

## THE EFFECT OF AMBIENT LIGHT ON BLIND CAVE CRAYFISH: SOCIAL INTERACTIONS

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### A B S T R A C T

This study investigated the social behaviors of blind cave-adapted crayfish and compared them to the behaviors of sighted crayfish. Because blind cave crayfish display phototactic behavior, presumably mediated by the caudal photoreceptors in the sixth abdominal ganglion, we tested whether light, a disturbance in the crayfish's normal cave environment, altered their normal social behaviors. Observations were made in infrared or dim-white light to quantify social interactions. Exposure to white light reduced the amount of interaction time as compared to infrared light. The results revealed that blind crayfish did not exhibit behaviors usually associated with visual displays and posturing (i.e., the raised meral spread was absent). Same-sized individuals, previously housed in isolated conditions for two weeks, were paired. Both individuals tended to tail flip or move apart immediately after initial antennae contact. This is the same behavior observed within the natural cave environment. After repeated interactions between them, the rapid tail flip behavior became less frequent, while avoidance became an automatic reaction. Ethograms were constructed to determine the differences in the observed behaviors between the aggressive and submissive cave crayfish.

Crayfish have been used extensively to examine behaviors associated with social interactions. They have been shown to exhibit intraspecies dominance hierarchies (Bovbjerg, 1953, 1956; Issa *et al.*, 1999; Goessmann *et al.*, 2000), as well as interspecies dominance between native and invading species (Guiaşu *et al.*, 1996). Primary access to resources, such as a shelter, has been shown to affect survival rates within a species, which is particularly relevant to the aquaculture industry (Figler *et al.*, 1999). In addition, the reproductive readiness of males (i.e., form I or II) affects how they interact in social competition (Guiaşu and Dunham, 1997a, b). This establishment of social status represses aggressive interactions between individuals, reduces the amount of energy required to keep themselves alive, and therefore helps to stabilize the population over time (Lomnicki, 1988; Smith and Taylor, 1993).

Determining which sensory cues are utilized in various species of crayfish is of interest in determining the functional role of particular sensory adaptations. For instance, the use of vision has been demonstrated to have an effect on the dynamics of agonistic communication in the sighted crayfish, *Orconectes rusticus* (see Bruski and Dunham, 1987; Smith and Dunham, 1990). The role

of olfaction in lobsters (Breithaupt and Atema, 1993) and crayfish (Rutherford *et al.*, 1996; Zulandt-Schneider *et al.*, 1999) also has been established as a component of social interactions. Communication between individuals is an important aspect of the behavioral repertoire before, during, and after interactions. A visual signal, such as a meral spread, can be a strong enough cue to prevent any further interaction from taking place between various species of sighted crayfish.

Blind cave crayfish (*Orconectes australis packardii*) provide an opportunity to determine whether they exhibit the same posturing behaviors and sensory modalities commonly noted during interactions among sighted crayfish. They may have evolved behavioral patterns as well as morphological, physiological, behavioral, and evolutionary adaptations unique to their species. For example, particular sensory modalities may be enhanced in such cavernous species to help them survive in total darkness. Just as for epigeal crayfish, cave species inhabit a variety of niches (i.e., streams or pools), each with varied resources. Temperature, food availability, and environmental space can vary in the cave as a result of seasonal changes on the surface.

Some caves contain a variety of troglobitic species (e.g., animals which are obligatory

cave dwellers), including crayfish, which are endemic to a particular cave, whereas other troglobitic species are not as restricted. Of the cave crayfish, there are many species that show different degrees of troglobitic adaptational characteristics. For instance, *Procambarus erythrops* (see Mellon, 1977) still has some ommatidia and retinal morphology, although reduced. In contrast, the species we investigated, *Orconectes australis packardii* Rhoades, 1944, does not contain any ommatidia in the eyes (Cooper *et al.*, 2001a). It is assumed that other sensory structures, such as those used in olfaction, may have evolved increased functionality in cave-adapted crayfish (Cooper *et al.*, 2001a).

This study was designed to test whether blind male cave crayfish display a repertoire of social behaviors similar to that of sighted male crayfish. In particular, we looked for the presence or absence of the social display, which is well established in sighted crayfish studies. Previous observations had indicated a behavioral response to white light in this species (Li *et al.*, 2000; Li and Cooper, 2001; Kellie *et al.*, 2001). This study also examined the effect of white light on the behavioral repertoire and dynamics exhibited between pairs of male blind cave crayfish. To quantify their interactions for comparison with other social animals, ethograms were developed for the blind cave crayfish.

Preliminary findings of this study have been presented in abstract form (Huffman *et al.*, 1998; Li *et al.*, 1998; Li and Cooper, 1999).

## MATERIALS AND METHODS

Adult blind male cave crayfish, *Orconectes australis packardii* Rhoades, of various sizes (1.8–2.5-cm carapace length), were collected from Sloan's Valley Cave, near Somerset, Kentucky. Twelve animals were used in the first set of experiments to test individual behavioral reactions to light. In the second set of experiments, 16 freshly obtained crayfish were used. Upon arrival at the laboratory, the 16 crayfish were divided into 8 pairs of individuals with similar lengths. The carapace length differences between them was less than 0.3 cm. The members of each pair were marked (A1, A2 . . . H1, H2) with white fingernail polish for easy identification. To prevent pre-experiment interactions between individual animals, they were isolated in a dark room (14–15°C) within their own aquaria (34 × 21 × 23 cm; water depth 10–15 cm) for 10–14 d. They were fed twice a week with fish pellets.

