reduction in reproductive potential (Payne 1997). Future research should be directed at the food habits of native signal crayfish and non-native red swamp crayfish in Pine Lake. The use of stable isotope analysis in corroboration with gut content analysis would provide a robust approximation of crayfish diet (Bondar et al. 2005) to evaluate similarities and differences between the species, and to determine whether competition for food is occurring.

Differences in crayfish community structure can result from interactions at juvenile life stages. Previous studies have shown that juvenile non-native species can cause behavioral shifts in juvenile native species, ultimately leading to higher predation on the native species. For example, Mather and Stein (1993) provided evidence that juvenile native Sanborn's crayfish (*Orconectes sanbornii*) altered their behavior, e.g., became more active, fought more, and changed habitat, in the presence of larger, juvenile

non-native rusty crayfish. By increasing their activity, the juvenile Sanborn's crayfish exposed themselves to greater predation risk. Interspecific interactions at earlier life stages were not explored at Pine Lake, but would likely be a fruitful direction for additional research.

Differences in life history strategies also influence crayfish community structure (Reynolds 2002). The red swamp crayfish exhibits many *r*-adapted traits such as early maturity at small body size, high fecundity, rapid growth, and a relatively short life span (Gherardi 2006). The phenomenal reproductive capacity of the red swamp crayfish partially explains its success as an invasive, non-native species (Sakai et al. 2001; Gherardi 2006). In contrast, the signal crayfish exhibits many *K*-adapted traits such as iteroparous production of relatively few eggs, slow growth, and increased longevity (Reynolds 2002). These dissimilar life history strategies provide another explanation for the differences in relative abundance of the two species reported by Mueller (2007).

In summary, the results of the field experiments at Pine Lake were consistent with much of the extant literature regarding the influence of body size, chelae length, sex, and reproductive status on the outcome of agonistic interactions among crayfish. The results also demonstrated that, at the life stages used here, there was no clear advantage conferred upon non-native red swamp crayfish in competitive interactions over limited shelter that might lead to increased predation of native signal crayfish. Still, if the size distributions of the crayfishes used in this study accurately represent the current size distributions of the populations in Pine Lake, then the red swamp crayfish would likely have an advantage over the signal crayfish due to their larger size (Vorburger and Ribi 1999; Usio et al. 2001; Gherardi and Cioni 2004; Gherardi and Daniels 2004).

In conclusion, the displacement of one species by another is not a function of any single process, rather a number of mechanisms working in concert (Sakai et al. 2001; Mills et al. 2004). If the non-native red swamp crayfish is indeed displacing the native signal crayfish at Pine Lake as suggested by Mueller (2007), it is likely that additional mechanisms besides shelter competition are contributing to the process. Competition for food, interaction at juvenile life stages, and differences in life history strategies are possible mechanisms. Indirect facilitation effects are another. These occur, for example, when a non-native predator removes a native species that impedes the establishment of another non-native species (Lockwood et al. 2007). Adams et al. (2003) demonstrated indirect facilitative interaction through a series of field experiments in Oregon. The authors reported that non-native bluegill sunfish (*Lepomis macrochirus*) increased the survival and abundance of non-native bullfrogs (*Rana catesbeiana*) by preying on native dragonfly nymphs (*Aeshnid* sp.), a major predator of bullfrog tadpoles. Given the rich assemblage of non-native fishes at Pine Lake (Verhey and Mueller 2001), especially the species whose natural range overlaps that of the red swamp crayfish (e.g., largemouth bass and smallmouth bass), similar interactions might be occurring. A holistic approach to future research at Pine Lake will shed light on a very complex freshwater ecosystem that has been altered by the introduction of non-native red swamp crayfish.

