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Investigations into the relationship between behavioural tendencies and social status using the signal crayfish *Pacifastacus leniusculus* as a model organism

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1 Abstract

Many studies have shown that animals from a variety of taxa display behavioural tendencies which differ between individuals. If such tendencies are consistent over time and across contexts, they are generally referred to as personalities, temperaments or coping styles. Social conflict is believed to be one of the main factors leading to the evolution of animal personalities. Social conflict may favour the adoption of alternative behavioural options by individuals within a population, thus leading to differing personalities. In many animals, competition for resources leads to the establishment of social hierarchies, through agonistic encounters between conspecifics. Using the signal crayfish (*Pacifastacus leniusculus*) as a model organism this study investigated if crayfish of differing social status display different behavioural tendencies. To this end size-matched dominant and subordinate individuals were tested for boldness and activity at different time points. The behaviour was tested one day before, immediately after, one day after and six days after an agonistic encounter, in order to test whether crayfish display behavioural tendencies which are consistent over time and expressed in different behaviours and to determine whether pre-existing behavioural tendencies predispose individuals to a certain social status or only emerge as a result of status acquisition. The results show dominant and subordinate individuals differed significantly in their defensive behaviour. Subordinates also showed a high degree of consistency in their response to a predatory stimulus but dominants showed no consistency. In addition there was a negative correlation between the amount of low-offensive behaviour displayed during the agonistic encounter and the response to the predatory stimulus during the behavioural trials. Individuals which showed more low-offensive behaviour showed a weaker response to the predatory stimulus. Furthermore, individuals which showed more high offensive behaviour during the agonistic encounter had also spent more time walking on the day prior to the encounter.

2 Introduction

2.1 Consistent individual differences in behavioural tendencies

Since the turn of the millennium, an increasing number studies have demonstrated that non-human animals -from higher level vertebrates such as primates (Capitanio 1999) to lower level invertebrates such as anemones (Briffa & Greenaway 2011) - display behavioural tendencies that differ between individuals (Stamps & Groothuis 2010). If such tendencies are consistent over time and affect behaviour across contexts they are generally referred to as personalities, temperaments or coping styles (Stamps & Groothuis 2010). Animals differ in a range of behavioural traits including for example aggressiveness (Huntingford 1976), boldness which describes a willingness to take risks (Sinn *et al.* 2008) and sociability (Cote & Clobert, 2007) and these trait dimensions roughly correspond to similar dimensions found in humans (Gosling & John 1999). Different traits are often correlated across contexts and a suite of correlated behaviours forms a behavioural syndrome (Sih *et al.* 2004). For example boldness and aggression are often correlated, with individuals who are bolder also tending to be more aggressive (Huntingford 1976).

It is should be noted that the terms personality, coping style, temperament and behavioural syndrome are not always used in the same manner in the literature. For example Mowels *et al.* (2012 pp 1-2) state that personalities are “consistent between-individual differences in the expression of individual behavioural traits”. In contrast, Stamps and Groothuis (2010 p1) describe personality as “underlying behavioural tendencies that differ across individuals, that are consistent within individuals over time, and that affect the behaviour that is expressed in different contexts”. Thus, according to Stamps and Groothuis, a key criterion of personality is not just consistency across time, but also across behavioural contexts (cited by Gheradhi *et al.* 2012 who offer a helpful discussion of ambiguity in terminology; see also Reale *et al.* 2010 on this issue). A similar situation also applies to the terms used to describe behavioural traits. So for example, certain behaviours such as exploration or neophobia should strictly be considered measures of boldness but can sometimes be described as being behavioural traits in their own right (e.g. Reale *et al.* 2007). In this study the definition of personality outlined by Stamps and Groothuis is used. The term behavioural tendency is used to describe individual differences which are not necessarily consistent over time or across contexts.

2.1.1 The importance of animal personalities

The importance of animal personalities is becoming increasingly recognised (Reale *et al.* 2010), since they affect many different aspects of behaviour and have profound implications - both positive and negative - for individual fitness (Smith and Blumstein 2008). Personality affects access to food (with bold individuals in a social group often gaining more food than shy individuals and maintaining higher growth rates (Ward *et al.* 2004)), survival (some individuals tending to show lower levels of predator avoidance than others, making them more vulnerable to predation (Jones & Godin 2011)), and reproductive success (with certain personality traits being preferred for selection by females (Godin & Dugatin 1996)). In addition, personality is linked to dispersal (Cote *et al.* 2010, Cote & Clobert 2007, Nilsson *et al.* 2010), learning ability (Guilette *et al.* 2010) and social interactions (Kurvers 2009, Scheid & Noe 2010). There are therefore many fitness consequences associated with different personalities but the relationships can be complex and are often context dependent. A personality trait which conveys a fitness advantage in one context may not necessarily provide this in another. So, for example, one study on salmonid fish (Huntingford & Adams 2004) showed that aggressive individuals gained more food when in a tank but, when in a stream, shyer individuals gained more food. Similarly, another study showed that bold, aggressive trout grew faster in simple habitats, but not in more complex ones (Hojesjo 2004.)

Personality not only affects behaviour and has implications for fitness, it has also been shown to be reflected in underlying physiology. There is evidence to show differences in, plasma cortisol levels and neuroendocrine responses (Koolhaas 1999) linked to personality differences. Indeed, individuals which respond actively to challenges, either confronting them or fleeing them (proactive coping style, Koolhaas *et al.* 1999), generally show lower plasma cortisol levels and high sympathetic nervous system activity. On the other hand, individuals that respond passively to challenges (reactive coping style Koolhaas *et al.* 1999) tend to show high plasma cortisol levels and a high parasympathetic nervous system activity.

2.1.2 The adaptive significance of animal personalities

One of the main reasons why the issue of animal personalities has generated so much research interest is because it appears to contradict the principle of natural selection. Behaviour tends to be more flexible than other traits (Hazlett 2005) and behavioural plasticity - the ability to modify one's behaviour to respond most appropriately to any situation should be beneficial (Wolf et al 2007). Consequently the occurrence of consistent behavioural tendencies is somewhat counterintuitive, as they bias an individual towards a specific behavioural response regardless of the situation. It has been suggested that animal personalities arise due to constraints on behavioural plasticity (Dall *et al.* 2004, Briffa *et al.* 2008). A prerequisite for plasticity is the ability accurately to acquire and process sensory information in order to respond appropriately to a situation. The cost of investment into the sensory and neural machinery necessary for information acquisition and processing may however be high, particularly in unpredictable environments, thus limiting individuals in their capacity for plasticity (Hazlett 1995, Dall *et al.* 2004). For example, in an aggressive context it may not always be possible to predict the behaviour of the opponent. Thus there may be selective pressure on individuals to adopt a consistent strategy for agonistic encounters to avoid paying the cost of plasticity. Moreover, selection for consistency may be enforced by positive feedback and experience plays an important role in this (Dingemanse & Wolf 2010). Thus, individuals which respond aggressively in agonistic contests and win, and which apply a consistent approach and continue to win, will over time improve their fighting skills enabling them to win even more fights; this, in turn, increasing their disposition to respond aggressively in future encounters. Constraints on plasticity may therefore help to explain why behaviour tends to be stable across time rather than plastic. Notwithstanding the above, one main question concerning animal personalities is why individual differences exist that are consistent over time and across contexts. Assuming that optimal behavioural responses exist for different contexts why don't all individuals show the same response (within the limits of their capabilities) for a given behavioural context?