### Light Responsiveness

Experiments were performed to determine whether *Orconectes australis packardii* Rhoades is sensitive to white light (i.e., possesses caudal photoreception), as has been

shown for the unsighted cave crayfish *Cambarus setosus* (see Larimer, 1966). Among epigeal species of crayfish there are photoreceptors that are associated with the ventral nerve cord within the abdomen (Prosser, 1934; Edwards, 1984). These light-receptive neurons may have a dual role as an interneuron for mechanosensory signals as well as light detection (Galeano, 1976). Twelve animals (six in each group) were observed under either white ( $n = 6$ ) or infrared ( $n = 6$ ) light in small tanks (36 × 25 × 14 cm; water depth 10 cm), with half of the tank covered with thick, black plastic. A dark shadow was produced for almost half the tank area, with a slight semi-shadow produced at the transition zone along the illuminated and dark sides of the tank.

### Experimental Conditions for Behavioral Interactions

The observation chamber was the same type of aquarium within which the animals were isolated, and the physical conditions encountered during each trial of testing were similar. Only the lighting condition varied. For each trial, the testing chamber was illuminated by either a dim-white light or an infrared light. Both individuals of one of the eight pairs of blind crayfish were simultaneously placed inside the chamber. The crayfish were allowed to adapt to the chamber for at least 2 h prior to being recorded by a video camera. This period was necessary to allow the crayfish to achieve a settled behavior, as defined in Bruski and Dunham (1987). Their interactions were recorded by a video camera for at least 1 h. Following each trial, the animals were separated and returned to their original compartments. Between trials, the testing chamber was washed twice to eliminate the possible effect of substances exuded from the animals tested previously. All eight pairs were tested within one week of each other under one lighting condition, and then tested under the alternate lighting condition two weeks later.

White light was supplied by a 60-Watt incandescent bulb in a removable lamp placed 2 m above the observation tank. Each crayfish was kept under these lighting conditions individually for at least 1 h prior to the trial pairing.

The interactions of crayfish under infrared light were recorded with an infrared sensing CCD camera (Toshiba, model IK-537A) fitted with a zoom lens (Pentax TV, zoom 8–48 mm) and a video cassette recorder (Panasonic, time lapse SVHS, model AG6T20). Lighting was fitted with a filter to provide a red light (Edmond Scientific, IR only, model # D43,951). Recordings were analyzed using direct viewing of the video data on a television screen (17-in diagonal).

### Description of Behaviors

In order to quantify agonistic behavior of blind crayfish, it was separated into discrete fundamental elements/behavioral acts that could be reliably identified by other observers. The selection of behavioral acts was based on previous descriptions of agonistic behavior in stomatopods (*Gonodactylus bredini*; Dingle, 1969), crayfish (*Orconectes rusticus*; Bruski and Dunham, 1987), and American lobster (*Homarus americanus*; Scrivener, 1971). The selected behavioral acts are as follows:

**Antennae Whip:** One or both antennae sweep downward over the anterior portion of the other animal.

**Approach:** A direct and obviously oriented advance toward another animal.

**Bout:** The period beginning when one or both animals in a pair approach the other, ending when one or both crayfish retreat.

Chase: Pursuit by one animal of the other animal.

Chelae Touch: An animal touches, slightly strikes, or pushes the other with its opened chelae.

Interaction: An exchange of behavioral acts by the two crayfish in a pair.

Lateral Spread: A simultaneous outward spreading of the open chelae (Dingle, 1969).

Lunge: A short and rapid advance toward the other animal with chelae spread out. The same as a "Rush" but shorter in duration.

Meet: A chance encounter that brings the rostral aspects of the body of two crayfish closer than the combined antenna lengths of the two individuals. This occurs when crayfish move around in either a natural or a laboratory setting.

Motionless: A crayfish remaining still, except for an occasional antenna flick and movement of the mouthparts.

Retreat: Rapid and abrupt moving away from the other animal quickly walking backward, running forward or turning away.

Rush: Abrupt and rapid advance of an animal with both open chelae spread out. The same as a "Lunge" but longer in duration.

Stretch Out: Two crayfish spread their opened chelae far apart and face each other.

Tail Flip: Abrupt and rapid contraction of the abdomen that propels an animal backwards.

Thrust: An animal thrusts or slaps the other with one or both open claws. For the blind crayfish, the whole animal abruptly moves forward and strikes the other with both open chelae stretched out and held in horizontal position.

#### Data Collection and Analysis

With observation tanks half covered, the exposed region was illuminated with either white or infrared light to examine a regional preference. Every 2 min a recording was made of the location of the animal within the tank. When the animal was observed in the exposed region a score of 1 was given as compared to -1 for the covered region. If the animal was in the transition zone a score of zero was recorded. Paired *t*-tests were performed to compare whether the animal preferred to stay in covered or illuminated regions,  $n = 6$  and  $\alpha = 0.05$ .

The agonistic behavior of blind cave crayfish was quantified from the videotape recordings as follows: The total 50-min observation period, monitored separately for each crayfish, was divided into 5-s intervals. The occurrence or absence of the 13 behavioral acts within each of these intervals was recorded. For quantitative analysis, these 13 behavioral acts can be collapsed into four categories, based on previous studies (Rubenstein and Hazlett, 1974; Huber and Kravitz, 1995): ambivalent behavior (meet, motionless, antennae whip); initiation behavior (lateral spread, approach, chase, lunge, rush); physical interaction (chelae touch, stretch out, thrust); and avoidance behavior (retreat, tailflip).