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APPENDIX

RESULTS OF REPLICATED G TESTS FOR ENCLOSURE EXPERIMENTS

Table A.1. Results of replicated tests of goodness of fit (G test) and results of unplanned tests of the homogeneity of replicates tested for goodness of fit (G test) for enclosure experiment 1 using mixed-species pairs of native signal crayfish (*Pacifastacus leniusculus*) and non-native red swamp crayfish (*Procambarus clarkii*) from Pine Lake, Sammamish, Washington. In this experiment, contestants were similar-sized female crayfish. The difference between carapace lengths of contestants was $\leq 10\%$. Data are number of times a crayfish was observed occupying the only shelter within an enclosure during nine observation bouts over three days. P -values < 0.05 indicate pairs of crayfish that differed significantly from the null hypothesis of equal use of the shelter by signal crayfish and red swamp crayfish. Crayfish pairs sharing a letter formed a homogenous group of winners that was dominated by one or the other species. Each homogenous group differed significantly (G test, $P < 0.0001$) from the null hypothesis of equal use of the shelter by signal crayfish and red swamp crayfish.

Enclosure	Signal crayfish ♀	Red swamp crayfish ♀	df	G	P
1 ^a	7	0	1	9.7041	0.0018
3 ^a	8	0	1	11.0903	0.0009
4 ^a	9	0	1	12.4766	0.0004
5 ^a	7	0	1	9.7041	0.0018
6 ^a	8	0	1	11.0903	0.0009
7 ^a	9	0	1	12.4766	0.0004
8 ^a	9	0	1	12.4766	0.0004
9 ^a	6	1	1	3.9624	0.0465
10 ^b	1	8	1	6.1977	0.0128
11 ^b	0	9	1	12.4766	0.0004
12 ^a	9	0	1	12.4766	0.0004
13 ^b	0	9	1	12.4766	0.0004
14 ^b	1	8	1	6.1977	0.0128
15 ^b	3	6	1	1.0194	0.3127
16 ^b	0	9	1	12.4766	0.0004
Total G			15	146.3025	< 0.0001
Pooled G	77	50	1	5.7842	0.0162
Heterogeneity G			14	140.5183	< 0.0001

Table A.2. Results of replicated tests of goodness of fit (G test) and results of unplanned tests of the homogeneity of replicates tested for goodness of fit (G test) for enclosure experiment 2 using mixed-species pairs of native signal crayfish (*Pacifastacus leniusculus*) and non-native red swamp crayfish (*Procambarus clarkii*) from Pine Lake, Sammamish, Washington. In this experiment, contestants were similar-sized male crayfish. The difference between carapace lengths of contestants was $\leq 10\%$. Data are number of times a crayfish was observed occupying the only shelter within an enclosure during nine observation bouts over three days. P -values < 0.05 indicate pairs of crayfish that differed significantly from the null hypothesis of equal use of the shelter by signal crayfish and red swamp crayfish. Crayfish pairs sharing a letter formed a homogenous group of winners that was dominated by one or the other species. Each homogenous group differed significantly (G test, $P < 0.0001$) from the null hypothesis of equal use of the shelter by signal crayfish and red swamp crayfish.

Enclosure	Signal crayfish ♂	Red swamp crayfish ♂	df	G	P
1 ^b	0	7	1	9.7041	0.0018
2 ^a	9	0	1	12.4766	0.0004
3 ^a	9	0	1	12.4766	0.0004
4 ^{a, b}	5	4	1	0.1113	0.7386
5 ^a	9	0	1	12.4766	0.0004
6 ^a	9	0	1	12.4766	0.0004
7 ^a	8	0	1	11.0903	0.0009
8 ^a	9	0	1	12.4766	0.0004
9 ^a	8	0	1	11.0903	0.0009
10 ^b	0	3	1	4.1589	0.0414
12 ^a	9	0	1	12.4766	0.0004
13 ^b	0	7	1	9.7041	0.0018
14 ^b	0	8	1	11.0903	0.0009
15 ^a	7	0	1	9.7041	0.0018
16 ^b	0	7	1	9.7041	0.0018
Total G			15	151.2174	< 0.0001
Pooled G	82	36	1	10.9374	0.0009
Heterogeneity G			14	140.2800	< 0.0001

