The Journal of Experimental Biology 215, 1210-1217

doi:10.1242/jeb.066191

RESEARCH ARTICLE

Development of agonistic encounters in dominance hierarchy formation in juvenile crayfish

Daisuke Sato and Toshiki Nagayama*
Department of Biology, Faculty of Science, Yamagata University, Yamagata 990-8560, Japan
*Author for correspondence (nagayama@sci.kj.yamagata-u.ac.jp)

Accepted 30 November 2011

SUMMARY

We have characterized the behavioural patterns of crayfish during agonistic bouts between groups of crayfish of four different body lengths (9–19, 20–32, 41–48 and 69–75 mm) to characterize changes in the patterns of agonistic encounter during development. The behaviour of both dominant and subordinate animals was analysed by single frame measurement of video recordings. Behavioural acts that occurred during agonistic bouts were categorized as one of seven types: capture, fight, contact, approach, retreat, tailflip and neutral. Dominant–subordinate relationships were formed between juvenile crayfish as early as the third stage of development. Patterns of agonistic bouts to determine social hierarchy became more aggressive during development. The dominant–subordinate relationship was usually determined after contact in crayfish of less than 20 mm and 20–32 mm in length, while several bouts of fights were necessary for crayfish of 41–48 and 69–75 mm in length. Furthermore, social hierarchy was formed more rapidly in small crayfish. In larger animals, the number of approaches by dominant animals that promoted retreat in subordinate animals increased after the establishment of the winner–loser relationship. In smaller crayfish, in contrast, no measurable changes in these behaviour patterns were observed before and after the establishment of the winner–loser relationship. With increasing body size, the probability of tailflips decreased while that of retreats increased as the submissive behavioural act of subordinate animals.

Key words: crayfish, dominance hierarchy, agonistic behaviour, development, juvenile.

INTRODUCTION

Crayfish as well as other crustaceans form social dominance hierarchies (Bovbjerg, 1953; Bovbjerg, 1956; Lowe, 1956; Bruski and Dunham, 1987; Edwards et al., 2002; Fujimoto et al., 2011). Dominants have access to good shelter, food and a mating partner, while subordinates can avoid severe injury. Thus, hierarchy formation and its maintenance serve to maintain social stability and enable peaceful populations. When two previously unacquainted crayfish encounter each other, conflict occurs immediately and a dominant–subordinate relationship is determined by a number of agonistic bouts. Physical differences such as size and weight are the major determining factor for victory as the larger crayfish usually wins in agonistic encounters (Berrill and Arsenault, 1984; Ranta and Lindstrom, 1992; Garvey and Stein, 1993; Rutherfold et al., 1995; Pavey and Fielder, 1996; Seebacher and Wilson, 2006). Although fights between crayfish of markedly different size are often short and of low intensity, fights between crayfish of similar size are more aggressive (Pavey and Fielder, 1996). Juvenile crayfish also show intraspecific aggression as early as the second stage of development, just after the first moult (Mason, 1970), and a social hierarchy is formed between them (Issa et al., 1999). Dominant juveniles are significantly correlated with increased access to food (Herberholz et al., 2007).

Patterns of behaviour are well known to change during the growth of animals (Lang et al., 1977). For example, when one crayfish approaches from the rear and touches the tailfan of another crayfish, that crayfish shows an avoidance reaction in which either one of two alternative patterns of response occurs – a dart or a turn – depending upon the size of the crayfish (Nagayama et al., 1986). The patterns of aggressive and/or defensive behaviours also change during postembryonic development (McDonald and Topoff, 1986). In golden hamsters, *Mesocricetus auratus*, puberty is marked by drastic changes in social behaviour, as agonistic behaviour matures from play fighting to adult aggression (Goldman and Swanson, 1975). Play fighting is a juvenile form of agonistic behaviour preceding adult aggression. This behaviour is initiated before puberty and gradually matures into adult aggression in late puberty. Juveniles are a lot more active than adults during agonistic encounters and perform more attacks and bites than adults (Goldman and Swanson, 1975; Wommack et al., 2003). The majority of bites performed by adults are focused on the lower belly and rump, while attacks and bites performed by juveniles during play fighting are mainly focused on the cheeks and face (Pellis and Pellis, 1988; Wommack et al., 2003). As for mature crayfish, juvenile crayfish also show intraspecific aggression. However, it is still unclear whether the pattern of agonistic encounters changes during development. In this study, we characterized the behavioural patterns during agonistic bouts in crayfish of four different size groups (9–19, 20–32, 41–48 and 69–75 mm in length from rostrum to telson).

MATERIALS AND METHODS

Four groups of crayfish *Procambarus clarkii* (Girard) of different body length (9–19, 20–32, 41–48 and 69–75 mm, from rostrum to telson) were used in this study. Male crayfish over 41 mm body length were obtained commercially and kept individually in separate opaque containers of 19×33×15 cm (width×length×height) filled with water to a depth of 10 cm for at least 2 weeks. Male and female juvenile crayfish of 10–32 mm in length were hatched in our laboratory from commercially obtained females and were collected at the third stage of development (after the second moult) and isolated in small water-filled plastic cups of 8 cm in diameter that were covered in black paper to prevent visual stimuli. In each group, crayfish were fed equal amounts of small food pellets twice a week and were last fed at least 3 days before the experiments began.

Experimental trials were carried out in a dimly lit laboratory at a room temperature of ~22°C. Two crayfish of similar size (length difference <10%) were selected and paired in a new opaque container of 26×38×24 cm (width×length×height) for crayfish of 69–75 mm, 12×20×8.5 cm (width×length×height) for crayfish of 41–48 mm, and 9×15×7 cm (width×length×height) for crayfish of less than 32 mm, filled with water to about half-depth. Prior to each trial, an opaque plastic barrier was placed in the centre of the tank to separate it into two areas. One crayfish was placed on each side of this barrier and allowed to acclimate for at least 10 min before the divider was removed.

The agonistic bouts of the crayfish were recorded using a video camera (Victor GZ-MG330-S, Yokohama, Japan) mounted on a tripod above the container, for 30 min. The behaviour of each crayfish was analysed separately for four consecutive 5 min periods (first period 0–5 min; second period 5–10 min; third period 10–15 min; fourth period 15–20 min) using single frame measurement to make an ethogram of each second. Behavioural acts that occurred during agonistic bouts were categorized as one of seven types: capture, fight, contact, approach, retreat, tailflip and neutral. Capture, fight, contact and approach were aggressive behavioural acts, while retreat and tailflip were submissive behavioural acts. Capture was defined as the behavioural act in which one crayfish held an opponent using its chelae. Fight was defined as the act in which both crayfish fought using their chelae. Contact was defined as the act in which both crayfish made physical contact without fighting, while approach was defined as the act in which one crayfish moved forward towards an opponent. Retreat was defined as the act in which one crayfish walked away from an approaching or attacking opponent, while tailflip was defined as the act in which one crayfish escaped using rapid tailflipping from an approaching or attacking opponent. Neutral was defined as other behavioural acts including pause, walking with no correlation to an opponent and no response. The winner–loser relationship was determined when the subordinate crayfish showed a retreat or tailflip following the dominant's approach at least three times in succession.