Each of the four behavioral categories was compared between the eventual winner and loser with a multivariate *t*-test (two-tailed) with  $d.f. = 7$ ,  $\alpha = 0.05$ . The effect of white light on the behaviors was assessed by the median number of acts per bout and compared with a paired *t*-test,  $d.f. = 7$ ,  $\alpha = 0.05$ . The median and maximum fighting durations were compared with a multivariate *t*-test,  $d.f. = 7$ ,  $\alpha = 0.05$ .

In these experiments, each animal may have a number of bouts within 50 min of observation time. The normalities of the distributions for either the number of acts

per bout or the bout durations were tested for each animal. If the normality tests passed, the means would have been chosen for later quantification and statistical tests. However, a few animals had a long duration in their fighting time, which resulted in skewed distributions in both the number of acts per bout and bout duration. Therefore, the means alone are not appropriate to use to represent the general trends of how long two animals would fight. The median value in the number of acts per bout and bout duration from each of the eight animals (winner or loser) were used instead. A mean of the medians was used for statistical purposes of a multivariate *t*-test (normality tests passed on the eight median values).

## RESULTS

### Phototactic Behavior

The results of this phototactic study demonstrated that indeed blind cave crayfish are sensitive to the white light as indicated by their tendency to remain in the shadow provided by a covering. In infrared light no preference for cover was observed. This was demonstrated by examining the location of separately tested individual crayfish every 2 min for a total of 110 min within the observation tank. Two representative cases of exposures to white light and infrared light are shown in Fig. 1A. If part of a crayfish's body was partially exposed to the lighted side or in the transition zone of a partial shadow, it was counted as exposed.

The locations for the 12 individuals (six in each group) in both lighting conditions at each 2-min interval were added to obtain the mean number of occurrences. Results indicated that when the animals were exposed to white light they preferred the shadow, paired *t*-test,  $t = 10.58$ ,  $d.f. = 5$ ,  $P < 0.0001$ ; there was no locational preference when animals were exposed to infrared light, paired *t*-test,  $t = 0.507$ ,  $d.f. = 5$ ,  $P = 0.634$ . The number of covered only and exposed only occurrences was also examined with the data on half-exposed animals discarded. The crayfish preferred to stay within the covered region (paired *t*-test,  $t = 9.70$ ,  $d.f. = 5$ ,  $P < 0.0001$ ) when exposed to white light and showed no preference for either side of the tank (paired *t*-test,  $t = 0.438$ ,  $d.f. = 5$ ,  $P > 0.6$ ) under infrared light (Fig. 1B).

### Ethological Description of Blind Crayfish Agonistic Behavior

*Exploratory Phase.*—When first introduced into the test chamber, the crayfish moved about the tank exhibiting exploratory behavior. This behavior involved fast walking movements along the boundary of the tank

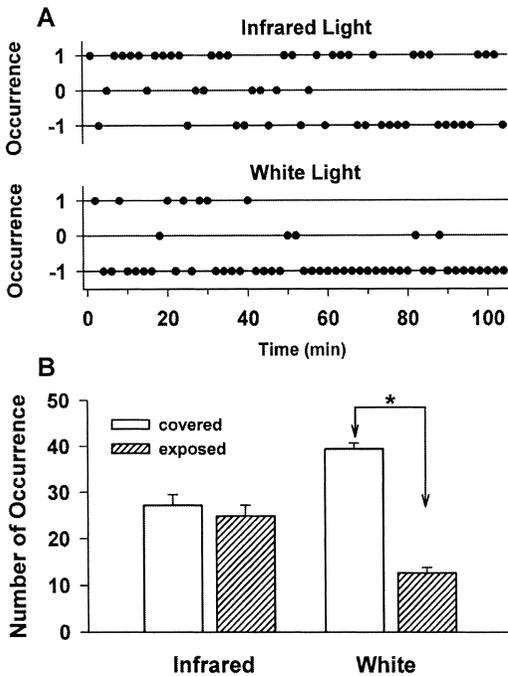


Fig. 1. Behavioral responses to white and infrared light. With observation tanks half covered, the exposed region was illuminated with either white or infrared light to examine a regional preference. Every 2 min an observation was made of the location of the animal within the tank. If the animal was observed in the exposed region a score of 1 was recorded as compared to -1 for the covered region. If the animal was in the transition zone, a score of zero was recorded. This type of analysis over time is shown in A for a representative tank exposed to infrared or white light. The preference of one side of the tank is readily demonstrated in the summed scores over the entire period during exposure to white light as compared to exposure to infrared light (B). The occurrences of half exposed animals were split into covered and exposed events for further analysis. The crayfish showed no side preference when exposed to infrared light, whereas white light induced the animals to seek the covered side of the tank.

with occasional movements to its center, while tapping objects with its chelae. One of the antennae usually remained in contact with the wall of the tank; the other flicked back and forth during initial exposure to the tank. The antennae flicking appeared to be coordinated or not coordinated with walking. When making a turn, one or both of the antennae swept to one side, followed by the whole animal turning in the same direction. During the exploratory behavior phase, the animals sometimes encountered or even bumped into each other. When this occurred, both animals would tail flip or quickly retreat from each other upon the first several encounters. Dur-

ing this period, both of the animals appear more interested in their new environment than in the interaction.

