

Crustacean Social Behavioral Changes in Response to Isolation

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Abstract. Periods of isolation during which animals have no social contact are common in the design of behavioral experiments. They are used, for example, to test memory and recognition responses, or to ensure a baseline condition before experimental manipulations commence. We investigated the effect of isolation periods on the aggressive behavior of matched pairs of the crayfish *Cherax destructor* in two contexts. The first experiment tested the effects of a period of isolation between two encounters. The second experiment tested the effects of isolation before an encounter by pairing one crayfish from a communal living environment with another crayfish from an isolated one. Fight outcome and aggression levels were analyzed, resulting in three conclusions about the social biology of *C. destructor*. First, encounters between familiar opponents are influenced by the outcome of the familiarization fight for about 2 weeks. Second, the level of aggression and the outcome of an encounter are affected over different time frames. Third, individuals that are isolated before an encounter can be disadvantaged. These data suggest that isolation, or events that occur during periods of isolation, affect multiple elements of social behavior in *C. destructor*. This suggestion has implications for the interpretation of previous results and future studies in crustaceans and other taxa.

Introduction

There is current interest in social behavior and the factors that affect interactions (*e.g.*, Whitehouse and Lubin, 2005; Hsu *et al.*, 2006; Komdeur, 2006; Sumpter, 2006). Decapod crustaceans are a key subject because investigations of their aggressive behavior permit study of physiological, behav-

ioral, and ecological issues (Breithaupt and Atema, 2000; Perry *et al.*, 2000; Gherardi and Pieraccini, 2004; Figler *et al.*, 2005; Bergman *et al.*, 2006; Edwards and Spitzer, 2006). This is largely because they typically have simple nervous systems and stereotyped aggressive behaviors that have been documented in numerous species (*e.g.*, Bovbjerg, 1953; Rubenstein and Hazlett, 1974; Bruski and Dunham, 1987; Huber and Kravitz, 1995). In addition, studies have also tested the effects of serotonin levels on aggression (Yeh *et al.*, 1996; Antonsen and Paul, 1997; Huber *et al.*, 1997), and the effects of psychostimulants (Panksepp and Huber, 2004).

Some of the studies of aggressive behavior examine the social or hierarchy structures that can occur when small groups of animals are housed close together (*e.g.*, Bovbjerg, 1953, 1956; Issa *et al.*, 1999). The ensuing encounters result in winning and losing animals, and the more interactions won the more dominant an animal becomes. These winning and losing outcomes create a history of social experience that can influence future behavior (*e.g.*, Goessmann *et al.*, 2000; Daws *et al.*, 2002; Bergman *et al.*, 2003). Closely associated with the effects of experience is recognition. For a number of crustacean species, victory in contests is determined by dominance status; animals recognize the status of the opponent (*e.g.*, Breithaupt and Atema, 1993; Karavanich and Atema, 1993; Zulantz Schneider *et al.*, 2001; Gherardi and Daniels, 2003). There is also evidence that social behavior around familiar animals is different from behavior around unfamiliar ones, which suggests that some decapods can recognise individuals (*e.g.*, Karavanich and Atema, 1998; Crook *et al.*, 2004; Gherardi and Tiedemann, 2004; Gherardi and Atema, 2005; Detto *et al.*, 2006).

Isolation is an important consideration in experimental design. Investigations often set out to establish a basal level of aggressive activity between pairs of animals by using

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isolation prior to matched contests. Behavioral changes in rematches represent the influence of prior experience (*e.g.*, Daws *et al.*, 2002; Bergman *et al.*, 2003) or recognition or memory of the prior combatant or its status (*e.g.*, Breithaupt and Atema, 1993; Karavanich and Atema, 1998; Zulandt Schneider *et al.*, 2001). Animals are also usually isolated between matches. When we compared studies of recognition and aggressive behavior in crustaceans, we found substantial variation in how long the effect of previous encounters affects subsequent encounters (5–30 days, Table 1). This variation is interesting because it appears to demonstrate different cognitive abilities between different crustacean species, but it is also potentially problematic because studies use isolation methods that are based on this variable time frame (Table 2).

Few studies test the hypothesis that this period of isolation could itself influence behavior. Some evidence suggests that this is an important consideration in crabs (Courchesne and Barlow, 1971); however, its influence in other groups such as crayfish and lobsters, which are commonly used as behavioral and physiological models (*e.g.*, Yeh *et al.*, 1996; Panksepp and Huber, 2004; Issa and Edwards, 2006), is limited (Rutishauser *et al.*, 2004; Gherardi and Atema, 2005). The matter is pertinent because methodologies vary isolation periods within the same species and also between crustacean groups (Table 3). There are also studies that maintain animals in groups prior to experiments (*e.g.*, Rubenstein and Hazlett, 1974; Winston and Jacobson, 1978; Obermeier and Schmitz, 2003) or in partially isolated

groups (*e.g.*, Song *et al.*, 2006—visual and physical isolation but not chemical). Studies on sexual recognition do not seem to isolate animals (*e.g.*, Rufino and Jones, 2001; Díaz and Thiel, 2003), presumably because of the need to establish pair bonds.

We hypothesized that behavior in social interactions of freshwater crayfish, *Cherax destructor*, would be different after periods of isolation. We targeted two social situations. First we tested the social interactions that occur when there is isolation between encounters. Contests between pairs of crayfish were staged such that each pair had two encounters separated by a period of isolation that was systematically varied. Second we tested whether behavior changed when the isolation occurred before an encounter. For these contests, one encounter was staged for a given pair of crayfish in which one opponent was from a social living environment and the other from an isolated tank where there was no opportunity for social interactions.