Statistical analysis was carried out using Student's *t*-test if data were normally distributed, or using Mann–Whitney rank sum test if they were not (SigmaPlot v11).

RESULTS

Agonistic bouts in crayfish of <20 mm

To examine the behaviour of crayfish in agonistic encounters, an ethogram of the dominant and subordinate crayfish was drawn. Fig. 1 shows two examples of the first 10 min of the agonistic bouts between a pair of juvenile crayfish of <20 mm in length. After the divider was removed in Fig. 1A, crayfish A walked forward towards crayfish B. Crayfish B walked backwards to retreat immediately following contact with crayfish A. Crayfish A walked further forward towards crayfish B, causing crayfish B to use a tailflip to escape from crayfish A. Both crayfish then paused for about 200 s before crayfish A approached again. Crayfish B tailflipped immediately following the approach of crayfish A. We therefore determined that crayfish A was dominant and that crayfish B was subordinate, and this social hierarchy was formed around 120 s after pairing, in this example. Crayfish A thus initiated the approach and became dominant. In the second example shown in Fig. 1B, crayfish D, which approached first, was beaten in the following bouts by crayfish C. After pairing, crayfish D approached crayfish C. Crayfish C responded to contact by crayfish D with a tailflip to escape. Both crayfish then paused for about 20 s, before crayfish D approached again. Crayfish C tailflipped immediately following contact with crayfish D. About 1 min following pairing, crayfish C approached crayfish D, causing crayfish D to retreat. Subsequently, only crayfish C made approaches, and when it did so, crayfish D responded with either a retreat or a tailflip. In this case, we determined that crayfish C was dominant and crayfish D was

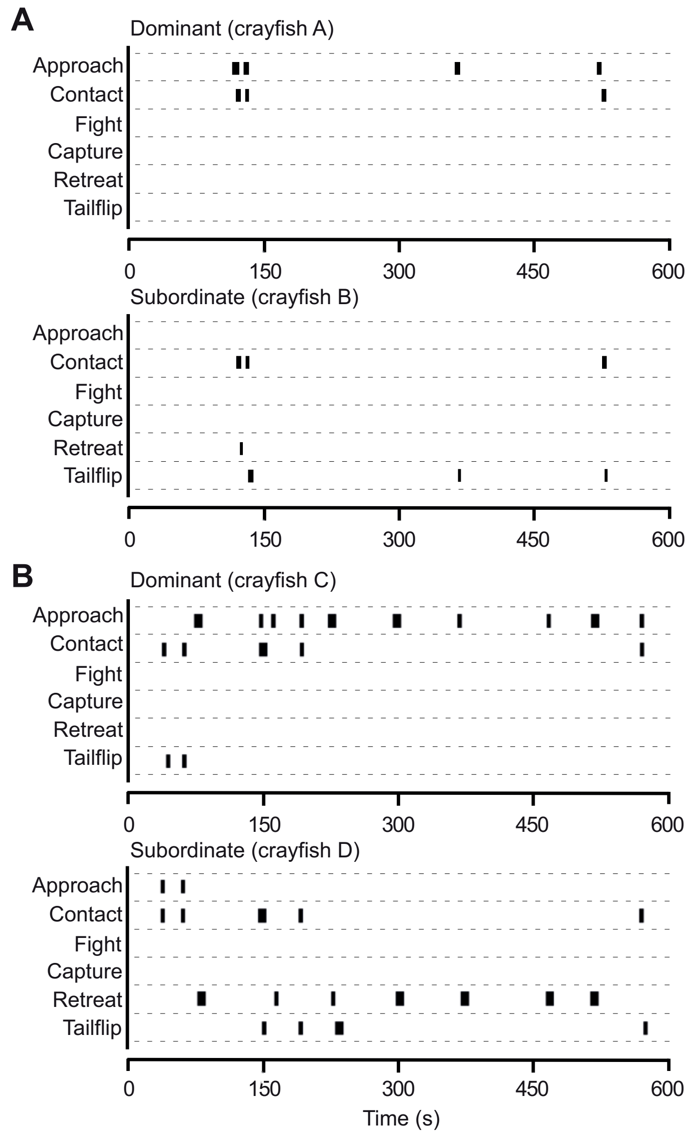

Fig. 1. Two examples of an ethogram of the agonistic bouts between a pair of juvenile crayfish <20 mm in body length. (A) The crayfish that initiated the approach became dominant. (B) The crayfish that initiated the approach was beaten in the following bouts by its opponent. The behaviour of both dominant and subordinate animals was analysed from single frame measurement of video recordings for each second. Behavioural acts (approach, contact, fight, capture, retreat and tailflip) are plotted for the first 10 min of the agonistic bouts.

subordinate, and this social hierarchy was formed around 145 s after pairing.

In agonistic bouts between 10 pairs of animals of <20 mm, the dominant–subordinate relationship was formed within 37–535 s (198.3±50.5 s, mean ± s.e.m.). The mean (±s.e.m.) length of dominant animals was 14.4±0.93 mm while that of subordinates was 13.9±0.92 mm (mean ± s.e.m.). The larger crayfish won in 6 pairings, but the smaller crayfish won in 3 out of 9 pairings (in one pairing, animals were of similar length). The crayfish that initiated the approach became dominants in 8 out of 10 pairings (Fig. 1A, Fig. 2A). In the remaining 2 pairings, as shown in Fig. 1B, the crayfish that initiated the approach was beaten in the following bouts by their opponents. The aggressiveness of agonistic bouts did not escalate (Fig. 2B). The fight response in which both crayfish tried to grasp each other using their chelae was observed in only 3 out of 10 pairings during the first 5 min period of the agonistic bouts (Fig. 2B, top). Both crayfish fought once (N=2) or twice (N=1) with a very short duration of 2–17 s (Fig. 2B, bottom). Subordinates responded with a retreat or tailflip when the dominant animals approached, and escaped with a tailflip when they were touched by the approaching dominants (Fig. 2C).