*Adaptive Phase.*—Following familiarization, when the animals met head on, they occasionally paused, extended the chelipeds with a slight flexion ( $\sim 45^\circ$  from being stretched straight out) at the meropodite-carpopodite joint, and faced the opponent. If one animal was approached by another from behind or from the side following familiarization, it usually rapidly turned to face the opponent with open chelipeds. In both types of interaction, the chela gaped at angles approximately  $70^\circ$  to  $85^\circ$ , which is extreme, and the chelipeds were stretched far apart (lateral spread) and held at an angle of  $10^\circ$  to  $30^\circ$  above the horizontal plane. If neither animal retreated from the other, one or both advanced and initiated a physical contact. Both adopted a posture in which the open chelipeds were stretched out without actually being interlocked with those of the other, with the animals facing each other. Both animals remained motionless except for the occasional slow antennae whip and the movement of maxillipeds. The movements of the pleopods were not monitored in this study.

After a brief time confronting each other, animals moved back and forth and began to push against each other with their open chelae. One animal may have withdrawn after several repetitions of pushing, either by walking backwards quickly, by running aside, or even by tail flipping away from its opponent. Upon retreating, the other crayfish may have tried to pursue or chase the opponent, often using a lunge response. If there was no pursuit, both animals continued to explore their environment until the next encounter. If neither one retreated, the fight escalated. Abruptly, one or both crayfish would thrust forward with open chelae. This was sometimes followed by rapidly bringing the chelae together while trying to pinch and tear at the other. When performing a thrust, the animal advanced abruptly with its open chelae held rigid. In most cases, one animal tail flipped away from its opponent while the other animal rushed around the tank, appearing to try to find its opponent. This rush may have lasted as long as 10 seconds. The crayfish which quickly retreated from the combat usually remained quiescent on the substrate even

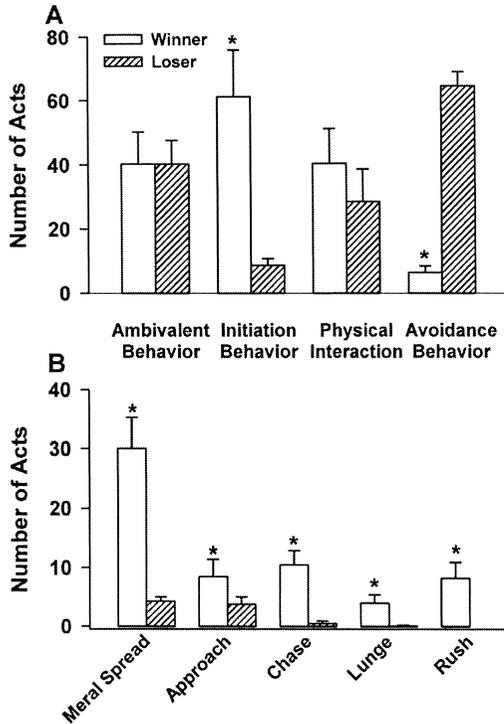


Fig. 2. The mean number of acts from each of the four behavioral categories (ambivalent behavior, initiation behavior, physical interaction, and avoidance behavior) were compared between the eventual winner and loser under the infrared lighting condition (A). No significant difference between the winner and loser was observed for the ambivalent behavior and for the physical interaction. The eventual winner performs more initiation behavior and less avoidance behavior than the loser. The number of initiation acts (lateral spread, approach, chase, lunge, rush) was further analyzed (B). The winner performed more initiation behavior than the loser during fights for each type of the behavior measured.

while the opponent may have rushed by. In some cases, the dominant bumped into the retreating crayfish, which again performed one or more tail flips to move away from the other.

*Winner or Loser.*—A crayfish could be easily classified as an eventual winner or loser based on the number of retreats, lunges, and rushes. Behaviors of the individuals within a pair over the entire interaction could be segregated as a winner's or a loser's behavior (Fig. 2, Table 1). Because we had already shown in the first phototactic study that the exposure of white light altered the animal's choice of environment, we wanted to examine if such exposure to white light might override the time spent socially interacting, thus affecting particular components of the temporal so-

Table 1. The behavioral differences between the eventual winner and loser. The mean number of each behavioral act is shown for the experiments under infrared and white lighting conditions.

Behavioral category	Behavioral act	Infrared		White	
		Winner	Loser	Winner	Loser
Ambivalent behavior	Meet	22.0	25.8	22.2	30.7
	Motionless	13.5	11.0	10.3	15.2
	Antennae whip	4.8	3.5	2.8	2.3
Initiation behavior	Lateral spread	30.0	4.3	34.2	3.0
	Approach	8.5	3.8	8.2	4.0
	Chase	10.5	0.5	9.5	0.5
	Lunge	4.0	0.1	4.3	0.0
	Rush	8.2	0.1	8.7	0.0
Physical interactions	Chelae touch	25.2	17.0	14.8	9.7
	Stretch out	10.0	10.2	4.8	4.2
	Thrust	5.2	1.3	4.0	0.3
Avoidance behavior	Retreat	5.7	55.2	5.3	52.0
	Tail flip	0.8	9.3	1.0	10.5

cial ethograms. Therefore, we conducted the same type of interaction experiments under the different lighting conditions as in the phototactic studies and constructed ethograms for eight pairs of crayfish. The behavioral act of rushing forth after a contact usually occurred only late during the total interaction time and was independent of white or infrared light exposure. During infrared or white light exposure one of the partners always showed a predominant act of tail flipping immediately after a contact. This act also increased in occurrence over time for the loser. Thus, this act served as a temporal indicator of the animal that would eventually be the loser. The individuals that showed an increase in tail flipping behavior rarely showed the behaviors of rushing or chasing after the opponent or lunging at the opponent during an interaction.