One theory which has been put forward to explain the coexistence different personalities within one population comes from Bergmueller and Taborsky (2009). They suggest that inter-individual conflict due to competition for resources is a key factor in the evolution of animal personalities. As engaging in conflict is often costly (Huntingford & Turner 1987) selection should favour the avoidance of conflict by individuals. Bergmueller and Taborsky (2009) propose that in an environment where competition is high, individuals will seek alternative behavioural options when available. For instance, if the majority of individuals in a population are foraging in one particular patch, then it is advantageous for an individual to seek out a new patch in which to forage as this would reduce competition for food and increase its foraging success. Selection would favour those individuals who are willing to seek out the new patch and their number would increase in the population. However, with increasing numbers of individuals dispersing to the new foraging patch, the level of competition would also increase, leading to a faster depletion of food resources in the new patch and therefore decreased foraging success. Eventually the level of competition would become so high that dispersal would no longer be advantageous. In such circumstances the costs of dispersal, including energy expenditure and risk of predation, would begin to outweigh the benefits and selection would swing in favour of individuals who don't disperse. Thus, natural selection can favour the coexistence of different behavioural strategies in individuals of the same population, due to the benefits of conflict avoidance.

One important question this raises is what pre-disposes individuals to certain behavioural strategies. Innate behavioural differences are likely to exist between individuals due to, early life experience or physical condition (Bergmueller & Taborsky 2009). Thus, some individuals may be more prone to explore their environment than others and therefore might be the ones who are willing to seek out a new foraging patch, whilst others may prefer to stay behind. Pre-existing behavioural differences can lead to individuals responding differently to the same environmental challenge and their particular behavioural specific response will in turn expose them to different environmental challenges for which they will require particular behavioural traits in order to deal with the circumstances linked to their social environment (Bergmueller & Taborsky 2009).

2.2 Behavioural tendencies and social rank

Choosing alternative behavioural options can reduce the amount of conflict between conspecifics, however if conflict is not resolved by these means, competition for resources leads to aggressive interactions between conspecifics (Huntingford & Turner). In many animals such social conflict leads to the formation of dominance hierarchies through repeated agonistic interactions between individuals (e.g. Herberholz *et al.* 2007). A dominance relationship between two individuals is Evidence of a dominance relationship between two individuals is can be said to exist when the results of encounters are consistent, i.e. the outcome is that the same individual prevails over the other. (Drews 1993). A dominance hierarchy describes a group of individuals where dominance relationships exist between all members in the group. (Chase *et al.* 2002). Moreover the term social status is used when referring to a relationship between two individuals, whereas social rank refers to individuals' position within a group (Drews 1993).

2.2.1 The ecological significance of social rank

It is generally believed that the rank of an individual within a hierarchy determines its access to resources. Higher ranked individuals appear to get more access to food resources (Herberholz *et al.* 2007, Ficken *et al.* 1990), safe foraging patches (Desroshers 1989) and shelter (Martin & Moore 2007). Additionally, they seem to receive more attention from potential mates (Reed *et al.* 1997) and achieve a higher number of copulations (Haley *et al.* 1994). Generally these benefits translate into greater reproductive success for dominant individuals than for subordinates, whose access to these resources tends to be more limited (Martin & Moore 2007).

The position of an individual in a dominance hierarchy has significant implications. With regard to ecological conditions, social niche theory predicts that individuals of differing social rank will develop different behavioural tendencies to cope with the challenges peculiar to their rank. For example, a positive relationship might be expected between dominance and aggression, since aggressive individuals are more likely to win agonistic encounters against non-aggressive individuals. Once attained, a status of dominance may need to be defended and this is likely to pre-dispose an individual to continued aggressive interactions (Moore 2007). Consequently, one would expect dominant individuals to maintain high levels of aggression. Subordinate individuals, on the other hand, might develop greater exploratory tendencies in order to compensate for their limited access to resources (Barta & Giraldeau 1998).

2.2.2 The relationship between social rank and behavioural tendencies

The relationship between social rank and behavioural tendencies is one that has not received a great amount of research attention thus far; nevertheless several studies have identified links between social rank and personality. In black-capped chickadees, dominant individuals were found to engage in more agonistic encounters than subordinates (Ficken *et al.* 1990). Similarly, in great tits, individuals who initiated more fights also became dominant (Verbeek *et al.* 1996). In rainbow fish, more aggressive individuals were also found to have a higher rank than their less aggressive counterparts. In addition, general activity levels and the latency associated with exploration of a novel area and approaching a novel object – both measures of boldness – were correlated with male hierarchy rank (Colleter & Brown 2011); higher ranked males were found to be bolder and more active than those of lower rank. Foraging behaviour has also been shown to differ with social status. In house sparrows, dominant individuals spent less time searching for food and more time scrounging off subordinates (Liker & Barta 2000). Since dominants have a higher competitive ability they are able to steal food off subordinates. Food intake was however not related to social rank. However, in another study behavioural predictions based on social rank did not hold true. Since subordinate individuals often have to wait on dominants before gaining access to a feeding site, their feeding success is less predictable and one would expect subordinates to have a greater need to cache food as a precaution against starvation (Pravosudov *et al.* 2003). Yet in mountain chickadees it was found that subordinates cached less food and were less successful at cache retrieval than dominants (Pravosudov *et al.* 2003). The authors suggest that the relationship between caching and social status, may depend on environmental conditions.

As indicated above, the fitness implications of personality are often context dependent. This is no less so in the relationship between behavioural tendencies and social rank. For instance, in great tits, fast exploring juveniles were found to be more aggressive than slow exploring juveniles and, consequently, won more fights when in single dyadic encounters (Verbeek *et al.*1996). However, when they engaged in fights in a larger group, it was the slow exploring individuals which on average won more fights and became dominant. Fast explorers were more aggressive, yet also took more risks during fights, potentially making them more prone to lose (Verbeek 1998). Similar results were found in another study on great tits (Dingemanse and de Goede 2004). Fast exploring males were dominant over slow exploring males but only if those males were also territorial. The dominance-personality relationship was precisely the opposite in non-territorial juveniles. The authors suggest that fast explorers are less able to cope with social defeat and should seek to flee the social group in order to recover. Territorial males can flee to their territories in order to recover, but non-territorial males lack that option and may be forced to stay with the group for protection from predation. The loss of dominance status through social defeat probably also leads to the loss of access to resources. Fast exploration may be linked to a life-history strategy that seeks to maximise resource acquisition in order to reproduce as soon as possible (Wolf *et al.* 2007). Thus, for non-territorial males, social defeat may represent a much more serious issue than for territorials.