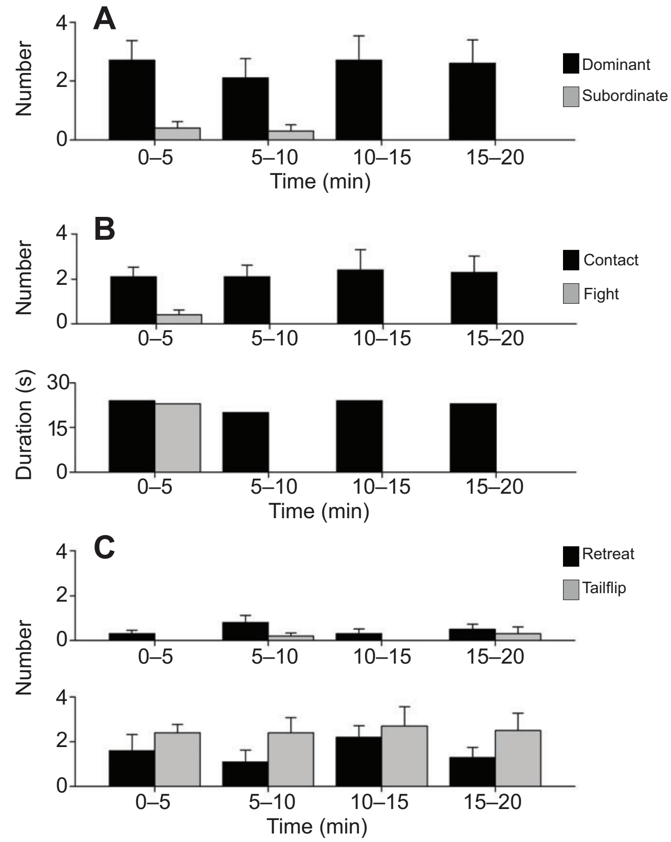

Fig. 2. Agonistic bouts of crayfish <20 mm in body length. (A) The number of approaches of dominant and subordinate animals during four consecutive 5 min periods. (B) The number (upper graph) and duration (lower graph) of contacts and fights in pairings during four consecutive 5 min periods. (C) The number of retreats and tailflips of dominant (upper graph) and subordinate (lower graph) animals during four consecutive 5 min periods. Bars represent means + s.e.m. from 10 pairs of dominant and subordinate animals.

Agonistic bouts of 20–32 mm crayfish

In agonistic bouts between 10 pairs of 20–32 mm crayfish, the dominant–subordinate relationship was formed within 39–698 s (234.4±66.9 s, mean ± s.e.m.) of pairing. The mean (±s.e.m.) length of dominant animals was 27.3±0.9 mm (mean ± s.e.m.), while that of subordinates was 26.9±1.0 mm (mean ± s.e.m.). The larger crayfish won in 8 out of 10 pairings, which was statistically significant (P<0.05, Student's t-test). There was no advantage for the crayfish that initiated the approach in terms of becoming dominant (Fig. 3A), as the crayfish that approached first won the agonistic bouts in only 6 out of 10 pairings.

After the divider was removed, either of the crayfish walked forward towards an opponent and made contact. These agonistic encounters escalated to a fight of 13.3±8.9 s (mean ± s.e.m.) duration in 6 out of 10 pairings in the first 5 min period (Fig. 3B). The number of approaches by dominant crayfish was relatively constant throughout the four 5 min periods of agonistic bouts, but that by subordinates decreased gradually in successive periods (Fig. 3A). When the subordinates approached the dominants, both crayfish fought after contact and the dominants beat the subordinates. In the fourth 5 min period, subordinates rarely approached the dominant animals (Fig. 3A), resulting in almost no opportunity to fight (Fig. 3B). Subordinates avoided the approach of dominants with either a retreat or a tailflip (Fig. 3C).

Agonistic bouts of 41–48 mm crayfish

In agonistic bouts between 11 pairs of 41–48 mm crayfish, the dominant–subordinate relationship was formed within 134–673 s (381.5±51.6 s, mean ± s.e.m.) of pairing. The mean (±s.e.m.) length of dominant animals was 45.3±0.68 mm, while that of the subordinates was 44.8±0.57 mm (mean ± s.e.m.). The larger crayfish won in 7 out of 10 pairings (in one pairing the animals were of similar length). The crayfish that initiated the approach became dominant in 7 out of 11 pairings. Statistically, there was no advantage to this in terms of winning.

Fig. 4 shows an example of agonistic bouts between a pair of crayfish of 41–48 mm in length. Ethograms of the dominant and subordinate crayfish were drawn over a period of 20 min. The first encounter between crayfish E and F started around 200 s after the divider was removed from the tank. Crayfish E walked forward towards crayfish F and the agonistic bout escalated from contact to a fight. Eventually, crayfish F escaped from crayfish E using a tailflip. After a pause of 200 s, crayfish E approached and made contact with crayfish F, and crayfish F immediately retreated. Crayfish E then approached crayfish F again and fought for 18 s, resulting in crayfish F performing a tailflip. Subsequently, as crayfish E repeatedly approached crayfish F, this resulted in retreat by crayfish F.

The number of approaches of both the dominants and subordinates was similar in the first 5 min period as the dominant–subordinate relationship was not determined (Fig. 5A). The number of contacts and fights increased in the second 5 min period (Fig. 5B). The aggressiveness of agonistic bouts escalated and the mean (±s.e.m.) duration of the fight in the this period was 90.6±21.3 s. After the hierarchy was established, the number and duration of fights decreased in the third and fourth periods (Fig. 5B). In addition, in subordinates, the number of approaches considerably decreased (Fig. 5A) while the number of retreats and tailflips increased (Fig. 5C) from the second period of the bout.

Agonistic bouts of 69–75 mm crayfish

In agonistic bouts between 10 pairs of 69–75 mm crayfish, the dominant–subordinate relationship was formed rather variably from 41 to 1488 s (829.6±166.8 s, mean ± s.e.m.) after pairing. The mean (±s.e.m.) length of dominant animals was 73.2±0.53 mm, while that

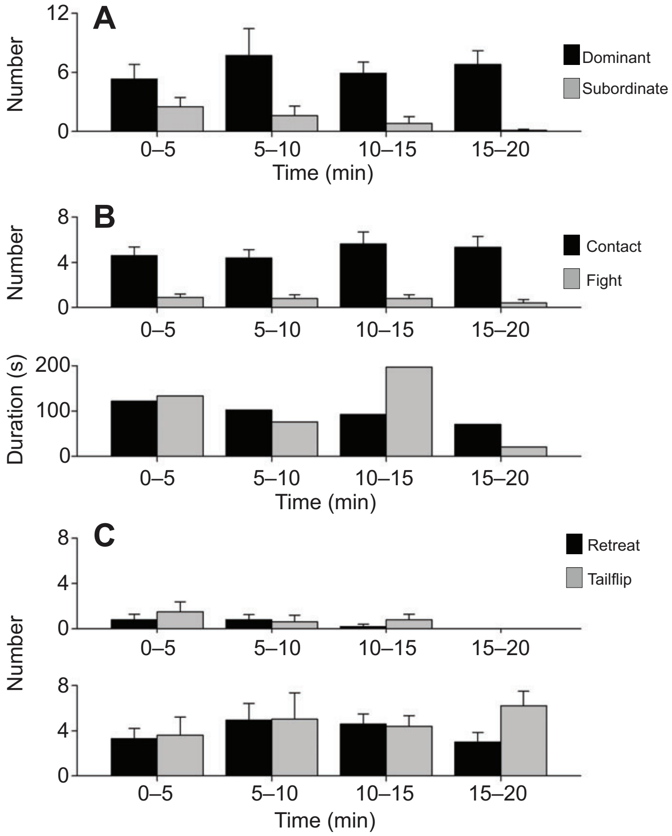

Fig. 3. Agonistic bouts between crayfish of 20–32 mm in length. (A) The number of approaches of dominant and subordinate animals during four consecutive 5 min periods. (B) The number (upper graph) and duration (lower graph) of contacts and fights in pairings during four consecutive 5 min periods. (C) The number of retreats and tailflips of dominant (upper graph) and subordinate (lower graph) animals during four consecutive 5 min periods. Bars represent means + s.e.m. from 10 pairs of dominant and subordinate animals.