The 13 behavioral acts within each of the observation intervals under the different lighting were placed into the four behavioral categories and were compared between the eventual winner and loser (Table 1). Because the total number of acts varied between the two experimental lighting conditions, the percentage of each act was also tabulated for ease in comparative analysis. The data indicated that the total number of acts was fewer for the groups exposed to white light when compared to those exposed to infrared light. Subtle differences were becoming apparent in the ethograms of pairs exposed to white or infrared light.

To examine whether the overall distribution pattern of the four behavior categories

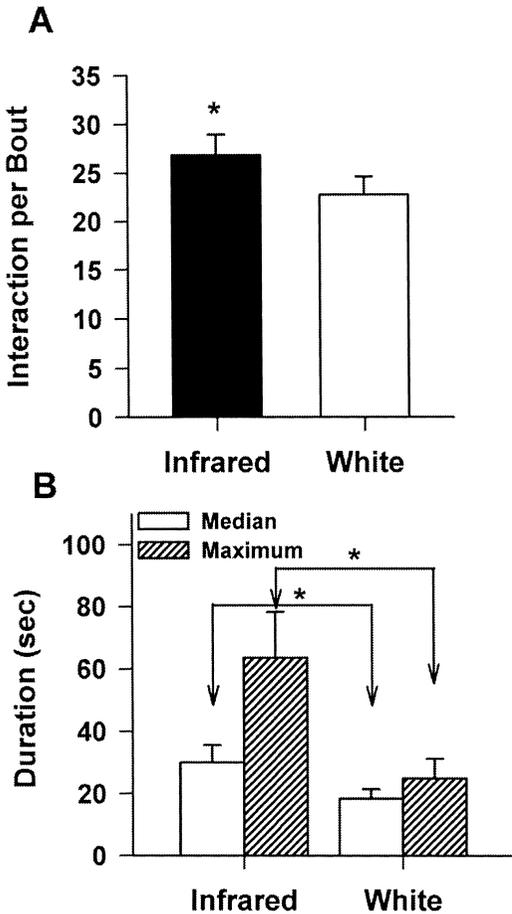


Fig. 3. White light effects on blind crayfish agonistic behavior. (A) the median number of acts per bout was significantly reduced, and the median and maximum fighting durations (B) also decreased while exposed to white light.

were altered under different lighting conditions, the number of occurrences for each behavior category was compared between infrared and white light condition for both winner and loser. Neither of the pair members changed their behavioral patterns while exposed to white light ( $\chi^2_3 = 3.47$ ,  $P > 0.4$  for winners and  $\chi^2_3 = 1.40$ ,  $P > 0.7$  for losers, Fig. 2B). Also, no significant differences were observed for the ambivalent behavior and physical interaction between the winner and loser (multivariate  $t$ -test,  $d.f. = 7$ ,  $P > 0.7$  and  $P > 0.5$  respectively). The eventual winner performed more initiation (multivariate  $t$ -test,  $d.f. = 7$ ,  $P < 0.001$ ) and less avoidance behavior (multivariate  $t$ -test,  $d.f. = 7$ ,  $P < 0.001$ ) than the loser.

Because there were some differences between the winners and losers, the initiation acts were further subdivided for analysis. Ini-

tiation behaviors in the past have been examined in a variety of crustacean species and can be used as indices to predict who will eventually win the fight (Rubenstein and Hazlett, 1974; Glass and Huntingford, 1988; Guiaşu and Dunham, 1997a, b, 1998; Figler *et al.*, 1995, 1999). Initiation acts were very apparent during the initial contact between two individuals, although some of the winners conducted more of a particular act than did other individual winners. The acts analyzed were as follows: lateral spread, approach, chase, lunge, and rush. The winner performed more initiation behaviors than did the loser during fights, (multivariate  $t$ -test,  $d.f. = 7$ ,  $P < 0.005$ ) for each type of behavior (Fig. 2B).

In another attempt to examine for differences in the interactions of pairs during white or infrared light exposure, the mean number of physical interactions per each contact bout were compared. During such bouts there could be several thrust movements and chela touches, which usually resulted in pushing contests. These types of interaction were counted and averaged among the groups under the various lighting conditions. The bout frequency remained unchanged for either of the two lighting conditions (paired  $t$ -test,  $d.f. = 7$ ,  $P = 0.17$ ). However, the median number of interactions per bout decreased when the animals were exposed to white light (paired  $t$ -test,  $d.f. = 7$ ,  $P < 0.01$ ) (Fig. 3A). The median and maximum fighting durations for the groups exposed to white or infrared light also decreased (multivariate  $t$ -test,  $d.f. = 7$ ,  $P < 0.001$ , respectively) when the animals were exposed to white light (Fig. 3B).

## DISCUSSION

The results of this study indicate that *O. australis packardii* are negatively phototactic and that they exhibit social behaviors that can be quantified to distinguish "winners" and "losers" using various criteria, derived from an ethogram. In addition, the duration of interactions is shorter when the animals are exposed to white light as compared to infrared. Some of the social behaviors between blind cave crayfish are quite different from those of sighted crayfish. For example, we did not observe the wide meral spread in this species as commonly seen for sighted species. However, if the cave crayfish were contacted from the front they would open their chelae, which would appear to create a block from direct assault.