Materials and Methods

Animals

Crayfish (*Cherax destructor* Clark) were sourced from a commercial supplier and placed in fiberglass tanks (120 × 50 × 20 cm). The husbandry room was at a constant temperature (18 ± 1 °C) and on a 12 h/12 h light/dark cycle (experiments between 1 and 6 h after the onset of light). Feeding and cleaning occurred weekly, and testing was scheduled the same time after feeding for various treatment groups. About 15–20 crayfish, 3–5 cm carapace length, were kept in each tank and maintained for at least 1 week before experiments. Experimental animals were in good condition with appendages intact. They had not molted within 1 week prior to use, and there was no indication of molting after experimentation. They were matched into pairs that later interacted in staged agonistic encounters.

Pairing crayfish

Carapace and claw length were measured with callipers. Animals from different tanks were randomly chosen and paired primarily on the basis of carapace length and secondarily on claw length. The size variation within each pair was less than 10% carapace length to exclude the possibility that size advantages would affect the outcome of the encounters (Pavey and Fielder, 1996; Daws *et al.*, 2002). Crayfish were individually marked with correction fluid (Pental, Victoria, Australia) on the dorsal carapace before the experiment commenced to distinguish them from one another.

Encounters

The two experiments involved staged encounters between pairs of individuals of *C. destructor*. These behavioral in-

Table 1

Examples of the variation in length of time that memory or experience affects aggressive behavior in crayfish and other crustaceans

Species	Isolation ¹	Reference ²
Freshwater crayfish		
<i>Astacus astacus</i>	5 d	Huber <i>et al.</i> , 1997
<i>Cambarus</i> spp.	1 wk	Guiasu and Dunham, 1999
<i>Orconectes rusticus</i> ³	1 wk	Zulandt Schneider <i>et al.</i> , 2001
<i>Procambarus clarkii</i> ³	2 wk	Daws <i>et al.</i> , 2002
<i>Cherax destructor</i>	2 wk	This study
Other crustaceans		
<i>Pagurus longicarpus</i>	4 d	Gherardi and Atema, 2005
<i>Homarus americanus</i>	4 d	Rutishauser <i>et al.</i> , 2004
<i>Homarus americanus</i>	1 wk	Karavanich and Atema, 1998
<i>Gonodactylus bredini</i>	2 wk	Caldwell, 1992
<i>Pagurus samuelis</i>	30 d	Courchesne and Barlow, 1971

¹ Values listed are the time at which significant differences in behavior were last detected, indicating that recognition or memory had subsided (d, days; wk, weeks).

² Fights staged over short periods, where effects of memory, status, or experience reliably persist, are not included in the table (*e.g.*, 30 min [Caldwell, 1985]; 1 day [Berkey and Atema, 1999; Rahman *et al.*, 2001; Breithaupt and Eger, 2002]; 2 days [Crook *et al.*, 2004]). Not all studies extended research beyond the isolation period listed.

³ Cited as unpublished observations, or with no data provided.

Table 2

Examples of studies that base isolation methods on results from other research

Study	Study organism	Cited organism
Zulandt Schneider <i>et al.</i> , 2001	<i>Orconectes</i>	<i>Cambarus</i> (Guiasu and Dunham, 1999) <i>Homarus</i> (Karavanich and Atema, 1998)
Breithaupt and Eger, 2002	<i>Procambarus</i> , <i>Astacus</i> , <i>Austropotamobis</i> , <i>Pacifasticus</i> , <i>Orconectes</i>	<i>Homarus</i> (Karavanich and Atema, 1998)
Gherardi and Daniels, 2003	<i>Procambarus</i>	<i>Orconectes</i> (Zulandt Schneider <i>et al.</i> , 2001)
Crook <i>et al.</i> , 2004	<i>Cherax</i>	<i>Procambarus</i> (Daws <i>et al.</i> , 2002) <i>Homarus</i> (Karavanich and Atema, 1998) <i>Orconectes</i> (Zulandt Schneider <i>et al.</i> , 2001)
Bergman and Moore, 2005	<i>Orconectes</i>	<i>Cambarus</i> (Guiasu and Dunham, 1999) <i>Homarus</i> (Karavanich and Atema, 1998)
Johnson and Atema, 2005	<i>Homarus</i>	<i>Homarus</i> (Karavanich and Atema, 1998)

teractions are common when crayfish and other crustaceans meet (*e.g.*, *Gonodactylus festae* [Caldwell, 1985]; *Homarus americanus* [Karavanich and Atema, 1998]; *C. destructor* [Crook *et al.*, 2004]; *Pagurus longicarpus* [Gherardi and Tiedemann, 2004]). Contests were staged in glass aquaria (37 × 18 × 22 cm, filled to a depth of ~10 cm with tap water) on a gravel substrate. The external sides of the aquaria were spray painted to exclude external visual stimuli. Each pair was placed into a tank with the opponents separated by a clear barrier. To reduce handling effects, they were given 2–3 min to acclimatize (Baird *et al.*, 2006). The barrier was removed and the pair was allowed to interact for a standard time of 1 h. Tanks were rinsed after each pairing. Encounters were videotaped with a black-and-white CCD camera (Jaycar, Victoria, Australia) connected to a VCR (Panasonic, Victoria, Australia).

Experiments

Isolation between encounters. To investigate the time frame over which isolation could affect social behavior, we varied the isolation period between bouts of fighting. Three sets of two-round contests were conducted, each involving 20 pairs

of size-matched crayfish (Fig. 1A). In the first round, animals in each pair interacted to establish a dominance relationship. They were then returned to their individual tanks. A second round rematched the crayfish after an isolation period of 1, 2, or 3 weeks to determine whether the relationship had changed.