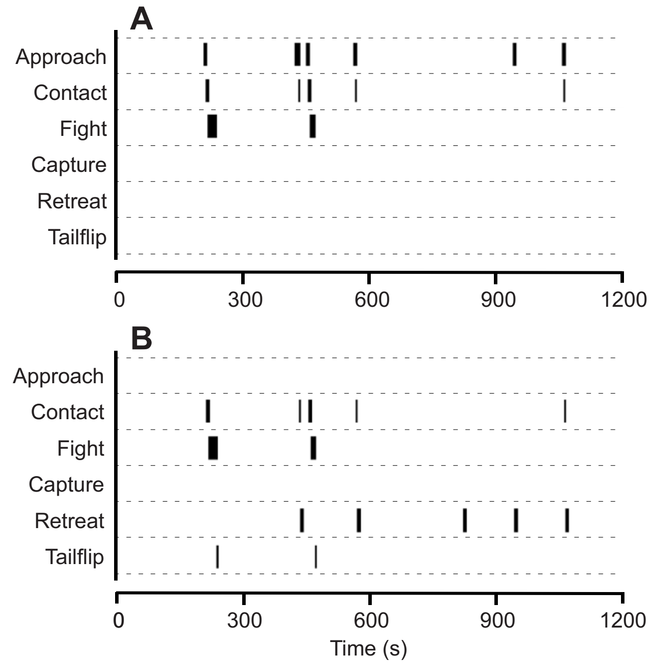

Fig. 4. An ethogram of the agonistic bouts between a pair of crayfish of 41–48 mm in body length. Behaviour of both dominant (crayfish E, A) and subordinate (crayfish F, B) animals was analysed from single frame measurement of video recordings for each second. Behavioural acts (approach, contact, fight, capture, retreat and tailflip) are plotted for 20 min of the agonistic bouts.

of subordinates was 70.8±0.57 mm (mean ± s.e.m.). The larger crayfish won in all 9 pairings (in one pairing the crayfish were of similar length). The crayfish that initiated the approach won the agonistic bouts in 5 out of 10 pairings, but there was statistically no advantage in terms of winning.

In the first 5 min of the pairing, both crayfish approached, resulting in a fight (Fig. 6A,B). The agonistic bouts escalated in the second and third 5 min periods and the number of approaches, contacts and fights increased. The duration of a fight also increased from 49.2±17.7 s (mean ± s.e.m.) in the first 5 min period to 75.3±25.9 s (mean ± s.e.m.) in the second. Furthermore, capture by the dominant crayfish using chelae was observed in 1 out of 10 pairings in the first 5 min, 5 pairings in the second 5 min and 2 pairings in the third and fourth 5 min periods. In the second period, the mean (±s.e.m.) duration of capture was 95.5±65.9 s, which was longer than the mean capture duration for the other periods (26.9 s in the first, 6.8±.4 s in the third and 7.1±5.0 s in the fourth 5 min period). After the dominant–subordinate relationship was determined, the number of approaches by the subordinates decreased (Fig. 6A), while retreats and tailflips of the subordinates increased (Fig. 6C), corresponding to the increase in the number of approaches by the dominant animals (Fig. 6A). In the fourth period, the approach of the dominants usually elicited retreat by the subordinates before contact occurred (Fig. 6C).

Comparison of agonistic bouts in each group

Fig. 7 shows the length of time in which the dominant–subordinate relationship was determined. The median of each group was 123.5 s in crayfish <20 mm, 161.5 s in 20–32 mm crayfish, 369.0 s in 41–48 mm crayfish and 838.5 s in 69–75 mm crayfish. When crayfish were smaller in length, the time to form a hierarchy was shorter. The dominant–subordinate relationship of crayfish <20 and 20–32 mm in length was formed significantly faster than that of crayfish of 41–48 and 69–75 mm in length ($P<0.05$; Mann–Whitney rank sum test). Although crayfish of 41–48 mm in length formed a hierarchy apparently more rapidly than crayfish of 69–75 mm in length, there was no difference statistically ($P=0.073$; Mann–Whitney rank sum test).

The agonistic encounters escalated as the crayfish became larger (Fig. 8). The total number of approaches during agonistic encounters of 20 min in crayfish <20 mm in length was 10.1±1.9 (mean ± s.e.m.) and the number of contacts was 8.9±1.9 (Fig. 8A). The number of approaches of crayfish of 20–32 mm in length increased to 25.7±5.7 and the number of contacts increased to 19.9±2.6 (Fig. 8A). In both sizes of crayfish, approach was usually followed by contact. In crayfish of 41–48 mm in length, the number of approaches was 8.4±1.5 while the number of contacts was 2.9±0.9 (Fig. 8A). In crayfish of 69–75 mm in length, the number of approaches increased to 22.2±4.7 while the number of contacts was 4.5±1.0 (Fig. 8A). The occurrence probability between approach and contact was statistically different in crayfish of 41–50 mm and of 69–75 mm in body length ($P<0.05$; Mann–Whitney rank sum test). The number of fights also increased from 0.4±0.2 in crayfish <20 mm in length to 2.9±0.5 in crayfish of 20–32 mm, 4.4±0.9 in crayfish of 41–50 mm, and 5.1±0.9 in crayfish of 69–75 mm in length (Fig. 8B). The occurrence probability of a fight in crayfish of both 41–50 mm and 69–75 mm was significantly higher