It is anatomically possible for this blind species to actually produce a wide meral spread if an experimenter spreads the chelipeds, but also we have observed the blind crayfish exhibiting a wide meral spread over its body within a cave environment in shallow pools. The crayfish would raise their chelae to the surface of the pool and rapidly pull the chelae downwards. Although it looked to us as if they were pulling organisms downward that were possibly floating on the surface, we did not observe any small animals on the water's surface. It remains to determine the function of this type of behavior, but for our immediate purposes it demonstrates that they do have the ability to produce a wide meral spread, which is not performed during social interaction. Another difference in behavior, that at least is different from the sighted *P. clarkii*, are the rapid tail flips by both individuals in the cave species upon initial contact.

In social interactions of *P. clarkii*, one individual may rapidly tail flip away from the other, but it is not common for both partners to simultaneously perform such an act (Bruski and Dunham, 1987; Spohn *et al.*, 1997). Based on the location in which these cave crayfish were collected, they are usually found in low population densities, which is an indication of limited competition. Possibly the avoidance strategy of tail flips is a consequence of few social interactions. Obviously, more behavioral investigations need to be conducted on these uniquely evolved blind cave crayfish within their natural environment.

In the laboratory conditions, the predominance of tail flips in one of the individuals was usually an early indication of its subordination. In this species of crayfish, individuals which initially show a submissive behavior rarely switch over to a search or rush mode after an interaction, which suggests that an individual may prefer a particular behavioral state, at least during our observational period. Observations also suggest that the pair member that exhibits the greater exploratory behavior when placed into the novel environment is less likely to tail flip upon initial contact, but this observation has not been fully quantified (Kellie *et al.*, 2001). It would also be of interest to examine social hierarchy behaviors using larger groups of these cave crayfish to index each individual within

a group as to the level of assertiveness and submissiveness related to size, weight, and physical activity and exploratory behavior. For instance, it is known that in groups of *P. clarkii* there can be a superdominant individual, and after establishment of a dominance hierarchy within a group, the number of agonistic interactions will decrease over time (Issa *et al.*, 1999; Goessmann *et al.*, 2000). The results that we obtained in a confined aquarium within the laboratory are relevant in the cave crayfish species we used because we obtained them from small pools within the cave. Comparisons to *P. clarkii* (see Issa *et al.*, 1999) or *Astacus astacus* (Goessmann *et al.*, 2000) may not be as relevant because it is known that the aggressive interactions are more frequent in confined environments than in the field for other crustacean species. However with *Cambarus robustus*, it appears the same agonistic behavioral acts are performed in either a laboratory setting or in the field (Guiaşu and Dunham, 1997a).

The duration of the agonistic encounters varied between individuals, from both animals rapidly producing tailflips without any further pursuit of each other, to one individual continuing with tailflips while the other would rush forward in search of the opponent. In some cases the individual that tailflipped away would be discovered. Upon being discovered, the submissive individual would again tailflip away. This pursuit would sometimes be repeated a few times before the aggressor no longer continued to seek out the opponent. The total amount of time spent in such social interactions was reduced in the presence of white light. This finding suggests that the white light disturbs the crayfish, which alters their level of interest in social interactions. This is also supported by the fact that the animals prefer the dark over lighted regions of their environment. Thus, this indicates that internal states of an individual can be altered by environmental variables such as light.

Larimer (1966) showed that the cave crayfish *Cambarus setosus* was sensitive to light, most likely as a function of the caudal photoreceptor. In a different troglobitic crayfish species, *P. erythrops*, Mellon (1977) showed that this particular species has retained ommatidia. However, in *O. australis packardii*, cross section of the eye cap and microscopic

assessment revealed that this species is devoid of ommatidia (Cooper *et al.*, 2001a). In spite of the lack of sensory structures in the eye associated with visual perception, an ability to perceive light may still have been possible. Monitoring of electrical discharges within the eye cap of *P. clarkii* and *O. australis packardii* during light flashes further indicated that the cave crayfish are not able to perceive light via the residual eyes (Cooper *et al.*, 2001a). Thus, the caudal photoreceptors are likely the means of sensory assessment of the environmental light. Light exposure as well as other sensory cues in this species of cave crayfish have been shown to result in an increased heart rate, which in some cases can persist for some time (Li *et al.*, 2000).

The neural mechanisms underlying social interactions are influenced by the sensory information that can be utilized. Combative interactions with other crayfish may be less likely to occur if an opponent is sensed earlier, providing more time to flee. The behavioral postures displayed in lobsters and crayfish are known to provide visual cues in establishing social status. In the sighted crayfish, vision provides this ability (Bruski and Dunham, 1987; Smith and Dunham, 1990; Huber and Kravitz, 1995). It is not yet known if the blind cave crayfish have an enhanced ability to better sense each other through tactile or chemical cues (Li *et al.*, 2000). It is known that there are more olfactory projection neurons that arise from cluster ten within the central brain and project into the eye stalk within adult *O. australis packardii* as compared to an adult sighted crayfish (*P. clarkii*) (Cooper *et al.*, 2001a). No functional studies have yet been conducted to determine if cave crayfish have better olfactory abilities as compared to species that also use vision. Recently, studies have investigated whether *O. australis packardii* have an enhanced ability to learn an environment spatially as compared to *P. clarkii* (Li *et al.*, 1998; Li and Cooper, 2001).