Table A.3. Results of replicated tests of goodness of fit (G test) and results of unplanned tests of the homogeneity of replicates tested for goodness of fit (G test) for enclosure experiment 3 using mixed-species pairs of native signal crayfish (*Pacifastacus leniusculus*) and non-native red swamp crayfish (*Procambarus clarkii*) from Pine Lake, Sammamish, Washington. In this experiment, contestants were similar-sized female signal crayfish and male red swamp crayfish. The difference between carapace lengths of contestants was $\leq 10\%$. Data are number of times a crayfish was observed occupying the only shelter within an enclosure during nine observation bouts over three days. P -values < 0.05 indicate pairs of crayfish that differed significantly from the null hypothesis of equal use of the shelter by signal crayfish and red swamp crayfish. Crayfish pairs sharing a letter formed a homogenous group of winners that was dominated by one or the other species. Each homogenous group differed significantly (G test, $P < 0.0001$) from the null hypothesis of equal use of the shelter by signal crayfish and red swamp crayfish.

Enclosure	Signal crayfish ♀	Red swamp crayfish ♂	Df	G	P
1 ^{a, b}	6	3	1	1.0194	0.3127
2 ^b	1	8	1	6.1977	0.0128
3 ^a	9	0	1	12.4766	0.0004
4 ^a	9	0	1	12.4766	0.0004
5 ^a	7	0	1	9.7041	0.0018
6 ^a	9	0	1	12.4766	0.0004
7 ^a	8	0	1	11.0903	0.0009
8 ^a	9	0	1	12.4766	0.0004
9 ^{a, b}	3	4	1	0.1433	0.7050
10 ^a	7	0	1	9.7041	0.0018
11 ^b	0	9	1	12.4766	0.0004
12 ^{a, b}	3	1	1	1.0465	0.3063
14 ^a	7	0	1	9.7041	0.0018
15 ^{a, b}	4	2	1	0.6796	0.4097
16 ^a	7	0	1	9.7041	0.0018
Total G			15	121.3763	< 0.0001
Pooled G	89	27	1	34.9297	< 0.0001
Heterogeneity G			14	86.4467	< 0.0001

Table A.4. Results of replicated tests of goodness of fit (G test) and results of unplanned tests of the homogeneity of replicates tested for goodness of fit (G test) for enclosure experiment 4 using mixed-species pairs of native signal crayfish (*Pacifastacus leniusculus*) and non-native red swamp crayfish (*Procambarus clarkii*) from Pine Lake, Sammamish, Washington. In this experiment, contestants were similar-sized male signal crayfish and female red swamp crayfish. The difference between carapace lengths of contestants was $\leq 10\%$. Data are number of times a crayfish was observed occupying the only shelter within an enclosure during nine observation bouts over three days. P -values < 0.05 indicate pairs of crayfish that differed significantly from the null hypothesis of equal use of the shelter by signal crayfish and red swamp crayfish. Crayfish pairs sharing a letter formed a homogenous group of winners that was dominated by one or the other species. Each homogenous group differed significantly (G test, $P < 0.0001$) from the null hypothesis of equal use of the shelter by signal crayfish and red swamp crayfish.

Enclosure	Signal crayfish ♂	Red swamp crayfish ♀	df	G	P
1 ^b	0	8	1	11.0903	0.0009
2 ^b	0	9	1	12.4766	0.0004
3 ^a	9	0	1	12.4766	0.0004
4 ^a	7	0	1	9.7041	0.0018
5 ^a	7	0	1	9.7041	0.0018
6 ^b	3	6	1	1.0194	0.3127
7 ^b	1	8	1	6.1977	0.0128
8 ^b	0	9	1	12.4766	0.0004
9 ^a	8	0	1	11.0903	0.0009
10 ^b	0	9	1	12.4766	0.0004
11 ^a	9	0	1	12.4766	0.0004
12 ^a	9	0	1	12.4766	0.0004
13 ^b	0	9	1	12.4766	0.0004
14 ^b	0	8	1	11.0903	0.0009
15 ^b	0	9	1	12.4766	0.0004
16 ^b	0	9	1	12.4766	0.0004
Total G			16	172.1861	< 0.0001
Pooled G	53	84	1	7.0757	0.0078
Heterogeneity G			15	165.1104	< 0.0001