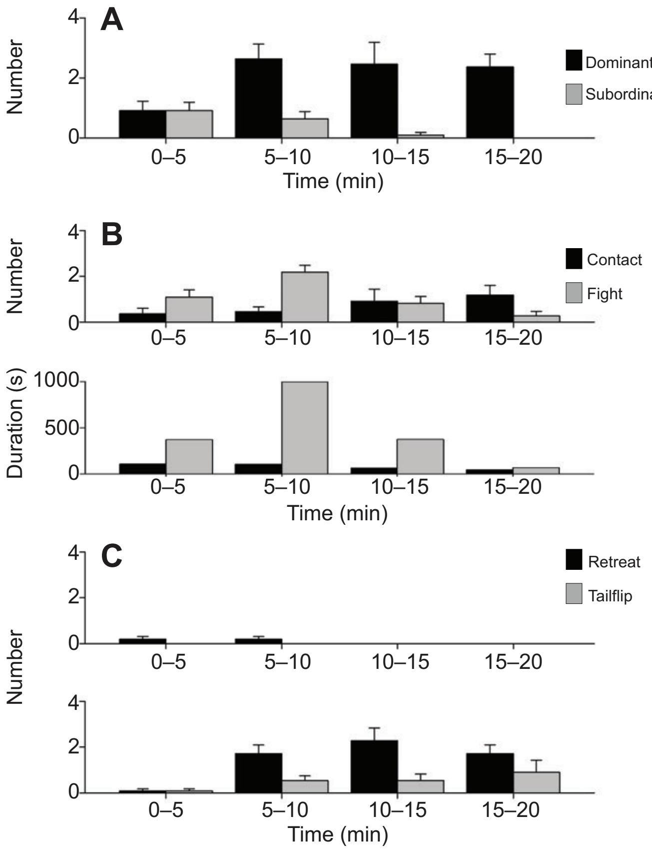

Fig. 5. Agonistic bouts of crayfish of 41–48 mm in body length. (A) The number of approaches of dominant and subordinate animals during four consecutive 5 min periods. (B) The number (upper graph) and duration (lower graph) of contacts and fights in pairings during four consecutive 5 min periods. (C) The number of retreats and tailflips of dominant (upper graph) and subordinate (lower graph) animals during four consecutive 5 min periods. Bars represent means + s.e.m. from 11 pairs of dominant and subordinate animals.

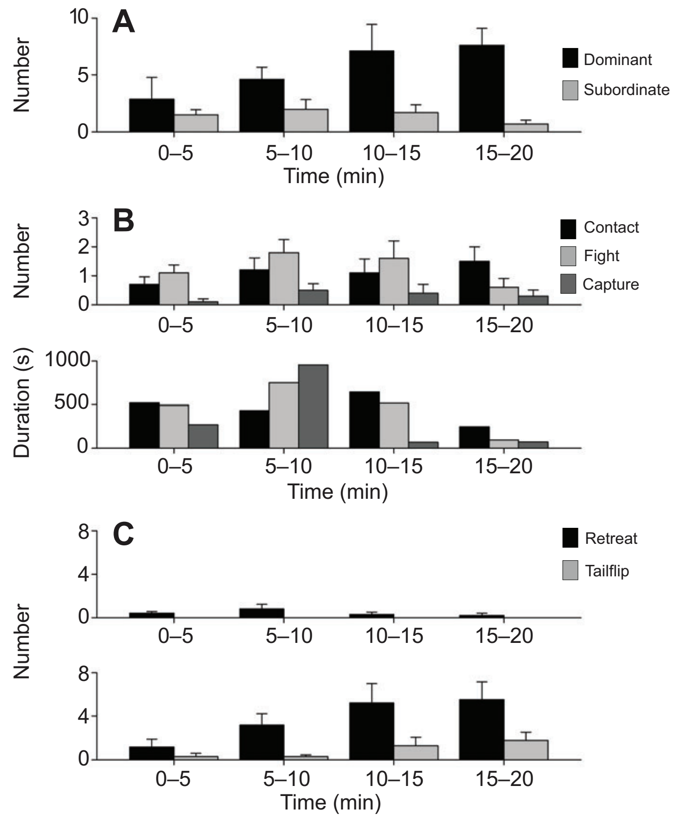

Fig. 6. Agonistic bouts of crayfish of 69–75 mm in body length. (A) The number of approaches of dominant and subordinate animals during four consecutive 5 min periods. (B) The number (upper graph) and duration (lower graph) of contacts and fights in pairings during four consecutive 5 min periods. (C) The number of retreats and tailflips of dominant (upper graph) and subordinate (lower graph) animals during four consecutive 5 min periods. Bars represent means + s.e.m. from 10 pairs of dominant and subordinate animals.

than that of crayfish <20 and 20–32 mm in length (P<0.001 compared with crayfish <20 mm and P<0.05 compared with crayfish of 20–32 mm in length; Mann–Whitney rank sum test). Crayfish of 20–32 mm in length also showed a fight response more frequently than crayfish <20 mm in length (P<0.005; Mann–Whitney rank sum test). Furthermore, the duration of individual fights also increased as animals became larger in size (Fig. 8C). In crayfish <20 mm in length, the mean duration was 5.8±3.75 s. The mean duration of a fight in crayfish of 20–32 mm in length was 14.7±4.07 s, while that in crayfish of 41–50 mm was 37.6±4.32 s and 36.3±4.80 s in crayfish of 69–75 mm in length. The fight duration of crayfish of 41–50 and 69–75 mm in length was significantly longer that of crayfish <20 and 20–32 mm in length (P<0.01; Mann–Whitney rank sum test). The winner–loser relationship was readily determined after contact in crayfish of <20 and 20–32 mm in length. In crayfish of <20 mm in length, contact constituted 95.7% of the aggressive acts. In crayfish of 20–32 mm in length, fight was observed only in 12.8% of aggressive acts. In contrast, the occurrence of fights increased to 60.0% in crayfish of 41–48 mm in length and 46.8% in crayfish of 69–75 mm in length (Fig. 8D). Furthermore, a capture response (12.0%) was observed in crayfish of 69–75 mm in length.

The occurrence probability of submissive behavioural acts (retreat and tailflip) of the subordinates changed according to the size of the crayfish (Fig. 9). In crayfish of <20 and 20–32 mm in length, the tailflip occurred more frequently (61.7% in crayfish <20 mm and 52.1% in crayfish of 20–32 mm). As crayfish became larger, the occurrence probability of a tailflip decreased and that of a retreat occurred more frequently (73.6% in crayfish of 41–48 mm and 78.4% in crayfish of 69–75 mm in length).

DISCUSSION

In this study, we have demonstrated that juvenile crayfish show intraspecific aggression as shown previously (Bovbjerg, 1956; Mason, 1970) and that the dominant–subordinate relationship is formed between juvenile crayfish as early as the third stage of development (Issa et al., 1999). Patterns of agonistic bouts to determine social hierarchy showed an increase in the level of aggression from contacts to fights and capture during growth of the crayfish. Furthermore, submissive acts performed by subordinate animals changed from tailflip to retreat during development.