One important question regarding the relationship between personality and social rank is whether pre-existing behavioural tendencies predispose individuals to certain social ranks, or whether consistent behavioural tendencies are the result of social interactions. In the study by Verbeek *et al.* (1996), differences in exploratory behaviour were identified in 4 week old juveniles, prior to the establishment of dominance hierarchies, suggesting that in great tits at least, innate differences in behaviour exist which affect social status via aggression. A study in starlings also reported the existence of behavioural differences prior to dominance hierarchy establishment (Boogert *et al.* 2006). In zebrafish individual differences in boldness assessed prior to an agonistic encounter, could be used accurately to predict social status of individuals after only 10 minutes of testing (Dalbohm *et al.* 2011). Dominant individuals had spent more time in the centre of the tank during an open field test and also showed more activity during a novel object test compared to subordinates. In anemones winners of an agonistic encounter showed shorter startle response durations when tested before the encounter compared to losers. Furthermore research suggests that physiological differences as well as behavioural differences may also predict social status. A study in trout showed that higher metabolic rate predicted social status (McCarthy, 2001), similarly, plasma cortisol responses prior to social interactions also indicated social status in these fish (Sloman *et al.* 2001). Together these studies suggest that individual behavioural tendencies predispose individuals to a particular social rank. However, a number of different factors are believed to be important in social hierarchy formation and behavioural tendencies are just one. In fact, research suggests that although individual differences play a role, social dynamics may be ultimately more important in determining social rank (Goessman *et al.* 2000). In particular, winner and loser effects appear to play a crucial role. These have been identified in several taxa (Chase *et al.* 1994) and act as positive or negative feedback mechanisms on aggressive tendency. (Hsu & Wolf 2000) Yet the evidence, as to the magnitude of these effects, their relative importance (i.e. whether winning or losing has a stronger effect) and their duration varies between studies (Hsu & Wolf 1999, Chase 1994). Thus in some studies both winning and losing were found to have a significant effect on agonistic behaviour in subsequent encounters (Hsu & Wolf 2001), whereas in other studies, only loser effects appeared to alter agonistic behaviour (Francis 1983, Beacham & Newman 1987). Winner-loser effects are believed to alter individuals' perception of their fighting ability (Otronen 1990) In addition, it is possible that differences in winner-loser effects could be due to differences in how these effects were produced and measured. In any case, winner-loser effects, if present, clearly alter agonistic behaviour. They have the potential to create consistently different behavioural tendencies if they also affect behaviour in the long term. Previous research, albeit limited, suggests that agonistic experience does have an effect on other non-agonistic behaviour. A study on *Kryptolebias marmotus*, a type of mangrove rivulus, found that after winning experience, individuals were not just more aggressive, but also demonstrated better cognitive performance (Chang *et al.* 2012). They also appeared to show less exploratory tendencies, although this result is questionable as it contradicted the finding that more aggressive individuals were more exploratory. Van de Pol *et al.* (1982) also reported effects on cognitive ability. Rats that had been conditioned to a subordinate status showed defects in special orientation which persisted for up to 14 days. Barnard and Luo (2002) found differences in learning ability between dominants and subordinates after, but not before, acquisition of social rank; dominants showing greater learning ability than subordinates. Finally, one study on rats showed that the establishment of dominance relationships influenced exploratory behaviour of postpubertal and adult rats (Arawaka *et al.* 2006). The effect on subordinates was stronger than in dominants, with subordinates displaying less active exploratory behaviour than dominants.

The above findings demonstrate that the relationship between social rank and behavioural tendencies is complex. There is some evidence to suggest that behavioural tendencies predispose individuals to a certain social rank, but the importance of social experience in shaping personalities cannot be denied.

2.3 Crayfish as a model organism

2.3.1 Decapod crustaceans and behavioural tendencies

Decapod crustaceans are one of the most species rich invertebrate taxa, including lobsters, crabs, crayfish and shrimp and have been extensively used in behavioural research (Gheradi *et al.* 2012). Despite their popularity as study organisms, only a few studies thus far have investigated behavioural tendencies in these organisms. Briffa *et al.* (2008), who were the first to demonstrate personality in crustaceans, showed that hermit crabs differed consistently in the duration of their startle responses – a measure of boldness. They also differed in the extent to which they explored a new environment and investigated novel objects. Boldness was also found to differ between individuals in fiddler crabs (Reany & Blackwell 2007) and crayfish (Vainikka 2011). Furthermore, in fiddler crabs boldness correlated with aggressiveness, activity and mating success (Reany & Blackwell 2007). Similarly, in crayfish boldness was correlated with aggression and foraging activity (Pintor *et al.* 2008). Yet studies looking specifically at the link between behavioural tendencies and social status in crustaceans are still lacking.

2.3.2 Crayfish behaviour

Crayfish, in particular, are well known for their agonistic behaviour, which has been studied in several different species and they have become model organisms for the study of aggression and dominance. In the wild, crayfish live in burrows along streams and rivers and seem to occur in reasonably high densities (Edwards *et al.* 2003, Herberholz *et al.* 2007) and engage in agonistic encounters over resources with conspecifics (Herberholz *et al.* 2007). In laboratory conditions they readily engage in agonistic encounters, even in the absence of any tangible resource (Herberholz *et al.* 2007) and their behaviour can be easily described and quantified (Huber *et al.* 2002). Agonistic encounters typically follow a stereotypical pattern of behavioural elements which has been described in previous research and resembles agonistic interactions in other crusteacean decapods (Goessman *et al.* 2000). At the start of an encounter contestants approach each other, often flicking antennules and whipping antennae and performing a threat display with the major chelae, known as meral spread. If contestants are evenly matched, they will engage in physical combat, using their chelae initially for pushing then for grasping the opponent, and in very intense encounters in an unrestrained manner with a view to inflicting severe injury to their opponents (Moore 2007). The winners of such encounters become dominant whereas the losers become subordinate. Initial dominance relationships become reinforced through repeated dyadic interactions between individuals and dominance hierarchies are established. The key question is: what makes one individual dominant and the other subordinate? If there are obvious differences in body size or claw size between individuals, these tend to determine the outcome of a fight. (Moore 2007) Other factors such as strength, moult stage and sex also play a role. Differences in aggressiveness might play a role (Bovbjerg 1953). Environmental factors such as perceived resource value or prior residency in a shelter are believed to be important, yet their significance has not been examined in detail (Moore 2007). In contrast, however, it is known that social conditioning i.e. previous fighting experience is very important in determining social rank. Winner-loser effects as described in section 2.2.2 have been demonstrated to operate in crayfish. Individuals that have previously won an encounter are more likely to win again likewise individuals that have lost a fight are more likely to lose again. Winning initially decreases, individuals tendency to retreat during an encounter and then increases the willingness to escalate in subsequent encounters (Huber *et al.* 2001). The observation that initial interactions between unfamiliar opponents tend to be long and intense, but that subsequent interactions are shorter and also occur less frequently, confirms this (Goessmann *et al.* 2000).