Our explanation of the inherent differences in aggressiveness among individuals may be related to the levels of various neuromodulators in the hemolymph. There is correlative evidence in crustaceans of an individual being either aggressive or submissive in the social order as related to levels of biogenic amines (Huber *et al.*, 1997a, b; Huber and Delago, 1998; Sneddon *et al.*, 2000), partic-

ularly serotonin (5-HT), because the duration of agonist encounters can be altered by treating the animals with fluoxetine, a presumed blocker of the 5-HT transporter (Huber and Delago, 1998). However, because the 5-HT responsiveness can be pharmacologically altered over a relatively short time, likely because of up- or down-regulation of 5-HT receptors, this suggests that maintained aggression in the crayfish would unlikely be the result of chronically elevated levels of circulating 5-HT (Cooper *et al.*, 2001b; see also Listerman *et al.*, 2000; Strawn *et al.*, 2000). Such differences in behavior may occur more on a short-term hormonal basis or different levels of autonomic neuronal function (Schapker *et al.*, 2002). Recent evidence suggests that the past dogma of chronically elevated 5-HT in aggressive crustaceans may need some reconsideration (Doernberg *et al.*, 2001; Djokaj *et al.*, 2001).

In summary, *O. australis packardii* have the same general pattern of aggressive and submissive interaction as all other crayfish species examined. The behavioral acts are stereotypical enough to be classified into categories for quantifying purposes. Environmental disturbances, such as light, alter the degree of social interaction. The mechanisms are still unknown as to why individual cave crayfish show differences in assertiveness. As determined in other socially interacting animals there may be a means by which a blind crayfish can assess its resource holding potential, that is, its ability to fight or win an encounter. Apparently, the cave crayfish species are equipped with sensory structures to compensate for cave life in the dark. Such evolutionary sensory adaptations may provide evolutionary stable strategies.

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#### LITERATURE CITED

- Bovbjerg, R.V. 1953. Dominance order in the crayfish *Orconectes virilis* (Hagen).—Physiological Zoology 26: 173–178.

- . 1956. Some factors affecting aggressive behavior in crayfish.—*Physiological Zoology* 29: 127–136.
- Breithaupt, T., and J. Atema. 1993. Evidence for the use of urine signals in agonistic interactions of American Lobster.—*Biological Bulletin* 185: 318.
- Bruski, C. A., and D. W. Dunham. 1987. The importance of vision in agonistic communication of the crayfish *Orconectes rusticus*, I. An analysis of bout dynamics.—*Behaviour* 63: 83–107.
- Cooper, R. L., H. Li, L. Y. Long, J. Cole, and H. L. Hopper. 2001a. Anatomical comparisons of neural systems in sighted epigeal and troglobitic crayfish species.—*Journal of Crustacean Biology* 21: 360–374.
- , R. J. Chase, and J. Tabor. 2001b. Altered responsiveness to 5-HT at the crayfish neuromuscular junction due to chronic p-CPA and mCPP treatment.—*Brain Research* 916: 143–151.
- Dingle, H. 1969. A statistical and information analysis of aggressive communication in the mantis shrimp *Gonodactylus bredini* Manning.—*Animal Behaviour* 17: 561–575.
- Djokaj, S., R. L. Cooper, and W. Rathmayer. 2001. Effects of octopamine, serotonin, and cocktails of the two modulators on synaptic transmission at crustacean neuromuscular junctions.—*Journal of Comparative Physiology* 187: 145–154.
- Doernberg, S. B., S. I. Cromarty, R. Heinrich, B. S. Beltz, and E. A. Kravitz. 2001. Agonistic behavior in naive juvenile lobsters depleted of serotonin by 5,7-dihydroxytryptamine.—*Journal of Comparative Physiology* 187: 91–103.
- Edwards, D. H. 1984. Crayfish external photoreception I. Behavioral and motoneuronal responses to abdominal illumination.—*Journal of Experimental Biology* 109: 291–306.
- Figler, M. H., H. M. Cheverton, and G. S. Blank. 1999. Shelter competition in juvenile red swamp crayfish (*Procambarus clarkii*): the influence of sex differences, relative size, and prior residence.—*Aquaculture* 178: 63–75.
- , 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.
- Galeano, C. 1976. The caudal photoreceptor of crayfish. A review.—*Acta Physiologica Latinoamericana* 26: 13–29.
- Glass, C. W., and F. A. Huntingford. 1988. Initiation and resolution of fights between swimming crabs (*Lio-carcinus depurator*).—*Ethology* 77: 237–249.
- Goessmann, C., C. Hemelrijk, and R. Huber. 2000. The formation and maintenance of crayfish hierarchies: behavioral and self-structuring properties.—*Behavioral Ecology and Sociobiology* 48: 418–428.
- Guiasu, R. C., and D. W. Dunham. 1997a. Initiation and outcome of agonistic contests in male Form I *Cambarus robustus* Girard, 1852 crayfish (Decapoda, Cambaridae).—*Crustaceana* 70: 480–496.
- , and ———. 1997b. Agonistic interactions in male Form II *Cambarus robustus* Girard, 1852 crayfish (Decapoda, Cambaridae) and a comparison between male Form I and Form II intra-form contests.—*Crustaceana* 70: 720–736.
- , and ———. 1998. Inter-form agonistic contests in male crayfish *Cambarus robustus* (Decapoda, Cambaridae).—*Invertebrate Biology* 117: 144–154.
- , ———, and D. W. Barr. 1996. Inter-specific agonistic contests between male *Cambarus bartonii bartonii* (Fabricius 1798) and *Cambarus robustus* Girard 1852 (Decapoda, Cambaridae) and the possible competition between the two species in Ontario.—*Freshwater Crayfish* 11: 364–377.
- Huber, R., and A. Delago. 1998. Serotonin alters decisions to withdraw in fighting crayfish, *Astacus astacus*: the motivational concept revisited.—*Journal of Comparative Physiology A* 182: 573–583.
- , and E. A. Kravitz. 1995. A quantitative analysis of agonistic behavior in juvenile American lobster (*Homarus americanus* L.).—*Brain Behavior Evolution* 46: 72–83.
- , M. Orzeszyna, N. Pokorny, and E. A. Kravitz. 1997a. Biogenic amines and aggression: experimental approaches in crustaceans.—*Brain Behavior and Evolution* 50: 60–68.
- , K. Smith, A. Delgo, K. Isaksson, and E. A. Kravitz. 1997b. Serotonin and aggressive motivation in crustaceans: altering the decision to retreat.—*Proceedings of the National Academy of Sciences of the USA* 94: 5939–5942.
- Huffman, P., H. Li, J. L. Cole, H. L. Hopper, B. Peretz, and R. L. Cooper. 1998. The neuroecology of cave crayfish: social interactions. Abstract for Society for Neuroscience 468.9. [Unpublished.]
- Issa, F. A., D. J. Adamson, and D. H. Edwards. 1999. Dominance hierarchy formation in juvenile crayfish *Procambarus clarkii*.—*Journal of Experimental Biology* 202: 3497–3506.
- Kellie, S., J. Greer, and R. L. Cooper. 2001. Alterations in habituation of the tail flip response in epigeal and troglobitic crayfish.—*Journal of Experimental Zoology* 290: 163–176.
- Larimer, J. L. 1966. A functional caudal photoreceptor in blind cavernicolous crayfish.—*Nature* 21: 204–205.
- Li, H., and R. L. Cooper. 1999. The neuroecology of the blind cave crayfish: social interactions.—*American Zoologist* 38: 201A. [Abstract.]
- , and ———. 2001. Spatial familiarity in the blind cave crayfish, *Orconectes australis packardii*.—*Crustaceana* 74: 417–433.
- , P. Huffman, J. L. Cole, H. L. Hopper, and R. L. Cooper. 1998. The neuroecology of cave crayfish: Spatial learning among blind cave crayfish. Abstract for Society for Neuroscience 468.8. [Unpublished.]
- , L. Listerman, D. Doshi, and R. L. Cooper. 2000. Use of heart rate to measure intrinsic state of blind cave crayfish during social interactions.—*Comparative Biochemistry and Physiology* 127A: 55–70.
- Listerman, L., J. Deskins, H. Bradacs, and R. L. Cooper. 2000. Measures of heart rate during social interactions in crayfish and effects of 5-HT.—*Comparative Biochemistry and Physiology* 125A: 251–264.
- Lomnicki, A. 1988. *Population Ecology of Individuals*. Princeton University Press, Princeton.
- Mellon, DeF. 1977. Retention of oculomotor reflexes in blind cave-dwelling crayfish.—*Brain Research* 134: 191–196.
- Prosser, C. L. 1934. Action potential in the nervous system of the crayfish. II. Response to illumination of the eye and caudal ganglion.—*Journal of Cellular and Comparative Physiology* 4: 363–377.
- Rhoades, R. 1944. The crayfishes of Kentucky, with notes on variation, distribution and descriptions of