Isolation before encounters. To test whether isolation before an encounter affected aggressive behavior, crayfish that had been isolated were matched with opponents that had previous social experience. Animals were divided into two groups: isolates were kept one to a tank (2 liter plastic) and communals were kept in groups of ~15 to a tank (fiberglass 120 x 50 x 20 cm). Each isolate animal was matched with one crayfish from a communal tank ($n = 20$) (Fig. 1B). Four groups of isolates were used: 1, 2, 3, and 4 weeks isolation. To standardize their social experience, communal animals were ordered from the supplier sequentially so that they resided in the husbandry tanks for 1 week prior to pairing.

Observations

Winning and agonistic intensity have been analyzed in several studies on aggressive behavior in crayfish and other crustaceans (*e.g.*, Karavanich and Atema, 1998). We used similar criteria so that our results would be comparable to previous data from a range of crustacean species. The taped footage was analyzed by an observer without knowledge of which treatment was playing. The 1-h encounters produced several contests, or fights. Each contest was defined as a head-on interaction in which the crayfish were within one body length of each other. The initiator, the level of aggression, and the outcome of each fight were recorded. The initiator was the first crayfish to approach an opponent. The level of aggression was calculated as the highest scoring behavior listed in Table 4 during each contest. The loser was the individual that moved more than one body length away from its opponent (Goessmann *et al.*, 2000). For each pair, the numbers of wins and losses from the contests over the

Table 3

Examples of studies that isolate animals for different periods before experiments

Species	Isolation	Reference
<i>Procambarus clarkii</i>	1.5–9 d	Herberholz <i>et al.</i> , 2003
<i>Procambarus clarkii</i>	1 wk	Copp, 1986
<i>Procambarus clarkii</i>	1 mo	Yeh <i>et al.</i> , 1996
<i>Procambarus clarkii</i> ¹	1 mo	Issa <i>et al.</i> , 1999
<i>Procambarus clarkii</i>	3 mo	Issa and Edwards, 2006
<i>Orconectes rusticus</i>	5 d	Schroeder and Huber, 2001
<i>Gonodactylus festae</i>	10 d	Caldwell, 1979
<i>Astacus astacus</i>	8 wk	Goessmann <i>et al.</i> , 2000
<i>Homarus americanus</i> ¹	>6 mo	Huber and Kravitz, 1995

¹ No prior social experience—isolated as young.

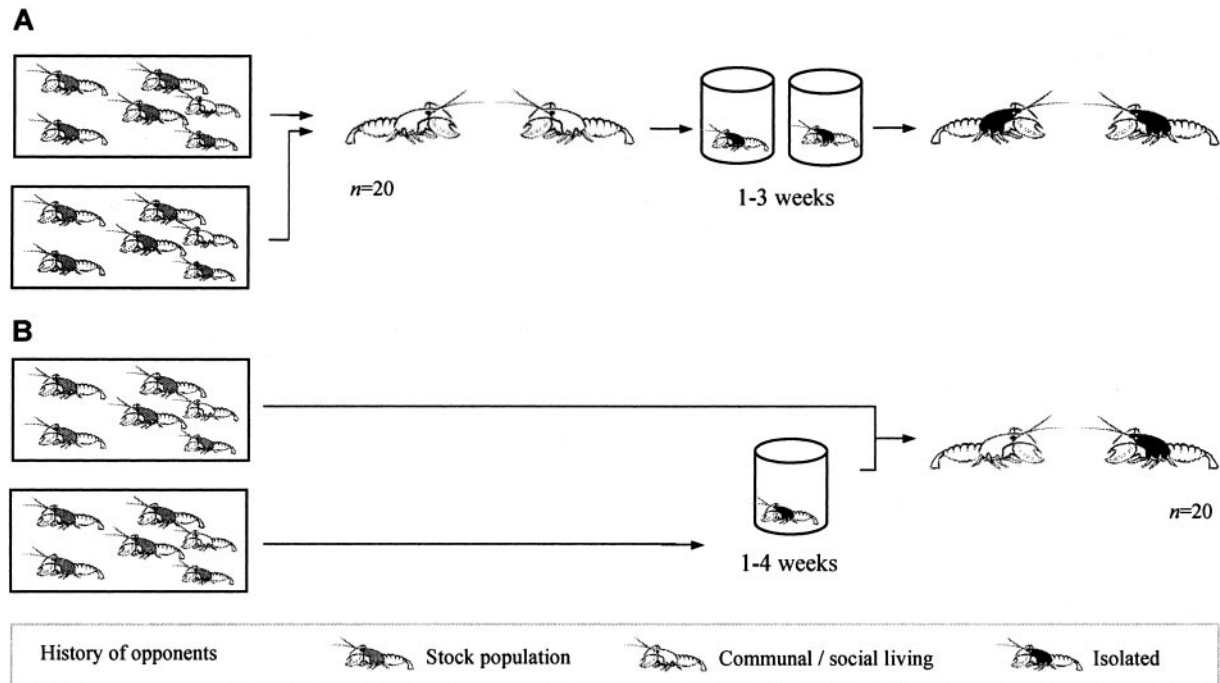


Figure 1. Experiments manipulated isolation periods between encounters (A), and before encounters (B). Size-matched pairs of *Cherax destructor* shown with isolation times for the treatment groups in each experiment.

1-h period were tallied, and the crayfish with the most wins was deemed the overall winner of the encounter.

Analysis

Isolation between encounters. The number of crayfish that won both encounters was tallied for each treatment. If there were no effect of the isolation periods on outcome, either crayfish in the pair would be equally likely to win both encounters irrespective of whether it won in the first round (Zulandt Schneider *et al.*, 2001). Therefore, a chi-square test was used to compare the number of animals that won both encounters, with an expected outcome of 50%. A Yates' correction was applied because there were only two possible outcomes: win in one round or both rounds (Sokal and

Rohlf, 1995). The mean aggression levels from the first and second encounters were also compared. If aggression intensity were not influenced by the isolation, levels would remain the same between the two rounds. A paired Student's *t*-test was used to analyze the difference between the two rounds for each of the three isolation periods. Only the pairs with the same winner in both encounters were included, because a switch in winners would already indicate no effect of isolation.