As initial social statuses are reinforced, so the behavioural characteristics of dominants and subordinates become obvious. Dominants continue to show low levels of aggression towards subordinates (Huber *et al.* 2001, Ameyaw-Akumfi 1979) who respond by showing lowered submissive postures or retreat (Ameyaw-Akumfi 1979). Dominants and subordinates also display differences in non-aggressive behaviour. For instance it has been shown that during and after a fight, dominants spend significantly more time digging burrows than subordinates, in which burrowing is inhibited (Herberholz *et al.* 2003). Dominants also respond aggressively to tactile stimulation, whereas subordinates show an avoidance response (Song *et al.* 2006). Attempts have been made to try to elucidate the neurochemical bases of these behavioural changes. In an early study by Livingston *et al.* (1980) researchers injected lobsters with the biogenic amine octopamine inducing a body posture which is typical for dominant animals, and with serotonin, inducing a posture typical for subordinates. This suggested an important role of these chemicals in the behaviour in these animals. Subsequent research demonstrated that serotonin - implicated in the role of aggression in a number of taxa (Edwards & Spitzter 2006) - affected the willingness to retreat during an agonistic encounter in lobsters and crayfish (Huber *et al.* 1997). Artificial infusion of serotonin reduced individuals' willingness to retreat and consequently lead to extended encounters. However it did not affect individuals' likelihood to initiate an attack or escalate during an encounter, neither did it affect the outcome of the encounter Huber *et al.* 1997, Huber & Delago 1998). Further research has shown that the properties of neuronal circuits involved in agonistic behaviour change as a result of social status acquisition. Thus, serotonin - implicated in the role of aggression in a number of taxa (Edwards & Spitzter 2006) - facilitates synaptic transmission in dominants and socially naïve individuals, yet inhibits it in subordinates. These changes take effect gradually over two weeks (Edwards & Spitzter 2006). Moreover dominance has been shown to increase the survival of neuronal precursors in juvenile crayfish (Song *et al.* 2007). These findings suggest that social experience may lead to lasting changes in behaviour and neurophysiology and thus could produce consistent individual differences in crayfish. Some specific research has shown that without reinforcement through repeated interactions, the winner effect only persists for up to 60 minutes (Bergmann *et al.* 2003). Song *et al.* (2006) found that subordinate crayfish only display an avoidance response to tactile stimulation in the presence of the dominant opponent which suggests that the loser effect has an equally short longevity. Moreover, social status in crayfish is readily reversible. Daws *et al.* (2002) exposed subordinates to winning experiences thereby conditioning them to become dominant in subsequent encounters, even against larger contestants and Graham & Herberholz (2009) demonstrated that established dominance relationships between two individuals could be disrupted by introducing a larger intruder. Social experience appears to be crucial not only to the formation, but also to the maintenance of behavioural differences.

2.3.3 The signal crayfish

This research study used the signal crayfish (*Pacifastacus leniusculus*) as a model organism to investigate the relationship between behavioural tendencies and social status. The signal crayfish is native to North America and was introduced into Europe in the 1960's and has since then spread throughout many freshwater ecosystems in many European countries (Lewis 2002). Signal crayfish are a major threat to the native crayfish populations due to competition and disease transmission (Lewis 2002). The insights from this study thus have potential implications for the treatment of this invasive species.

2.4. Research aims and objectives

The aim of this study was to ascertain whether dominant and subordinate crayfish showed different behavioural tendencies and if these tendencies were consistent over time and expressed in different behaviours.

The current study sought to address the following questions:

1) Do crayfish of differing social status display different behavioural tendencies?

2) Can future social status be predicted based on the behavioural tendencies displayed prior to an agonistic encounter?

3) Are the behavioural tendencies displayed by dominants and subordinates consistent over time?

4) Are different behavioural traits correlated with each other?

The hypotheses associated with the different research questions were:

1) Crayfish of different social status do display different behavioural tendencies.

   In order to assess this, behavioural trials were conducted with dominant and subordinate crayfish to test for differences in boldness and activity

2) Future social status can be predicted based on behavioural tendencies displayed prior to an agonistic encounter.

   In order to assess this, behavioural trials with dominant and subordinate crayfish were conducted before and after an agonistic encounter.

3) Behavioural tendencies displayed by dominants and subordinates are consistent over time.

   In order to assess this, behavioural trials were conducted four times in total. Once before the agonistic encounter and three times after the agonistic encounter

4) Different behavioural traits are correlated with each other.

   In order to assess this, different measures of boldness and activity were examined and correlation analysis was performed on these behavioural measures as well as on measures of aggression displayed during the agonistic encounter.

3 Methodology

3.1 Collection and housing of specimens

The crayfish used in all research activities were obtained in September 2011 and January 2012 from the same commercial supplier.

The research specimens were kept in same sex holding tanks (91.5 × 30 × 30 cm) in groups of up to 30 individuals per tank. They were fed twice per week on a diet of prawns from two supermarket chains. The crayfish were kept on a 10/14 h light/dark cycle at 15°C.

3.2 Rationale of the experimental design

Finding an appropriate experimental design proved to be something of a challenge. The following description outlines the conception of the final design and explains the underlying rationale.

Given that the aim of the research was to investigate the relationship between social status and behavioural tendencies, it was necessary to establish dominance relationships between individual crayfish, as well as test their behaviour in non-agonistic contexts. Since crayfish readily engage in agonistic encounters in the laboratory without any particular stimulus (Herberholz *et al.* 2007), establishing dominance between individual crayfish was not a major issue. The main challenge lay in finding appropriate behaviours to test observable behaviour in non-agonistic contexts. It is known that many crustaceans possess mechanoreceptors which can detect disturbances in water (Herberholz 2007). Thus, in the first experimental design the chosen behaviour to be tested was the response to an aversive mechanical stimulus. In order to achieve this, blindfolded crayfish were exposed to a strong mechanical stimulus, created by moving a Perspex board through the water, after emergence from a shelter. The response to the aversive stimulus was tested before and after an agonistic encounter, using separate groups of crayfish for each treatment. The latency of emergence from the shelter after exposure to the stimulus was analysed. However, the results did not yield any statistically significant differences between dominant and subordinate crayfish ($p = 0.867$; paired t-test, $p = 0.473$; paired t-test). Out of the 36 specimens tested, only 20 could be used in the analysis. One particular issue was the exposure of the experimental tank to vibrations during the procedure which would have affected the behaviour of the experimental subjects. Consequently, a less invasive design was sought that did not involve mechanical stimulation. Activity is a common behavioural measure for behavioural tendencies, therefore in the second design this was chosen as the behaviour to be investigated. Crayfish were tested in red light conditions in order to spare them from the stressful experience of being blindfolded. For the behavioural trials, crayfish were introduced into a shelter in a tank and the latency of emergence as well as their activity after emergence was assessed. Once again, no significant differences were found between dominants and subordinates in regards to their latency of emergence ($p = 0.240$; paired t-test $p = 0.098$; paired t-test). Due to the lack of differences in latency of emergence as well as time constraints activity was not analysed. The lack of significant results could have come from a number of sources. Inadvertent differences in handling of the crayfish could have had an effect. Additionally, there was no incentive for the crayfish to emerge from the shelter and explore the experimental tank. Since the red light conditions created an environment which was generally dark, with no obvious difference between inside the shelter outside the shelter, there may have been no particular motivation to remain in the shelter. Had this approach been pursued it would have made the results difficult to interpret.

The final design aimed to eliminate these potential experimental errors. Thus, in order to remove potential handling effects, crayfish were, as far as was practicable, manipulated with nets. On a few occasions, manual handling was unavoidable but as far as possible it was kept to a minimum. An additional feature of the final design was to test the activity of the crayfish in bright white light after they had been introduced into blacked out shelters. This was a deliberate element of the method in order to create a significant contrast between the two environments. Since crayfish are mainly nocturnal animals (Moore 2007), the bright light was intended to act as an aversive stimulus. A further measure introduced to aid successful experimental conditions was the addition of a standard amount of food odour into the water outside the shelter to act as an incentive for the crayfish under test to emerge from the shelter.

One important weakness of both previous designs was that they only addressed the first two research objectives. In order to test whether crayfish display behavioural tendencies which are consistent over time and expressed in different behaviours and to determine whether pre-existing behavioural tendencies predispose individuals to a certain social status or only emerge as a result of agonistic encounters, in the final design crayfish were tested for boldness and activity before, immediately after, one day and six days after an agonistic encounter.