Development of agonistic bouts during growth of crayfish

The relative size of animals has frequently been identified as the major determinant of dominance order in crustaceans, including crayfish (Bovbjerg, 1956; Rubenstein and Hazlett, 1974; Berrill and Arsenault, 1984; Ranta and Lindstrom, 1992; Pavey and

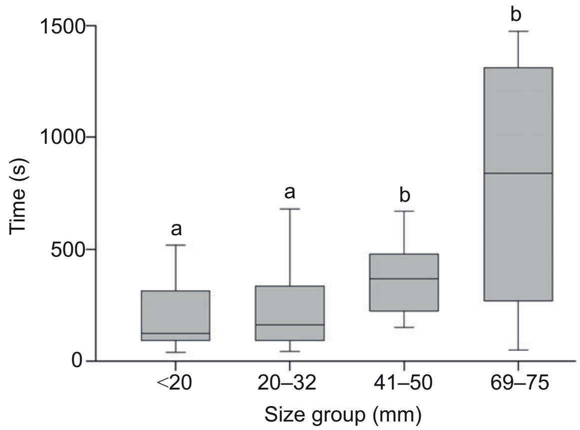

Fig. 7. The time during which the dominant–subordinate relationship was determined. Box plots show median (solid black line), interquartile range (box length), and minimum and maximum values (error bars) for the 10 pairs of crayfish of <20, 20–32 and 69–75 mm in length, and for the 11 pairs of the crayfish of 41–48 mm in length. Box plots with different letters are significantly different.

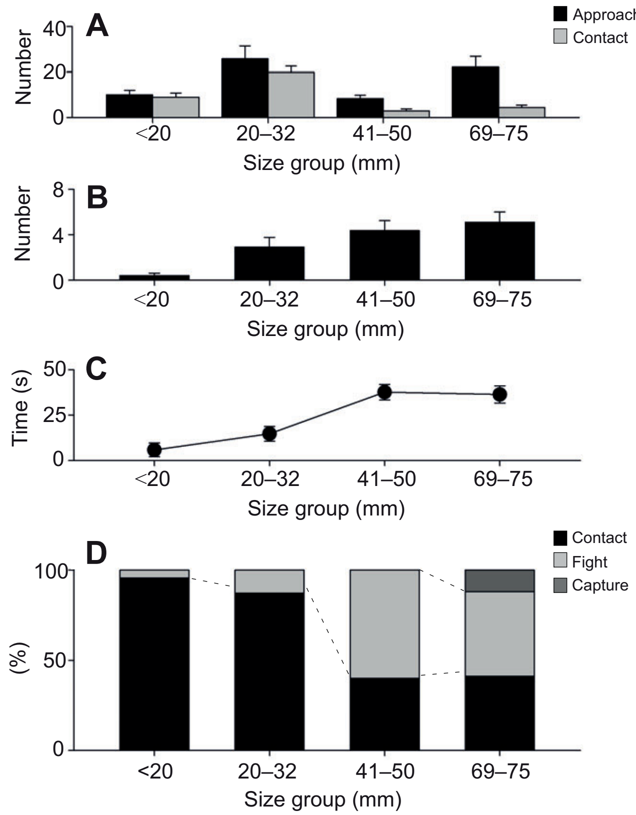

Fig. 8. Comparison of aggressive behavioural acts in each of the four size groups. (A) The number of approaches and contacts of dominant animals during 20 min agonistic bouts. (B) The number of fights during 20 min agonistic bouts. (C) The mean duration of individual fights in pairings during 20 min agonistic bouts. (D) The probability of occurrence of contact, fight and capture during 20 min agonistic bouts. Behavioural acts that showed approach alone were omitted in this figure. Bars represent means + s.e.m. from 10 pairs of crayfish of <20, 20–32 and 69–75 mm in length, and for 11 pairs of crayfish of 41–48 mm in length.

Fielder, 1996; Issa et al., 1999; Seebacher and Wilson, 2006; Herberholz et al., 2007). In our study, larger crayfish showed the same pattern, and won every pairing in the 69–75 mm body length group. In crayfish <20 mm, however, larger crayfish won only in 6 out of 9 pairings. Rubenstein and Hazlett have indicated that initiating the approach has advantages in terms of winning agonistic bouts when two animals are of a similar size (Rubenstein and Hazlett, 1974). This was also the case in our study, in which the crayfish making the first approach in the <20 mm body length group became dominant in 8 out of 10 pairings. However, there was no significant advantage for crayfish in the 20–32, 41–48 and 69–75 mm body length groups. The probability of winning for the crayfish making the first approach was 60%, 63.6% and 50%, respectively.

The first notable difference in agonistic bouts between smaller and larger juvenile crayfish was an escalation of aggressiveness during agonistic bouts in larger animals. The dominant–subordinate relationship was usually determined after contact in crayfish <20 and 20–32 mm in length, while several bouts of fighting were necessary for crayfish of 41–48 and 69–75 mm. In comparison with smaller crayfish, the number and duration of individual fights of larger animals increased significantly. Furthermore, the time in which the winner–loser relationship was determined was again different between smaller (crayfish <20 and 20–32 mm in length) and larger crayfish (41–48 and 69–75 mm). Social hierarchy was formed more rapidly in smaller animals. In larger crayfish, dominance hierarchy was developed through a series of fights. One crayfish would retreat from the fight and be deemed the loser of the bout. After a series of losses, we decided this crayfish was subordinate. Smaller juvenile crayfish, however, did not fight but could still be deemed either dominant or subordinate. Thus, it remains to be determined how smaller crayfish estimate the strength of their opponents without a series of fights and thus how they form dominant and subordinate status.

The second significant difference in agonistic bouts between smaller and larger animals was the performance of animals after determination of social hierarchy. In larger animals of 41–48 and 69–75 mm in body length, the number of contacts decreased. The number of approaches in dominant animals that promoted retreat by subordinate animals also increased after the establishment of the winner–loser relationship. In contrast, the number of contacts in smaller animals of <20 and 20–32 mm in length was fairly consistent in all four 5 min periods of the agonistic bouts. The number of approaches of dominant animals did not change significantly before and after the establishment of the winner–loser relationship, while that of subordinate animals decreased, especially in crayfish of 20–32 mm in length. As winning crayfish are more likely to win and losers more likely to lose subsequent conflicts (Daws et al., 2002; Bergman et al., 2003; Seebacher and Wilson, 2007; Zulandt et al., 2008; Graham and Herberholz, 2009), it is likely that some neural modulation underlying social hierarchy would occur in both dominant and subordinate crayfish.