Isolation before encounters. Victories of the isolated crayfish were tallied for each period of isolation (1, 2, 3, and 4 weeks). If there were no effect of prior living experience, then the isolated animals should win 50% of the encounters.

Table 4

*Fight behaviors and definitions for the contests observed during encounters between matched pairs of *Cherax destructor* (based on Bruski and Dunham, 1987; Atema and Voigt, 1995; Crook *et al.*, 2004)*

Behavior	Description	Aggression level
Meral Spread	Threat display, both claws extended up and out from body	1
Touch	Touch of claws on opponent's body	1
Punch	Continuous or intermittent punching of the claw(s) on the opponent's body	2
Claw Box	Backward or forward striking motion onto opponent	3
Claw Grasp	Clamping of claw(s) on opponent's body	4
Claw Scissor	Both claws rapidly crossing in front of the opponent's body in a scissor motion	4
Claw Rip	Rapid grasp or pull with a claw	5

A Yates' corrected chi-square test was used to compare the number of isolates winning in each of the four groups. There was only one encounter in this experiment, so aggression level was compared between the four treatments using the week 1 group as a comparison point for all other treatments. A one-factor ANOVA (isolation period) tested for an overall difference across the groups, and planned comparisons were applied between the week 1 group and each of the three remaining groups (Quinn and Keough, 2002).

Some crayfish molted or died during the isolation periods, and these were excluded from analysis. However, in all cases we used sample sizes comparable to those in previous crustacean studies (Bergman *et al.*, 2003; Gherardi and Atema, 2005) (final *n* given in brackets; see Results). Tests were two-sided, with alpha set at 0.05. Data were manipulated in Microsoft Excel (ver. 2000, Microsoft Corporation) and analyzed in Systat (ver. 11, Systat Software Inc.).

Results

The encounters followed a general pattern at the start of a trial. After the divider was raised, both opponents showed some exploratory behavior. They slowly walked around the perimeter of the tank and often contacted the glass with their bodies or antennae. Crayfish engaged in multiple fights during the 1-h encounter (median, 8 contest; range, 1 to 29 contests). The duration of each contest was generally less than 5 min but in some cases up to 35 min.

Isolation between encounters

Encounter outcome. The winner of the first encounter was significantly more likely to win the rematch when the isolation period was 1 or 2 weeks (Fig. 2A). For the week 1 isolation period, the first-round winner won 9 out of 10 round-two encounters ($n = 10$, $\chi^2 = 4.900$, $P = 0.027$). For

the 2-week period, the first-round winners won all of their second encounters ($n = 10$, $\chi^2 = 8.100$, $P = 0.004$). In the 3-week isolation period, the winner of the second round was unpredictable: winners of the first encounter won 8 and lost 3 of the rematches ($n = 11$, $\chi^2 = 1.455$, $P = 0.228$).

Level of aggression. There was a significant reduction in aggression levels for crayfish rematched after 1 week of isolation. Eight of 9 winners showed a reduction in fight scores ($n = 9$, $t = 2.578$, $P = 0.033$; Fig. 2B). No difference in aggression was detected between the encounters after 2 and 3 weeks isolation ($n = 10$, $t = 2.124$, $P = 0.063$; $n = 8$, $t = 0.267$, $P = 0.797$).

Isolation before encounters

Encounter outcome. Crayfish isolated for 1 week won fewer encounters than expected ($n = 18$, $\chi^2 = 4.500$, $P = 0.034$; Fig. 3A). For the 2-, 3-, and 4-week periods, isolated animals won as predicted, with outcomes not differing from 50% (2-wk: $n = 10$, $\chi^2 = 0.100$, $P = 0.752$; 3-wk: $n = 18$, $\chi^2 = 0.056$, $P = 0.813$; 4-wk: $n = 11$, $\chi^2 \sim 0.000$, $P > 0.999$; Fig. 3A).

Level of aggression. There was a significant difference in the mean fight scores between the four periods of isolation ($F = 4.142$, $P = 0.010$; Fig. 3B). The week 2 and week 3 treatments did not differ from the week 1 period ($F_{1 \text{ vs. } 2} = 3.719$, $P_{1 \text{ vs. } 2} = 0.059$; $F_{1 \text{ vs. } 3} = 0.373$, $P_{1 \text{ vs. } 3} = 0.544$). Fight scores increased in week 4 ($F_{1 \text{ vs. } 4} = 5.825$, $P_{1 \text{ vs. } 4} = 0.019$).

Discussion

The staged encounters indicate that two elements of social behavior, outcome and intensity of aggression, are

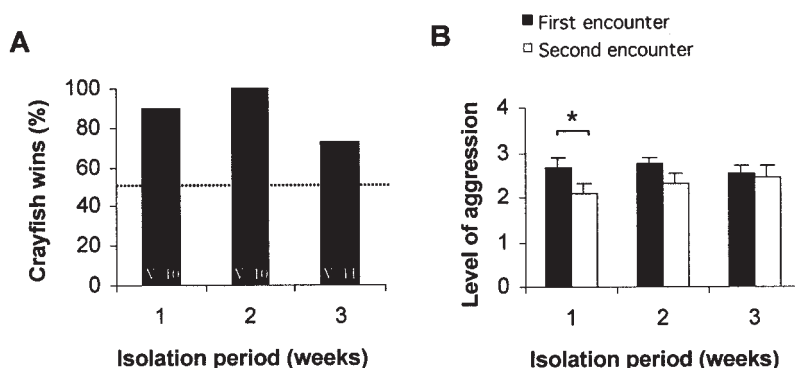


Figure 2. Summary of the effect of isolation between two encounters of matched *Cherax destructor*. (A) Percentage of crayfish that won both the first and second encounters. More crayfish than predicted by chance won both rounds after 1- and 2-week isolation intervals between the encounters. (B) Mean fight scores for the 2 encounters in each of the 3 treatments. There was a significant decrease in level in the 1-week isolation group. Data from encounters with the same outcome (1 wk: 90% of pairs, $n = 9$; 2 wk: 100%, $n = 10$; 3 wk: 73%, $n = 8$). Error bars +1 SEM. * indicates significance difference $P < 0.05$, see main text.