Table A.5. Results of replicated tests of goodness of fit (G test) and results of unplanned tests of the homogeneity of replicates tested for goodness of fit (G test) for enclosure experiment 5 using mixed-species pairs of native signal crayfish (*Pacifastacus leniusculus*) and non-native red swamp crayfish (*Procambarus clarkii*) from Pine Lake, Sammamish, Washington. In this experiment, contestants were small male signal crayfish and large male red swamp crayfish. The difference between carapace lengths of contestants was $> 10\%$. Data are number of times a crayfish was observed occupying the only shelter within an enclosure during nine observation bouts over three days. P -values < 0.05 indicate pairs of crayfish that differed significantly from the null hypothesis of equal use of the shelter by signal crayfish and red swamp crayfish. Crayfish pairs sharing a letter formed a homogenous group of winners that was dominated by one or the other species. Each homogenous group differed significantly (G test, $P < 0.0001$) from the null hypothesis of equal use of the shelter by signal crayfish and red swamp crayfish.

Enclosure	Small signal crayfish ♂	Large red swamp crayfish ♂	df	G	P
1 ^b	0	9	1	12.4766	0.0004
2 ^a	5	0	1	6.9315	0.0085
3 ^b	0	8	1	11.0903	0.0009
4 ^b	0	9	1	12.4766	0.0004
6 ^b	0	9	1	12.4766	0.0004
7 ^b	0	9	1	12.4766	0.0004
8 ^b	0	9	1	12.4766	0.0004
9 ^b	0	8	1	11.0903	0.0009
11.1 ^b	0	9	1	12.4766	0.0004
11.2 ^b	0	8	1	11.0903	0.0009
13 ^b	0	9	1	12.4766	0.0004
14 ^b	0	8	1	11.0903	0.0009
15 ^a	4	2	1	0.6796	0.4097
16 ^a	9	0	1	12.4766	0.0004
Total G			14	151.7857	< 0.0001
Pooled G	18	97	1	59.6368	< 0.0001
Heterogeneity G			13	92.1489	< 0.0001

Table A.6. Results of replicated tests of goodness of fit (G test) and results of unplanned tests of the homogeneity of replicates tested for goodness of fit (G test) for enclosure experiment 6 using mixed-species pairs of native signal crayfish (*Pacifastacus leniusculus*) and non-native red swamp crayfish (*Procambarus clarkii*) from Pine Lake, Sammamish, Washington. In this experiment, contestants were small female signal crayfish and large female red swamp crayfish. The difference between carapace lengths of contestants was $> 10\%$. Data are number of times a crayfish was observed occupying the only shelter within an enclosure during nine observation bouts over three days. P -values < 0.05 indicate pairs of crayfish that differed significantly from the null hypothesis of equal use of the shelter by signal crayfish and red swamp crayfish. Crayfish pairs sharing a letter formed a homogenous group of winners that was dominated by one or the other species. Each homogenous group differed significantly (G test, $P < 0.0001$) from the null hypothesis of equal use of the shelter by signal crayfish and red swamp crayfish.

Enclosure	Small signal crayfish ♀	Large red swamp crayfish ♀	df	G	P
2.1 ^b	1	7	1	5.0620	0.0245
2.2 ^b	0	9	1	12.4766	0.0004
3 ^b	0	9	1	12.4766	0.0004
4 ^a	7	2	1	2.9419	0.0863
5 ^b	0	8	1	11.0903	0.0009
7 ^b	0	9	1	12.4766	0.0004
8 ^b	0	9	1	12.4766	0.0004
9 ^b	0	9	1	12.4766	0.0004
10 ^b	0	9	1	12.4766	0.0004
12 ^b	0	9	1	12.4766	0.0004
14 ^b	0	9	1	12.4766	0.0004
15 ^b	0	8	1	11.0903	0.0009
16 ^b	0	8	1	11.0903	0.0009
Total G			13	141.0882	< 0.0001
Pooled G	8	105	1	98.8643	< 0.0001
Heterogeneity G			12	42.2239	< 0.0001