3.3 Specimens used

A total of 32 male crayfish were used for the experiments. They were between 71 and 90 mm in total length and had chelae ranging from 32 to 47 mm in lenght. They were measured with 150mm calipers (Draper 4817P) and numbered for identification purposes on their carapaces with Tipp-Ex.

3.4 Experimental set up

Behavioural experiments were conducted in a temperature controlled room from February – April 2012. The room temperature was kept at 15-17°C for all experiments.

The experimental setup was as follows: three glass aquaria were setup on a bench in the experimental room. One tank (45.5 x 24.6 x 24 cm), used for the agonistic encounters was placed in a position on the bench separate from the other two. Two red-light lamps were placed either side of it for illumination during agonistic encounters. The observer faced its long side. The tank was divided into two halves using an opaque board held in place by ridges either side of the tank (Fig. 1).

Fig. 1 Tank used for agonistic encounters as described in section 3.4

The other two tanks (left tank: 45.5 x 24.4 x 23.7 cm, right tank: 45 x 25 x 25.8 cm), used for the behavioural trials, were placed side by side with the long sides facing each other. The outer sides of these tanks were blacked out to prevent crayfish from seeing the observer during experiments. In addition, a Styrofoam board was placed between the two tanks to prevent crayfish in one tank from being affected by the experimenter's action in the other tank (Fig. 2). The substrate of the agonistic tank was a plastic board (42.4 x 23.5x 40 x 19.5x 4 cm) covered with black velvet material. The substrate for the behavioural tanks were plastic boards (left tank: 42 x 23.5 x 39.9 x 21.5 x 2.9 cm, right tank: 41.9 x 23.5 x 39.6 x 21.5 cm) covered with cream pillowcase material. In each of the behavioural tanks a Plexiglas shelter (17 x 12.3 x 4.8 cm) was placed in the near left hand corner. The shelters were covered with black plastic material to prevent light from entering them (Fig. 2).

Fig. 2 Tanks used for the behavioural trials viewed from above and as described in section 3.4

For the experiments, the tanks were filled with aerated tap water, stored in a rain tub. The rain tub was refilled regularly, following depletion of water. All tanks were filled to a height of 12cm. The water temperature in the tanks tended to range from about 14.7-16.1°C.

3.5 Treatment of animals prior to experiment

The crayfish to be used as research specimens were paired roughly according to size, though one of the pair was always chosen to have slightly larger claw size than the other in order to ensure a clear outcome in the agonistic encounter. They were subsequently isolated five days before the beginning of an experiment. Each specimen was placed in clear plastic isolation boxes filled with tap water from the experimental room and kept in a separate temperature controlled room. Since specimens had previously been kept in large holding tanks in which they would engage in agonistic encounters with conspecifics and decapod crustaceans have been shown to have the capacity for dominance or individual recognition Karavanich & Atema 1998), the rationale for isolation was to ‘erase’ the specimens' memory of prior social experience. The temperature of the isolation room was held constant at 15°C and it was maintained in a permanent normal laboratory lighting regime. The water temperature in the isolation boxes ranged between 16.1-16.9°C.

3.6 Experimental procedure

For each pair of crayfish, four behavioural trials were conducted as well as one agonistic encounter. The sequence of trials undertaken with each pair was as following:

Day 1: the first behavioural trial, a day before an agonistic encounter

Day 2: the agonistic trial followed immediately by the second behavioural trial.

Day 3: the third behavioural trial, a day after the agonistic encounter.

Day 8: the fourth and final behavioural trial, six days after an agonistic encounter.

During the procedure, specimens were fed two days before the first behavioural trial, after the third behavioural trial and two days before the last behavioural trial. Since up to three pairs of crayfish were tested on the same day, feeding generally took place after all trials for one day had been completed. The water in their Tupperware boxes was changed immediately after the first behavioural trial and a day before the last behavioural trial.

For the first behavioural trial, a pair of crayfish was carried through to the experimental room in their isolation boxes. They were placed in front of the agonistic tank in red light conditions for five minutes acclimation time. After that they were taken out of their boxes using nets and each crayfish was positioned in a shelter in one of the tanks and the gate was placed in front of the opening. Bright white light was immediately switched on, the red lights switched off and the crayfish were left undisturbed for another five minutes. After this second period of five minutes, 1mil of prawn odour was introduced by pipette into each tank immediately in front of the shelter. The gates of the shelters were then removed. The aim was to attract the specimens out of their shelters into the bright environment. Prawn odour was obtained by defrosting a prawn (up to 3 hours prior to commencement of an experiment) and placing it in a plastic vial with warm tap water.

Four minutes after a crayfish had emerged from its shelter a plastic board (41.9 x 23.2 x 39.5 x 21.5cm) was moved over the tank to create a large shadow covering the specimen and then removed. This methodology was to mimic a predatory stimulus. All the behavioural trials were supported by a video suite comprising: a video camera (Sony Hyper HAD B&W video camera CCD-IRIS), video recorder (Sony time lapse video cassette recorder) and a TV monitor (JVC). Recordings were made of each trial from the moment of the introduction of the crayfish into the shelter, until 30 minutes after removal of the gates from the shelter. After the first behavioural trial, the reverse of the process described above was used to return the specimens to the isolation room. On those occasions when crayfish had remained in their shelter, the shelter was lifted out of the tank and placed directly into the isolation box and they were left to crawl out of the shelter into the box before transportation back to the isolation room.

For each pair of specimens, one day after the first behavioural trial, the agonistic encounter was carried out in order to establish social status. The crayfish were carried into the experimental room and introduced into the agonistic tank; each specimen in one half. - They were allowed to acclimatise for 15 minutes in red light conditions after which time the divider was lifted and the crayfish were allowed to interact, for at least 30 minutes. If after 30 minutes the crayfish were still fighting, or there was no clear winner, they were allowed to continue until they either separated or there was a clear winner. The agonistic encounter was terminated by placing the divider back into position. All agonistic encounters were recorded using a tripod mounted camcorder (Tripod: Manfrotto 390; camcorder: Sony Digital video camera recorder). After the agonistic encounter the specimens were transferred back briefly to their isolation boxes and from there immediately into a shelter in the behavioural tanks under red light conditions for the second behavioural trial. The tank in which an individual crayfish was placed was alternated for each behavioural trial so as to reduce any confounding effects, such as the slight differences in position and light conditions between the tanks. After the last trial crayfish were either returned to the holding tanks or kept in isolation boxes for other experiments.

3.7 Analysis

To undertake detailed analysis, the video recordings were transferred to DVD using a converter (Sony VRD -MC6 multifunction DVD recorder). The behavioural trials were used to examine boldness and activity. Two different behaviours were analysed to assess the characteristic of boldness. Latency of emergence from the shelter was one factor. This was measured by timing the period taken from the removal of the gate from the shelter to the time crayfish came out of the shelter. Specimens were considered to be out of the shelter when the third walking leg was visible on at least one side. In order to maintain a consistent approach, since specimens sometimes emerged from their shelter partially before retreating back, an emergence was only counted if it led to a full body emergence (i.e. the complete body would have to be seen outside the shelter). The second criteria for boldness was defined as the reaction of the specimens to the predatory stimulus. Nine different categories of response were observed and crayfish were ranked for boldness according to the category. The responses are listed in Table 1 in order from least bold to most bold. In some trials it was not possible to ascertain the reaction of the crayfish to the stimulus due to the manner in which the board was applied. These cases were excluded from the analysis.