- species and subspecies.—American Midland Naturalist 31: 111–149.
- Rubenstein, D. L., and B. A. Hazlett. 1974. Examination of the agonistic behaviour of the crayfish *Orconectes virilis* by character analysis.—Behaviour 50: 193–216.
- Rutherford, P. L., D. W. Dunham, and V. Allison. 1996. Antennule use and agonistic success in the crayfish *Orconectes rusticus*.—Crustaceana 69: 117–122.
- Schapker, H., T. Breithaupt, Z. Shuranova, Y. Burmistrov, and R. L. Cooper. 2002. Heart rate and ventilatory measures in crayfish during environmental disturbances and social interactions.—Comparative Biochemistry and Physiology A 131: 397–407.
- Scrivener, J. C. E. 1971. Agonistic behavior of the American lobster, *Homarus americanus*.—Fisheries Research Board of Canada, Technical Report 235: 1–113.
- Smith, M. R., and D. W. Dunham. 1990. Chela posture and vision: compensation for sensory deficit in the crayfish *Orconectes propinquus* (Girard) (Decapoda, Camaridae).—Crustaceana 59: 309–313.
- Smith, I. P., and A. C. Taylor. 1993. The energetic cost of agonistic behaviour in the velvet swimming crab, *Necorauber (L.)*.—Animal Behaviour 45: 375–391.
- Sneddon, L. U., A. C. Taylor, F. A. Huntingford, and D. G. Watson. 2000. Agonistic behavior and biogenic amines in shore crabs *Carcinus maenas*.—Journal of Experimental Biology 203: 537–545.
- Spohn, B. G., W. S. Neckameyer, B. Peretz, and R. L. Cooper. 1997. Characterization of aggressive & submissive behavior among male crayfish related to the endogenous levels of 5-HT & other neuromodulators. Abstract for Society for Neuroscience 313.7. [Unpublished.]
- Strawn, J. R., W. S. Neckameyer, and R. L. Cooper. 2000. The effects of 5-HT on sensory, central and motor neurons driving the abdominal superficial flexor muscles in the crayfish.—Comparative Biochemistry and Physiology 127B: 533–550.
- Zulandt-Schneider, R. A., R. W. S., Schneider, and P. A. Moore. 1999. Recognition of dominance status by chemoreception in the red swamp crayfish, *Procambarus clarkii*.—Journal of Chemical Ecology 25: 781–794.

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