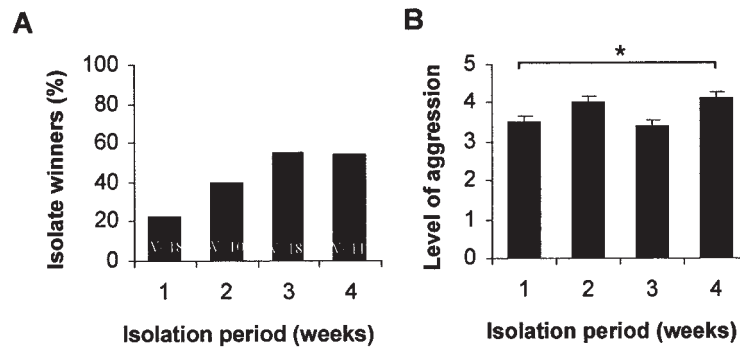


Figure 3. Summary of the effect of isolation before encounters—opponent from communal living against one from isolated environment. (A) Percentage of isolated crayfish that won. Fewer crayfish than predicted by chance won after being isolated for 1 week. (B) Mean fight scores for the encounters in each of the 4 treatments. There was a significant increase in level between the 1- and 4-week isolation groups. Error bars +1 SEM. * indicates significance difference $P < 0.05$, see main text.

different after periods of isolation when pairs of *Cherax destructor* fight. The findings demonstrate that fight outcome and intensity can change over different time frames, suggesting that the ability to recognize a conspecific, or the influence of experience on contests, was also altered. Whether individuals were isolated or housed with other crayfish before contests affected the outcome of encounters as well.

Isolation between encounters

When the familiar crayfish were rematched after 1 and 2 weeks of isolation, the behavior of the first encounter prevailed as the determining factor of the outcome in the second round. This indicates that individual recognition, status recognition, the effect of prior experience, or a combination of these processes occurred. These processes are known to exist in *C. destructor* and other crustaceans (e.g., Karavanich and Atema, 1998; Goessmann *et al.*, 2000; Zulant Schneider *et al.*, 2001; Breithaupt and Eger, 2002; Daws *et al.*, 2002; Bergman *et al.*, 2003; Crook *et al.*, 2004). This finding suggests that if an individual of *C. destructor* encountered the same individuals in the wild within short time frames, 1–2 weeks, social standing could be maintained, depending on the amount of additional social activity between encounters.

There was no difference in aggression levels in encounters after the 2- and 3-week isolation periods. In other species, it has been suggested that this is because animals no longer recognize each other (Hojesjo *et al.*, 1998; Herberholz *et al.*, 2001; Zulant Schneider *et al.*, 2001). The reduction in aggression after 1 week was not detected in the 2-week isolation, but the outcome of the encounters was still significantly biased toward the winner of the first encounter. We conclude from this that the effect of recognition or experience is fading by 2 weeks, and thus multiple behavioral elements—in this case outcome and intensity—can

change differently with respect to each other and the duration of the isolation. Reports of fight outcome in *Homarus americanus* suggest that this may also be the case in another species (experiment F in Karavanich and Atema, 1998).

The outcome of rematched encounters has been investigated in several crustacean species. The research suggests that two main processes govern the behavior in the rematch: experience effects and recognition. It is known that there are short-term effects of winning experience (e.g., 20 min in *Orconectes rusticus*—Bergman *et al.*, 2003; 24 h in *Procambarus clarkii*—Daws *et al.*, 2002). Whether our data reflect a winner effect or an experience effect, the effect of the 2-week period of isolation is substantially longer than previously demonstrated in crustaceans. In rematches between familiar pairs of *C. destructor* after an “experience equalization encounter” to control for status recognition and experience effects, behavioral outcomes were still dictated by the initial familiarization encounter (Crook *et al.*, 2004). This memory of the previous opponent is therefore also likely to explain the outcomes of the contests in our experiment. The possibility of an experience effect cannot, however, be completely discounted.

Isolation before encounters

Isolation affected the winning ability of *C. destructor*. Short-term isolation (the 1-week period) reduced the number of winning encounters by isolated crayfish. After 3 and 4 weeks, isolates were the overall winners about 50% of the time. This could be interpreted as a return to an unbiased situation in which neither of the two unfamiliar individuals was more likely to win than the other. It suggests that in the wild, short periods of isolation could decrease the probability that a crayfish would win. The amount of serotonin in a crayfish is known to affect retreat behavior (Edwards and Kravitz, 1997; Huber *et al.*, 1997), and it may be that isolation changes the concentration of this neurotransmitter.

Alternatively, individuals of *C. destructor* may need more time to exhibit normal aggressive behavior when placed in isolated environments or different-sized terrain (small containers in this case), because they explore novel surrounds and are affected by complexity in the topography (Basil and Sandeman, 2000; Baird *et al.*, 2006).

Aggression levels differed between the 1- and 4-week isolation groups, suggesting that being isolated in the wild may increase an individual's propensity to invest more energy into its next encounter. This could suggest that the effect of isolation, memory, or experience persists longer than 2 weeks; however, the levels overall were similar (~3.5–3.9, Fig. 3B) and at 4 weeks neither crayfish was advantaged in ability to win the fight. Therefore, this difference in aggression level may not be important, because either crayfish could obtain access to resources after the encounter in the wild.