Table 1 Categories of response for the reaction of specimens to the predatory stimulus

Extended tailflip	At least one tailflip and escape response
Tailflip	Brief single tailflip
Fast retreat	walking backward with >1 body lenght / 5s
Slow retreat	walking backward with <1 body lenght / 5s
Long immobility	Period of immobility > 10s
Medium immobility	Period of immobility between 3 and 10 sec
Short immobility	Period of immobility < 3s
No response	No obvious response
Meral spread	Claws open and upraised toward the stimulus

To assess the activity of the specimens during the behavioural trial, different behavioural elements were classified and the amount of time spent expressing each behaviour was assessed. For each specimen only the first four minutes after the emergence were analysed. In some cases it was not possible to analyse all four minutes.

These were excluded from the analysis. Table 2 lists the behaviours analysed and their definitions.

Table 2 Definition of behavioural elements analysed during behavioural trials

Walking	Crayfish outside of the shelter and moving, showing clear displacement of the body.
Retreat	walking backwards into the shelter retreats were only counted if the crayfish retreated at least up to their carapace into the shelter (i.e. the abdomen was not visible) and provided the crayfish had been outside of the shelter before
In shelter	Crayfish inside the shelter with no more than the second pair of walking legs was visible. (If the crayfish entered the shelter face forwards, then no more than the last pair of walking legs was allowed to be visible)
Duration of emergence	Measured from the moment crayfish were out of the shelter with the third walking leg visible on at least on side till the moment they were completely out of the shelter

The agonistic encounters were analysed by assessing two distinct aspects. First, the outcome of the fight was assessed. The winner and loser of a fight were determined by assessing the number of bouts respectively won or lost. A bout being defined as an interaction in which both contestants showed aggression of at least level 3. (For a definition of the agonistic levels see table 3). A bout was determined to have ended after the contestants had separated for at least 30 seconds. The specimen that initiated the separation by showing defensive behaviour (at least level -1) was considered to be the looser.) Second, the aggressive level of each contestant was determined. For this purpose, the assessment was based on consideration of time spent by each specimen showing defensive behaviour (levels -2 and -1), low aggressive behaviour (levels 1 to 3) and high aggressive behaviour (levels 4 and 5). Timings were recorded by using three timers (online stopwatch.com) on the computer screen whilst viewing the recording of the trials in slow motion (x 0.8 speed).

Unfortunately, for one of the encounters the last part of the video footage was lost and therefore could not be accurately assessed for aggressive levels. However, since dominant and subordinate status was still able to be assessed it is included in the rest of the results.

Table 3 Description of aggressive levels – adapted from Atema and Voigt (1995)

Aggressive level	Description	Behavioural elements
-2	Fleeing	Fast walking backwards, fast walking away, tail-flipping
-1	Avoidance	Walking backwards slowly, walking away slowly, turning away from opponent
0	No activity	Separate and no activity
A	Activity	Separate and walking
1	No physical contact (within 1 body length)	Approaching opponent, turning towards opponent, following opponent
2	No physical contact (threat display)	High on legs, meral spreading
3	Physical contact (claws not used to grasp)	Antenna touching, antenna whipping, claw touching, claw pushing, claw boxing, claw tapping
4	Physical contact (claws used to grasp)	Clamping of chela(e) onto opponents body
5	Unrestrained use of claws	Claw snapping, claw ripping

3.8 Statistical analysis

Statistical analysis was performed in order to address the research objectives, outlined in section 2.6. Most statistical analysis was performed using SigmaPlot 12.0. For the analysis of behavioural consistency SPSS 19 was used. Two-way repeat measures ANOVA were performed for the non-aggressive behavioural traits in order to test whether dominant and subordinate individuals displayed different behavioural tendencies. One-way repeated measures ANOVA was performed for the different levels of aggressive behaviour. Data were transformed as necessary (arcsin transformation for aggressive levels, log +1 transformation for other variables) in order to obtain a normal distribution. In cases where data did not meet the criteria for parametric analysis (i.e. normal distribution and equal variance) they were analysed using non-parametric alternative (Friedman repeated measures ANOVA on ranks).

To assess whether social status was predictable based on previous behaviour, individuals were classified into bold-active and shy-passive categories according to the behaviours they displayed in the trial prior to the agonistic encounter.

For the behaviours latency of emergence, number of retreats and duration of emergence behaviours, the median value was ascertained and this was used as the classification threshold. Individuals which showed a lower value than the median were classed as bold, whereas individuals which showed a higher value than the median were classified as shy. In respect of walking and shelter behaviours, individuals which spent more than 50% of the time walking or less than 50% of the time in the shelter were classified as bold, whereas individuals which spent 50% or less of the time walking and 50% or more of the time in the shelter were classified as shy. Chi-squared analysis was then performed on the data. In cases where data did not meet the criteria for Chi-squared analysis, a Fisher Exact test was performed.

In order to assess the temporal consistency in behaviour, Kendall's coeffiencient of concordance was performed for each of the behavioural traits. Dominants and subordinates were first analysed together and then separately in order to determine whether there were any differences in consistency between the two groups.

Finally, correlation analyses were performed for each of the non-aggressive behavioural traits as well as for the different levels of aggressive behaviour for each day of the experimental sequence. Once again dominants and subordinates were analysed together as well as separately. Due to the nature of the data, non-parametric Spearman correlations where generally performed, though where possible the parametric Pearson correlations where performed.

4. Results

4.1 Behavioural trials

4.1.1 Latency of emergence

There was some difference in the latency of emergence between the different days of testing ($p < 0.001$, Two way repeated measures ANOVA on ranks). Latency of emergence was significantly lower on day one compared to all the following days when dominants and subordinates were analysed together ($p < 0.001$, day two, $p = 0.031$ day three, $p = 0.001$ day 4; Two way repeated measures ANOVA with Holm-Sidak post-hoc test, Fig. 3). Multiple comparison tests (Holm-Sidak post-hoc analysis) further revealed that there was also a significant difference in the latency of emergence between day one and day two ($p = 0.031$; Two way repeated measures ANOVA with Holm-Sidak post-hoc analysis) as well as between day one and day four ($p = 0.016$; Two way repeated measures ANOVA with Holm-Sidak post-hoc analysis) for dominants. For subordinates, there was only a significant difference between the latency of emergence on day one and day two ($p = 0.002$; Two way repeated measures ANOVA with Holm-Sidak post-hoc analysis, Fig. 3) There was however no significant difference between dominants and subordinates with regards to the latency of emergence on any of the days ($p = 0.693$; Two way repeated measures ANOVA)