Table A.7. Results of replicated tests of goodness of fit (G test) and results of unplanned tests of the homogeneity of replicates tested for goodness of fit (G test) for enclosure experiment 7 using mixed-species pairs of native signal crayfish (*Pacifastacus leniusculus*) and non-native red swamp crayfish (*Procambarus clarkii*) from Pine Lake, Sammamish, Washington. In this experiment, contestants were large female signal crayfish and small female red swamp crayfish. The difference between carapace lengths of contestants was > 10%. Data are number of times a crayfish was observed occupying the only shelter within an enclosure during nine observation bouts over three days. P -values < 0.05 indicate pairs of crayfish that differed significantly from the null hypothesis of equal use of the shelter by signal crayfish and red swamp crayfish. Crayfish pairs sharing a letter formed a homogenous group of winners that was dominated by one or the other species. Each homogenous group differed significantly (G test, $P < 0.0001$) from the null hypothesis of equal use of the shelter by signal crayfish and red swamp crayfish.

Enclosure	Large signal crayfish ♀	Small red swamp crayfish ♀	df	G	P
1 ^a	9	1	1	7.3613	0.0067
2 ^a	9	0	1	12.4766	0.0004
3 ^a	8	0	1	11.0903	0.0009
4 ^a	9	0	1	12.4766	0.0004
5 ^b	0	9	1	12.4766	0.0004
6 ^{a, b}	5	3	1	0.5053	0.4772
7 ^a	7	1	1	5.0620	0.0245
8 ^b	0	9	1	12.4766	0.0004
9 ^a	7	1	1	5.0620	0.0245
10 ^a	6	1	1	3.9624	0.0465
11 ^a	9	0	1	12.4766	0.0004
12 ^a	9	0	1	12.4766	0.0004
13 ^{a, b}	2	0	1	2.7726	0.0959
14 ^b	0	9	1	12.4766	0.0004
15 ^b	0	9	1	12.4766	0.0004
16 ^{a, b}	6	6	1	0.0000	1.0000
Total G			16	135.6293	< 0.0001
Pooled G	86	49	1	10.2717	0.0014
Heterogeneity G			15	125.3576	< 0.0001

Table A.8. Results of replicated tests of goodness of fit (G test) and results of unplanned tests of the homogeneity of replicates tested for goodness of fit (G test) for enclosure experiment 8 using mixed-species pairs of native signal crayfish (*Pacifastacus leniusculus*) and non-native red swamp crayfish (*Procambarus clarkii*) from Pine Lake, Sammamish, Washington. In this experiment, contestants were large male signal crayfish and small male red swamp crayfish. The difference between carapace lengths of contestants was $> 10\%$. Data are number of times a crayfish was observed occupying the only shelter within an enclosure during nine observation bouts over three days. P -values < 0.05 indicate pairs of crayfish that differed significantly from the null hypothesis of equal use of the shelter by signal crayfish and red swamp crayfish. Crayfish pairs sharing a letter formed a homogenous group of winners that was dominated by one or the other species. Each homogenous group differed significantly (G test, $P < 0.0001$) from the null hypothesis of equal use of the shelter by signal crayfish and red swamp crayfish.

Enclosure	Large signal crayfish ♂	Small red swamp crayfish ♂	df	G	P
1 ^{a, b}	4	2	1	0.6796	0.4097
2 ^b	0	9	1	12.4766	0.0004
3 ^a	9	0	1	12.4766	0.0004
4 ^a	5	0	1	6.9315	0.0085
5 ^a	8	0	1	11.0903	0.0009
6 ^a	9	0	1	12.4766	0.0004
7 ^a	9	0	1	12.4766	0.0004
8 ^a	6	0	1	8.3178	0.0039
9 ^a	6	2	1	2.0930	0.1480
10 ^b	0	9	1	12.4766	0.0004
11 ^a	8	0	1	11.0903	0.0009
12 ^b	0	9	1	12.4766	0.0004
13 ^a	8	1	1	6.1977	0.0128
14 ^a	7	0	1	9.7041	0.0018
15 ^a	8	0	1	11.0903	0.0009
16 ^a	9	0	1	12.4766	0.0004
Total G			16	154.5312	< 0.0001
Pooled G	96	32	1	33.4879	< 0.0001
Heterogeneity G			15	121.0433	< 0.0001