The results appear to indicate an interaction between effects of isolation and communal living. The communal opponents were maintained in groups. There is evidence in other species of crayfish that social structures will form when multiple crayfish are in proximity; however, this has been demonstrated only in small groups (*e.g.*, 4–5 animals, Bovbjerg, 1953; Issa *et al.*, 1999). Our observations in these larger groups suggest that *C. destructor* will generally not have dominant status because interactions are common and status is therefore reasonably even. If the communal status were a strong factor, then we would have expected the isolates to win more fights because they would be fighting against predominantly submissive animals. This was not the case: isolates won fewer fights in the first week.

Isolation and social behavior

Our results differ from some prior research because they suggest that recognition, experience, or both affect encounters for about 2 weeks. Some studies suggest that less time eliminates the effects of prior experience in some species (*Cambarus* sp., 1 week [Guiasu and Dunham, 1999]; *H. americanus*, 1–2 weeks [Karavanich and Atema, 1998]; *Orconectes rusticus*, 1 week [Zulandt Schneider *et al.*, 2001]; more detail in Table 1). Our results closely resemble unpublished observations cited in Daws *et al.* (2002) of 2 weeks for *P. clarkii*. The ecology of the study animals may explain different outcomes in different species. There is no reason to expect all species to be the same in this respect. Three study species are the hermit crab *Pagurus longicarpus*, the crayfish *C. destructor*, and the lobster *H. americanus*. Isolation affects behavior between familiar opponents for 4 days, 2 weeks, and 1–2 weeks for each species, respectively (Gherardi and Atema, 2005; this study; Karavanich and Atema, 1998). *P. longicarpus* forms aggregations along the shore, and the members remain in proximity, so individuals commonly encounter one another (Gherardi

and Atema, 2005) and may have no need to remember opponents for long periods of time. *C. destructor* and *H. americanus*, on the other hand, form sparsely populated communities and lead more solitary lives (Karnofsky *et al.*, 1989). They would therefore require longer memory retention because of the need to travel farther and for longer to encounter fellow members. If this type of reasoning is applied, it suggests that different species trade off the need to maintain neighborly contacts according to their environment and lifestyle.

Alternatively, the interpretation of the data may also explain cross-species differences like that previously mentioned between *C. destructor*, *H. americanus*, and *P. longicarpus*. For example, the inference that *H. americanus* encounters are no longer influenced by prior experience after 1–2 weeks (Karavanich and Atema, 1998) is from data on fight intensity (essentially equivalent to our fight scores). We based our conclusions on intensity and outcome because both could affect *C. destructor* in the wild. As with other crustaceans, intensity reflects energy investment; outcome reflects access to resources, mates, and territories (*e.g.*, Capelli and Munjal, 1982; Figler *et al.*, 1995, 2005; Issa and Edwards, 2006). Our findings suggest that part of the differences reported in the literature could be due to the amount of social interaction determined by the experimental designs.

There is evidence that isolation influences social behavior of another crustacean, the hermit crab *Pagurus samuelis*. Isolates win more encounters after they have had no social contact for up to 30 days (Courchesne and Barlow, 1971), which suggests that the influence of isolation may be stronger in *C. destructor*. We also observed a decrease in the ability to win, as opposed to the increase shown in *P. samuelis*, so isolation appears to be affecting aggressive behavior differently across species. However, in the hermit crab experiment, the authors pooled data across different isolation periods; we did not in our analysis.

Our results indicate that an animal's social behavior can change as a result of periods of isolation or communal contact with conspecifics. This finding has implications for the design of future studies of the mechanisms involved in recognition and memory. It counsels caution in the use of isolation as a means of establishing a baseline state of physiological or behavioral experiments because the period of isolation can itself be a factor.