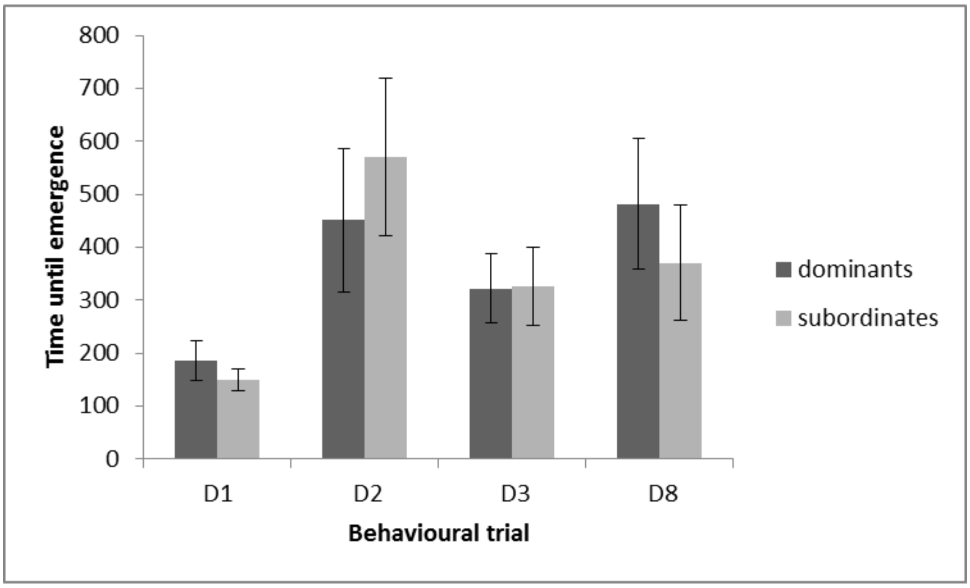

Fig. 3 Latency of emergence for dominants and subordinates in each behavioural trial (D1 = one day prior to the agonistic encounter, D2 = immediately after the agonistic encounter, D3 = one day after the agonistic encounter, D8 = six days after the agonistic encounter) Error bars indicate standard error of the mean (SEM). Data were transformed (log + 1) for the analysis but raw data used for illustration.

There were five occasions throughout the experimental procedure when individuals didn’t emerge from the shelter and thus their behaviour could not be analysed. On day one (prior to the agonistic encounter) all individuals emerged within 599 seconds. On day two (immediately after the agonistic encounter), three individuals did not emerge whilst all others emerged within 1788 seconds. On day three (one day after the agonistic encounter) all individuals emerged within 1111 seconds. Finally on day eight (five days after the agonistic encounter), two individuals did not emerge whilst all others emerged within 1225 seconds.

4.1.2 Walking

There was no significant difference with regards to the time spent walking between dominants and subordinates throughout the sequence of behavioural trials (p = 0.467 Two way repeated measures ANOVA) and there was also no overall difference between the days of testing (p = 0.103 Two way repeated measures ANOVA)

4.1.3 Number of retreats

There was no significant difference with regards to the number of retreats that dominants and subordinates showed throughout the sequence of behavioural trials (p = 0.982 Two way repeated measures ANOVA) and there was also no overall difference between the days of testing (p = 0.085 Two way repeated measures ANOVA)

4.1.4 Shelter

There was no significant difference with regards to the time spent in the shelter between dominants and subordinates throughout the sequence of behavioural trials (p = 0.959; Two way repeated measures ANOVA) and there was also no overall difference between the days of testing (p = 0.447 ANOVA)

4.1.5 Duration of emergence

There was some difference in the duration of emergence between the different days of testing (p = 0.001, Two way repeated measures ANOVA, Fig. 4). Duration of emergence was significantly lower on day one compared to days three (p < 0.001) and four (p = 0.032) when dominants and subordinates were analysed together (Two way repeated measures ANOVA with Holm-Sidak post-hoc test,), However when analysed separately, Holm-Sidak post-hoc analysis revealed a significant difference in the duration of emergence between day one and day three only, for dominants as well as subordinates (p = 0.034, dominants p = 0.042, subordinates; Two way repeated measures ANOVA with Holm-Sidak post-hoc analysis, Fig. 4).

There was however no significant difference with regards to the time taken to emerge from the shelter between dominants and subordinates throughout the sequence of behavioural trials (p = 0.432; Two way repeated measures ANOVA).

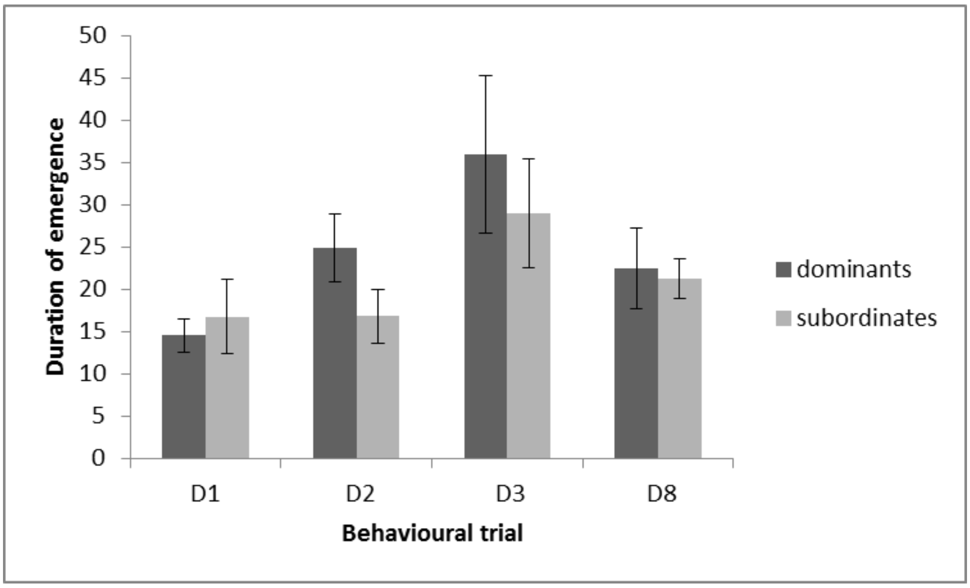

Fig. 4 Duration of emergence for dominants and subordinates in each behavioural trial (D1 = one day prior to the agonistic encounter, D2 = immediately after the agonistic encounter, D3 = one day after the agonistic encounter, D8 = six days after the agonistic encounter) Error bars indicate standard error of the mean (SEM). Data were transformed (log + 1) for statistical analysis but raw data used for illustration.

4.1.6 Response to the predatory stimulus

There was no significant difference with regards to the response to the predatory stimulus between dominants and subordinates throughout the sequence of behavioural trials (p = 0.819; Two way repeated measures ANOVA) and there was also no overall difference between the days of testing (p = 0.796; Two way repeated measures ANOVA)

4.2 Behavioural differences prior to the agonistic encounter

There was no statistically significant evidence to suggest that social status could be predicted based on the behaviours displayed before the fight. With regards to latency of emergence there was no difference in emergence time between dominant and subordinate individuals ($p = 0.724$; Chi-square test). Likewise there was no significant difference in the response to the predatory stimulus between dominants and subordinates ($p = 1$; Fisher exact test) 60% of dominants spent more time walking compared to 46.7% of subordinates ($p = 0.714$; Chi-square test) and 60% of dominants showed fewer retreats compared to 53.3% of subordinates ($p = 1$; Chi square test). Conversely, 86.7% of subordinates spent less time in the shelter compared to 60% of dominants ($p = 0.215$; Fisher exact test), also 50% of subordinates spent less time emerging from the shelter compared to 43.7% of dominants ($p = 0.724$; Chi square test).