Dominance hierarchies are formed in various species of arthropods including crickets (Alexander, 1961), spiders (Aspey, 1977), cockroaches (Ewing, 1974), lobsters (Fiedler, 1965), hermit crabs (Hazlett, 1968) and crayfish (Bovbjerg, 1953). Some species of vertebrates including mice (Long, 1972; Bronson and Marsden, 1973), golden hamsters (Goldman and Swanson, 1975), rhesus monkeys (Bernstein and Gordon, 1974) and birds (Gottier, 1968) also form dominance hierarchies. However, it is still unclear whether the pattern of agonistic encounters changes during development. Only in male golden hamsters has agonistic behaviour

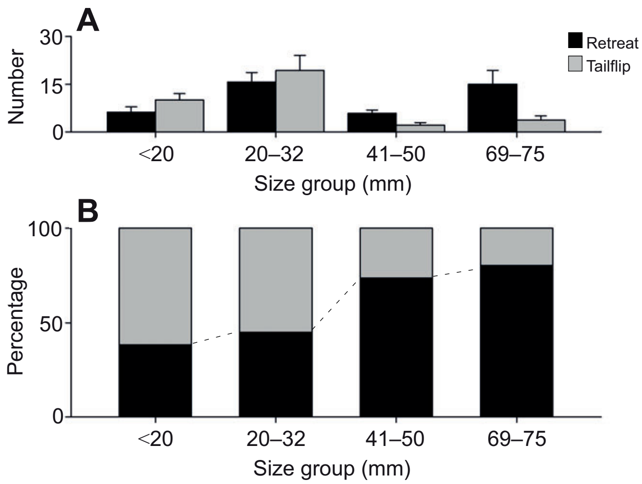

Fig. 9. Comparison of submissive behavioural acts in each of the four size groups. (A) The number of retreats and tailflips of subordinate animals for 20 min agonistic bouts. Bars represent means + s.e.m. from the 10 pairs of crayfish of <20, 20–32 and 69–75 mm in length, and for the 11 pairs of crayfish of 41–48 mm in length. (B) The probability of occurrence of retreat and tailflip during 20 min agonistic bouts.

been characterized as maturing during puberty, changing from play fighting to adult aggression (Goldman and Swanson, 1975). Juvenile play fighting is characterized by more repetitive attacks and contact bouts during agonistic interactions than that in adults (Goldman and Swanson, 1975; Wommack et al., 2003). In addition, hamsters target different parts on the body of opponents during play fighting and adult aggression. In juveniles, the attacks are mainly focused on the cheeks and face of the opponents. The majority of bites performed by adults are focused on the lower belly and rump. This transition in the focus of attacks occurs around mid-puberty as testicles start growing and serum testosterone levels start rising (Wommack et al., 2003). In this study, we found that more fighting occurred as juvenile crayfish became larger. This would be the case in hamsters as play fighting is initiated in early puberty (Goldman and Swanson, 1975). However, in this study we have not characterized agonistic encounters between mature adult crayfish, so further studies are needed to clarify the maturation of agonistic behaviour during sexual maturity of crayfish. As a vast number of behavioural studies have been carried out in crayfish (Fujimoto et al., 2011) and the neural circuits underlying various movements have been characterized in detail (Nagayama et al., 1994; Newland et al., 2000), crayfish would represent an ideal model to examine dominance hierarchy formation.

Changes in the pattern of submissive behavioural acts

In smaller juvenile crayfish, especially those <20 mm, tailflips occurred more frequently. As animals became larger, the occurrence probability of tailflips decreased and retreats occurred more frequently. In larger animals, we frequently observed that subordinate animals showed first a retreat and then an escape with tailflipping. This contrast in the repertoire of submissive behavioural acts between smaller and larger crayfish could be due to a different level of activation of the neural circuitry underlying tailflip. Two distinct types of tailflip, medial giant-mediated tailflips (MG-flip) and lateral giant-mediated tailflips (LG-flip), have been well characterized (Wine, 1984). The neural circuitry underlying LG-flips has also been analysed in detail (Wine and Krasne, 1972). The LGs are activated by parallel direct and indirect pathways from sensory afferents innervating hairs on the tailfan (Zucker, 1972; Newland et al., 1997; Araki and Nagayama, 2003; Araki et al., 2005). The LGs receive excitatory postsynaptic potentials (EPSPs) directly *via* electrical (α component) and chemical synapses (α' component) from mechanosensory afferents, and indirectly through sensory interneurones (β component). The amplitude of the EPSP in the α component in adult crayfish is insufficient to elicit LG spikes, resulting in the requirement for a higher intensity of stimulus to trigger an LG-flip, while in smaller crayfish the LG is excited by the α component alone and thus readily elicits a spike (Edwards et al., 1994a). During the growth of crayfish, the stimulus threshold of the α component is increased by changes in the synaptic integration of LGs (Edwards et al., 1994b). Thus, tailflips would be more readily elicited in smaller crayfish by approaches from or contacts with opponents.

ACKNOWLEDGEMENTS

We thank A. Kanai and Y. Momohara for technical assistance.

FUNDING

This work was supported by Grants in-Aid from the Ministry of Education, Science, Sport and Culture to T.N.