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Literature Cited

- Antonsen, B. L., and D. H. Paul. 1997.** Serotonin and octopamine elicit stereotypical agonistic behaviors in the squat lobster *Munida quadrispina* (Anomura, Galatheididae). *J. Comp. Physiol. A* **181**: 501–510.
- Atema, J., and R. Voigt. 1995.** Sensory biology and behavior. Pp. 313–348 in *The Biology of the Lobster*, *Homarus americanus*, J. Factor, ed. Academic Press, San Francisco.
- Baird, H. P., B. W. Patullo, and D. L. Macmillan. 2006.** Reducing aggression between freshwater crayfish (*Cherax destructor* Clark: Decapoda, Parastacidae) by increasing habitat complexity. *Aquat. Res.* **37**: 1419–1428.
- Basil, J., and D. Sandeman. 2000.** Crayfish (*Cherax destructor*) use tactile cues to detect and learn topographical changes in their environment. *Ethology* **106**: 247–259.
- Bergman, D. A., and P. A. Moore. 2005.** Prolonged exposure to social odours alters subsequent social interactions in crayfish (*Orconectes rusticus*). *Anim. Behav.* **70**: 311–318.
- Bergman, D. A., C. P. Kozłowski, J. C. McIntyre, R. Huber, A. G. Daws, and P. A. Moore. 2003.** Temporal dynamics and communication of winner-effect in the crayfish *Orconectes rusticus*. *Behaviour* **140**: 805–825.
- Bergman, D. A., C. N. Redman, K. C. Fero, J. L. Simon, and P. A. Moore. 2006.** The impacts of flow on chemical communication strategies and fight dynamics of crayfish. *Mar. Freshw. Behav. Physiol.* **39**: 245–258.
- Berkey, C., and J. Atema. 1999.** Individual recognition and memory in *Homarus americanus* male-female interactions. *Biol. Bull.* **197**: 253–254.
- Bovbjerg, R. V. 1953.** Dominance order in the crayfish *Orconectes virilis* (Hagen). *Physiol. Zool.* **26**: 173–178.
- Bovbjerg, R. V. 1956.** Some factors affecting aggressive behaviour in crayfish. *Physiol. Zool.* **29**: 127–136.
- Breithaupt, T., and J. Atema. 1993.** Evidence for the use of urine signals in agonistic interactions of the American lobster. *Biol. Bull.* **185**: 318.
- Breithaupt, T., and J. Atema. 2000.** The timing of signaling with urine in dominance fights of male lobsters (*Homarus americanus*). *Behav. Ecol. Sociobiol.* **49**: 67–78.
- Breithaupt, T., and P. Eger. 2002.** Urine makes the difference: chemical communication in fighting crayfish made visible. *J. Exp. Biol.* **205**: 1221–1231.
- Bruski, C. A., and D. W. Dunham. 1987.** The importance of vision in agonistic communication in fighting crayfish *Orconectes rusticus*. I. An analysis of bout dynamics. *Behaviour* **103**: 83–107.
- Caldwell, R. L. 1979.** Cavity occupation and defensive behaviour in the stomatopod *Gonodactylus festae*: evidence for chemically mediated individual recognition. *Anim. Behav.* **27**: 194–201.
- Caldwell, R. L. 1985.** A test of individual recognition in the stomatopod *Gonodactylus festae*. *Anim. Behav.* **33**: 101–106.
- Caldwell, R. L. 1992.** Recognition, signalling and reduced aggression between former mates in a stomatopod. *Anim. Behav.* **44**: 11–19.
- Capelli, G. M., and B. L. Munjal. 1982.** Aggressive interactions and resource competition in relation to species displacement among crayfish of the genus *Orconectes*. *J. Crustac. Biol.* **2**: 486–492.
- Copp, N. H. 1986.** Dominance hierarchies in the crayfish *Procambarus clarkii* (Girard, 1852) and the question of learned individual recognition (Decapoda, Astacidae). *Crustaceana* **51**: 9–24.
- Courchesne, E., and G. W. Barlow. 1971.** Effect of isolation on components of aggressive and other behaviour in the hermit crab, *Pagurus samuelis*. *Z. Vgl. Physiol.* **74**: 32–48.
- Crook, R., B. W. Patullo, and D. L. Macmillan. 2004.** Multimodal individual recognition in the crayfish, *Cherax destructor*. *Mar. Freshw. Behav. Physiol.* **37**: 271–285.
- Daws, A. G., J. Grills, K. Konzen, and P. A. Moore. 2002.** Previous experiences alter the outcome of aggressive interactions between males in the crayfish, *Procambarus clarkii*. *Mar. Freshw. Behav. Physiol.* **35**: 139–148.
- Detto, T., P. R. Y. Backwell, J. M. Hemmi, and J. Zeil. 2006.** Visually mediated species and neighbour recognition in fiddler crabs (*Uca mjoebergi* and *Uca capricornis*). *Proc. R. Soc. Lond. B* **273**: 1661–1666.
- Díaz, E. R., and M. Thiel. 2003.** Female rock shrimp prefer dominant males. *J. Mar. Biol. Assoc. UK* **83**: 941–942.
- Edwards, D. H., and E. A. Kravitz. 1997.** Serotonin, social status and aggression. *Curr. Opin. Neurobiol.* **7**: 812–819.
- Edwards, D. H., and N. Spitzer. 2006.** Social dominance and serotonin receptor genes in crayfish. *Curr. Top. Dev. Biol.* **74**: 117–199.
- Figler, M. H., M. Twum, J. E. Finkelstein, and H. V. S. Peeke. 1995.** Maternal aggression in red swamp crayfish (*Procambarus clarkii* Girard): the relation between reproductive status and outcome of aggressive encounters with male and female conspecifics. *Behaviour* **132**: 107–125.
- Figler, M. H., G. S. Blank, and H. V. S. Peeke. 2005.** Shelter competition resident male red swamp crayfish *Procambarus clarkii* (Girard) and conspecific intruders varying by sex and reproductive status. *Mar. Freshw. Behav. Physiol.* **38**: 237–248.
- Gherardi, F., and J. Atema. 2005.** Memory of social partners in hermit crab dominance. *Ethology* **111**: 271–285.
- Gherardi, F., and W. H. Daniels. 2003.** Dominance hierarchies and status recognition in the crayfish *Procambarus acutus acutus*. *Can. J. Zool.* **81**: 1269–1281.
- Gherardi, F., and R. Pieraccini. 2004.** Using information theory to assess dynamics, structure, and organization of crayfish agonistic repertoire. *Behav. Process.* **65**: 163–178.
- Gherardi, F., and J. Tiedemann. 2004.** Binary individual recognition in hermit crabs. *Behav. Ecol. Sociobiol.* **55**: 524–530.
- Goessmann, C., C. Hemelrijk, and R. Huber. 2000.** The formation and maintenance of crayfish hierarchies: behavioural and self-structuring properties. *Behav. Ecol. Sociobiol.* **48**: 418–428.
- Guiasu, R. C., and D. W. Dunham. 1999.** Aggressive interactions between the crayfishes *Cambarus bartonii bartonii* and *C. robustus* (Decapoda, Cambaridae): interspecific and intraspecific contests. *J. Crustac. Biol.* **19**: 131–146.
- Herberholz, J., F. A. Issa, and D. H. Edwards. 2001.** Patterns of neural circuit activation and behavior during dominance hierarchy formation in freely behaving crayfish. *J. Neurosci.* **21**: 2759–2767.
- Herberholz, J., M. M. Sen, and D. H. Edwards. 2003.** Parallel changes in agonistic and non-agonistic behaviors during dominance hierarchy formation in crayfish. *J. Comp. Physiol. A* **189**: 321–325.
- Hojesjo, J., J. Johnson, E. Peterson, and T. Jarvi. 1998.** The importance of being familiar: individual recognition and social behaviour in sea trout (*Salmo trutta*). *Behav. Ecol.* **9**: 445–451.
- Hsu, Y. Y., R. L. Earley, and L. L. Wolf. 2006.** Modulation of aggressive behaviour by fighting experience: mechanisms and contest outcomes. *Biol. Rev.* **81**: 33–74.
- Huber, R., and E. A. Kravitz. 1995.** A quantitative analysis of agonistic behavior in juvenile American lobsters. *Brain Behav. Evol.* **46**: 72–83.
- Huber, R., K. Smith, A. Delago, K. Isaksson, and E. A. Kravitz. 1997.** Serotonin and aggressive motivation in crustaceans: altering the decision to retreat. *Proc. Natl. Acad. Sci. USA* **94**: 5939–5942.
- Issa, F. A., and D. H. Edwards. 2006.** Ritualized submission and the reduction of aggression in an invertebrate. *Curr. Biol.* **16**: 2217–2221.
- Issa, F. A., D. J. Adamson, and D. H. Edwards. 1999.** Dominance hierarchy formation in juvenile crayfish *Procambarus clarkii*. *J. Exp. Biol.* **202**: 3497–3506.
- Johnson, M. E., and J. Atema. 2005.** The olfactory pathway for indi-