REFERENCES

Alexander, R. D. (1961). Aggressiveness, territoriality, and sexual behavior in field crickets (Orthoptera: Gryllidae). *Behaviour* **17**, 130-223.
Araki, M. and Nagayama, T. (2003). Direct chemically mediated synaptic transmission from mechanosensory afferents contributes to habituation of crayfish lateral giant escape reaction. *J. Comp. Physiol. A* **189**, 731-739.
Araki, M., Nagayama, T. and Sprayberry, J. (2005). Cyclic AMP mediates serotonin-induced synaptic enhancement of lateral giant interneuron of the crayfish. *J. Neurophysiol.* **94**, 2644-2652.
Aspey, W. P. (1977). Wolf spider sociobiology: I. Agonistic display and dominance–subordinance relations in adult male *Schizocosa crassipes*. *Behaviour* **62**, 103-141.
Bergman, D. A., Kozlowski, C. P., McIntyre, J. C., Huber, R., Daws, A. G. and Moore, P. A. (2003). Temporal dynamics and communication of winner-effects in the crayfish, *Orconectes rusticus*. *Behaviour* **140**, 805-825.
Bernstein, I. S. and Gordon T. P. (1974). The function of aggression in primate societies. *Am. Sci.* **62**, 304-311.
Berrill, M. and Arsenault, M. R. (1984). The breeding behaviour of a northern temperate orconectid crayfish *Orconectes rusticus*. *Anim. Behav.* **32**, 333-339.
Bovbjerg, R. V. (1953). Dominance order in the crayfish, *Orconectes virilis* (Hagan). *Physiol. Zool.* **26**, 173-178.
Bovbjerg, R. V. (1956). Some factors affecting aggressive behavior in crayfish. *Physiol. Zool.* **29**, 127-136.
Bronson, F. H. and Marsden, H. M. (1973). The preputial gland as an indicator of social dominance in male mice. *Behav. Biol.* **9**, 625-628.
Bruski, C. A. and Dunham, D. W. (1987). The importance of vision in agonistic communication of the crayfish *Orconectes rusticus*. I: an analysis of bout dynamics. *Behaviour* **103**, 83-107.
Daws, A. G., Grills, J., Konzen, K. and Moore, P. A. (2002). Previous experiences alter the outcome of aggressive interactions between males in the crayfish, *Procambarus clarkii*. *Mar. Fresh. Behav. Physiol.* **35**, 139-148.
Edwards, D. H., Fricke, R. A., Barnett, L. D., Yeh, S.-R. and Leise, E. M. (1994a). The onset of response habituation during the growth of the lateral giant neuron of crayfish. *J. Neurophysiol.* **72**, 890-898.
Edwards, D. H., Yeh, S.-R., Barnett, L. D. and Nagappan, P. R. (1994b). Changes in synaptic integration during the growth of the lateral giant neuron of crayfish. *J. Neurophysiol.* **72**, 899-908.
Edwards, D. H., Yeh, S.-R., Musolf, B. E., Antonsen, B. L. and Krasne, F. B. (2002). Metamodulation of the crayfish escape circuit. *Brain Behav. Evol.* **60**, 360-369.
Ewing, L. S. (1974). Hierarchy and its relation to territory in the cockroach (*Naupheta cineria*). *Behaviour* **32**, 152-174.
Fiedler, D. R. (1965). A dominance order for shelter in the spiny lobster *Jasus lalandei*. *Behaviour* **24**, 236-245.
Fujimoto, S., Hirata, B. and Nagayama, T. (2011). Dominance hierarchy-dependent behavioural plasticity of crayfish avoidance reactions. *J. Exp. Biol.* **214**, 2718-2723.
Garvey, J. and Stein, R. A. (1993). Evaluation how chela size influences the invasion potential of an introduced crayfish (*Orconectes rusticus*). *Am. Midl. Nat.* **129**, 172-181.
Goldman, L. and Swanson, H. H. (1975). Developmental changes in pre-adult behavior in confined colonies of golden hamsters. *Dev. Psychobiol.* **8**, 137-150.
Gottier, R. F. (1968). The dominance-submission hierarchy in the social behavior of the domestic chicken. *J. Genet. Psychol.* **112**, 205-226.
Graham, M. E. and Herberholz, J. (2009). Stability of dominance relationship in crayfish depends on social context. *Anim. Behav.* **77**, 195-199.
Hazlett, B. A. (1968). Effects of crowding on the agonistic behavior of the hermit crab *Pagurus bernhardus*. *Ecology* **49**, 573-575.

Herberholz, J., McCurdy, C. and Edwards, D. H. (2007). Direct benefits of social dominance in juvenile crayfish. *Biol. Bull.* **213**, 21-27.
Issa, F. A., Adamson, D. J. and Edwards, D. H. (1999). Dominance hierarchy formation in juvenile crayfish *Procambarus clarkii*. *J. Exp. Biol.* **202**, 3497-3506.
Lang, F., Govind, C. K., Costello, W. J. and Greene, S. I. (1977). Developmental neuroethology: changes in escape and defensive behavior during growth of the lobster. *Science* **197**, 682-685.
Long, S. Y. (1972). Hair-nibbling and whisker-trimming as indicators of social hierarchy in mice. *Anim. Behav.* **20**, 10-12.
Lowe, M. E. (1956). Dominance–subordinance relationships in the crayfish *Cambarellus shufeldtii*. *Tulane Stud. Zool.* **4**, 139-170.
Mason, J. C. (1970). Maternal–offspring behavior of the crayfish *Pacifastacus trowbridgii* (Stimpson). *Am. Midl. Nat.* **84**, 463-473.
McDonald, P. and Topoff, H. (1986). The development of defensive behavior against predation by army ants. *Dev. Psychobiol.* **19**, 351-367.
Nagayama, T., Takahata, M. and Hisada, M. (1986). Behavioural transition of crayfish avoidance reaction in response to uropod stimulation. *Exp. Biol.* **46**, 75-82.
Nagayama, T., Namba, H. and Aonuma, H. (1994). Morphological and physiological bases of crayfish local circuit neurones. *Histol. Histopath.* **9**, 791-805.
Newland, P. L., Aonuma, H. and Nagayama, T. (1997). Monosynaptic excitation of lateral giant fibers by proprioceptive afferents in the crayfish. *J. Comp. Physiol. A* **181**, 103-109.
Newland, P. L., Aonuma, H. and Nagayama, T. (2000). The role of proprioceptive signals in the crayfish escape circuit. *Zool. Sci.* **17**, 1185-1195.
Pavey, C. R. and Fielder, D. R. (1996). The influence of size differential on agonistic behaviour in the freshwater crayfish, *Cherax cuspidatus* (Decapoda: Parastacidae). *J. Zool. Lond.* **238**, 445-457.
Pellis, S. M. and Pellis, V. C. (1988). Play-fighting in the Syrian golden hamster *Mesocricetus auratus* Waterhouse, and its relationship to serious fighting during postweaning development. *Dev. Psychobiol.* **21**, 323-337.
Ranta, E. and Lindstrom, K. (1992). Power to hold sheltering burrows by juveniles of the signal crayfish, *Pacifastacus leniusculus*. *Ethology* **92**, 217-226.
Rubenstein, D. I. and Hazlett, B. A. (1974). Examination of the agonistic behavior of the crayfish *Orconectes virilis* by character analysis. *Behaviour* **50**, 193-216.
Rutherfold, P. L., Dunham, D. W. and Allison, V. (1995). Winning agonistic encounters by male crayfish *Orconectes rusticus* (Girard) (Decapoda, Cambaridae), chela size matters but chela symmetry does not. *Crustaceana* **68**, 526-529.
Seebacher, F. and Wilson, R. S. (2006). Fighting fit: thermal plasticity of metabolic function and fighting success in the crayfish *Cherax destructor*. *Funct. Ecol.* **20**, 1045-1053.
Seebacher, F. and Wilson, R. S. (2007). Individual recognition in crayfish (*Cherax disper*): the role of strength and experience in deciding aggressive encounters. *Biol. Lett.* **3**, 471-474.
Wine, J. J. (1984). The structural basis of an innate behavioral pattern. *J. Exp. Biol.* **112**, 283-319.
Wine, J. J. and Krasne, F. B. (1972). The organization of escape behaviour in the crayfish. *J. Exp. Biol.* **56**, 1-18.
Wommack, J. C., Taravosh-Lahn, K., David, J. T. and Delville, Y. (2003). Repeated exposure to social stress alters the development of agonistic behavior in male golden hamsters. *Horm. Behav.* **43**, 229-236.
Zucker, R. S. (1972). Crayfish escape behavior and central synapses. II. Physiological mechanisms underlying behavioral habituation. *J. Neurophysiol.* **35**, 621-637.
Zulandt, T., Zulandt-Schneider, R. A. and Moore, P. A. (2008). Observing agonistic interactions alters subsequent fighting dynamics in the crayfish, *Orconectes rusticus*. *Anim. Behav.* **75**, 13-20.