- vidual recognition in the American lobster *Homarus americanus*. *J. Exp. Biol.* **208**: 2865–2872.
- Karavanich, C., and J. Atema. 1993.** Agonistic encounters in the American lobster, *Homarus americanus*: do they remember their opponents? *Biol. Bull.* **185**: 321–322.
- Karavanich, C., and J. Atema. 1998.** Individual recognition and memory in lobster dominance. *Anim. Behav.* **56**: 1553–1560.
- Karnofsky, E. B., J. Atema, and R. H. Elgin. 1989.** Field observations of social behavior, shelter use, and foraging in the lobster, *Homarus americanus*. *Biol. Bull.* **176**: 239–246.
- Komdeur, J. 2006.** Variation in individual investment strategies among social animals. *Ethology* **112**: 729–747.
- Obermeier, M., and B. Schmitz. 2003.** Recognition of dominance in the big-clawed snapping shrimp (*Alpheus heterochaelis* Say 1818). Part I. Individual or group recognition? *Mar. Freshw. Behav. Physiol.* **36**: 1–16.
- Panksepp, J. B., and R. Huber. 2004.** Ethological analyses of crayfish behavior: a new invertebrate system for measuring the rewarding properties of psychostimulants. *Behav. Brain Res.* **153**: 171–180.
- Pavey, C. R., and D. R. Fielder. 1996.** The influence of size differential on agonistic behaviour in the freshwater crayfish, *Cherax cuspidatus* (Decapoda: Parastacidae). *J. Zool.* **238**: 445–457.
- Perry, W. L., D. M. Lodge, and G. A. Lamberti. 2000.** Crayfish (*Orconectes rusticus*) impacts on zebra mussel (*Dreissena polymorpha*) recruitment, other macroinvertebrates and algal biomass in a lake-outlet stream. *Am. Midl. Nat.* **144**: 308–316.
- Quinn, G. P., and M. J. Keough. 2002.** *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.
- Rahman, N., D. W. Dunham, and C. K. Govind. 2001.** Mate recognition and pairing in the big-clawed snapping shrimp, *Alpheus heterochaelis*. *Mar. Freshw. Behav. Physiol.* **34**: 213–226.
- Rubenstein, D. I., and B. A. Hazlett. 1974.** Examination of the agonistic behaviour of the crayfish *Orconectes virilis* by character analysis. *Behaviour* **50**: 193–216.
- Rufino, M. M., and D. A. Jones. 2001.** Binary individual recognition in *Lysmata debelius* (Decapoda: Hippolytidae) under laboratory conditions. *J. Crustac. Biol.* **21**: 388–392.
- Rutishauser, R. L., A. C. Basu, S. I. Cromarty, and E. A. Kravitz. 2004.** Long-term consequences of agonistic interactions between socially naïve juvenile American lobsters (*Homarus americanus*). *Biol. Bull.* **207**: 183–187.
- Schroeder, L., and R. Huber. 2001.** Fight strategies differ with size and allometric growth of claws in crayfish, *Orconectes rusticus*. *Behaviour* **138**: 1437–1449.
- Sokal, R. R., and F. J. Rohlf. 1995.** *Biometry*. Freeman and Company, New York.
- Song, C. K., J. Herberholz, and D. H. Edwards. 2006.** The effects of social experience on the behavioral response to unexpected touch in crayfish. *J. Exp. Biol.* **209**: 1355–1363.
- Sumpter, D. J. T. 2006.** The principles of collective animal behaviour. *Philos. Trans. R. Soc. B* **361**: 5–22.
- Whitehouse, M. E. A., and Y. Lubin. 2005.** The functions of societies and the evolution of group living: spider societies as a test case. *Biol. Rev.* **80**: 347–361.
- Winston, M. L., and S. Jacobson. 1978.** Dominance and effects of strange conspecifics on aggressive interactions in the hermit crab *Pagurus longicarpus* (Say). *Anim. Behav.* **26**: 184–191.
- Yeh, S. R., R. A. Fricke, and D. H. Edwards. 1996.** The effect of social experience on serotonergic modulation of the escape circuit of crayfish. *Science* **271**: 366–369.
- Zulandt Schneider, R. A., R. Huber, and P. A. Moore. 2001.** Individual and status recognition in the crayfish, *Orconectes rusticus*: the effects of urine release on fight dynamics. *Behaviour* **138**: 137–153.