4.3 Agonistic encounters

Concerning the analysis of agonistic encounters, all encounters yielded identifiable dominant and subordinate individuals. Further analysis of the agonistic encounters revealed that dominant individuals spent less time in defensive behaviour ($p = <0.05$; Friedman repeated measures ANOVA on ranks) than subordinates (Fig. 2). There was no significant difference between the groups with regards to low offensive behaviour ($p > 0.05$ Friedman repeated measures ANOVA on ranks) high offensive behaviour ($p > 0.05$ Friedman repeated measures ANOVA on ranks), though that might have been due to the fact that the time spent in high offensive behaviour was generally very low (values range from 0 – 0.018 s, relative time).

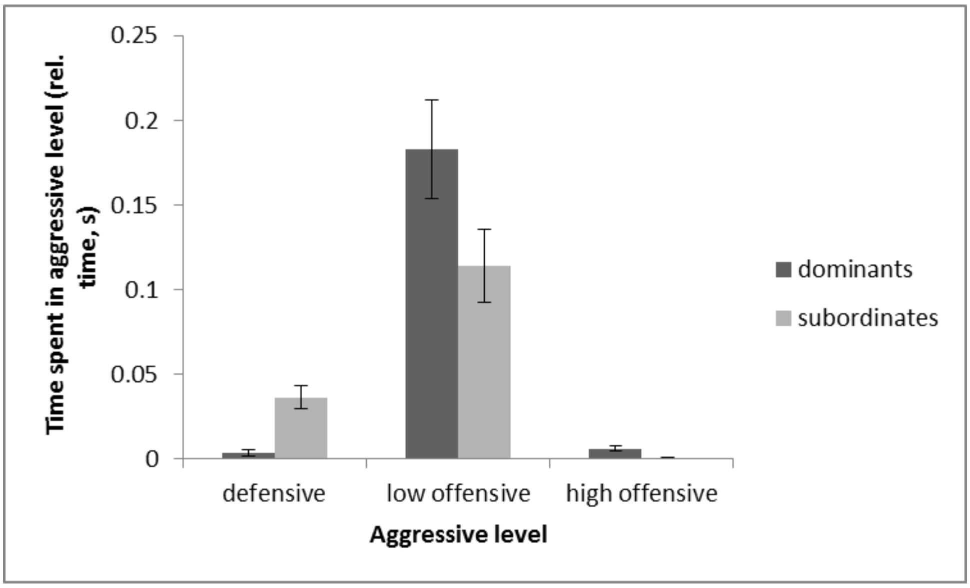

Fig. 5 Average time spent in each aggressive level by dominants and subordinates during the agonistic encounter. Shown is the relative time compared to total time of the agonistic encounter. Error bars indicate SEM. Data were transformed (arcsin) for the analysis but raw data used for illustration.

Since it was part of the methodology to pair up specimens with slightly different claw sizes, the difference in claw size was assessed. For this, left and right claw size was averaged for each individual, then the overall average claw size between dominants and subordinates was compared using statistical analysis. This revealed a significant difference between dominants and subordinates (p = 0.002; paired t-test, see also Appendix Table 5), with dominants having an average claw size of 43.071mm and subordinates having an average claw size of 39.143mm.

4.4 Temporal consistency in behaviour

Statistically significant consistency in behaviour was found in response to the aversive stimulus (p = 0.006, Kendall’s w 0.832; Kendall's Coefficient of Concordance). However when dominants and subordinates were analysed separately, only subordinates showed a significant consistency in response to the aversive stimulus (p = 0.013, Kendall’s w = 0.903, Tables 1 and 2), but not in any other behaviours, which suggests that the subordinates were responsible for the overall consistency in response to the aversive stimulus. As indicated in section 3.7, it was not always possible to ascertain the reaction of the crayfish to the stimulus. Consequently, out of the 128 times the response to the predatory stimulus was tested during the sequence of the experiments, only 86 could be analysed.

Table 4 Response of subordinate individuals to predatory stimulus. Same responses on different days highlighted with an asterisk (*)

Individual	D1	D2	D3	D8
A	8			9
B	9			
C	4*	4*	5*	5*
D	8	9*	5	9*
E	5*	6	5*	5*
F	8		5*	5*
G	7		5*	5*
H	4	3	5	
I	8			9
J	1			
K	5		8*	8*
L	1	1		6
M	7			
N	9*	9*	9*	8
O			9*	9*
P	9*	9*	5	

D1 = one day prior to the agonistic encounter, D2 = immediately after the agonistic encounter, D3 = one day after the agonistic encounter, D8 = six days after the agonistic encounter

9 = Extended tailflip; 8 =Tailflip; 7 = Fast retreat; 6 = Slow retreat; 5 =Long immobility; 4 = Medium immobility; 3 = Short immobility; 2 = No response; 1 = Meral spread

Table 5 Table showing response of dominant individuals to predatory stimulus. Same responses on different days highlighted with an asterisk (*)

Individual	Day 1	Day 2	Day 3	Day 8
A		5*	5*	7
B	7		9	
C	5	8		6
D	9	5*	5*	4
E		4*		4*
F				9
G	7	8	5	
H		5	8	9
I	9*	9*	6*	6*
J	5	9*	9*	9*
K		6	6	
L	2		5*	5*
M	9	5	8	
N		5	8	
O	9*		9*	9*
P	4		3	

D1 = one day prior to the agonistic encounter, D2 = immediately after the agonistic encounter, D3 = one day after the agonistic encounter, D8 = six days after the agonistic encounter

9 = Extended tailflip; 8 =Tailflip; 7 = Fast retreat; 6 = Slow retreat; 5 =Long immobility; 4 = Medium immobility; 3 = Short immobility; 2 = No response; 1 = Meral spread

4.5 Correlations between different behavioural traits and measures of aggression

Different aspects of behaviour were found to be significantly correlated with each other on several of the days of testing.

Statistical analysis revealed correlations between some of the behavioural traits measured during the behavioural trials on most of the days of testing.

On all days significant correlations were found between the amount of walking and number of retreats (day one: p = 0.0269; Spearman correlation, Table 6, Fig. 6; day two: p = 0.0151; Spearman correlation, Table 6; day three p = 0.00281; Spearman correlation, Table 6, day four p = 0.00252 Spearman correlation, Table 6) , as well as between the amount of walking and the time spent in the shelter (day one: p = 0.00185; Spearman correlation, Table 4, Fig. 7; day two: p = 0.0263; Spearman correlation, Table 4; day three p = 0.0202, Spearman correlation,Table 6; day four p = 0.000171; Spearman correlation,Table 6).

Further, on day two, significant negative correlations were found between the latency of emergence (p = 0.0404; Spearman Correlation,Table 6, Fig. 8) and the time spent walking, and between the number of retreats and the time spent in the shelter (p = 0.000289; Spearman correlation,Table 6, Fig. 9)

On day eight there was a significant positive correlation between the time spent in the shelter and the number of retreats (p = 0.00326; Spearman correlation, Table 6, Fig. 10) and a significant correlation between the number of retreats and the response to the predatory stimulus (p = 0.0381; Spearman correlation, Table 6)

In addition, statistical analysis also revealed correlations between measures of aggressive behaviour displayed during the agonistic encounter and behavioural traits.

On day one, a significant positive correlation (p = 0.0170; Spearman correlation, Table 6 and Fig. 11) between high offensive behaviour and the amount of walking was found and on day eight a significant negative correlation between low-offensive behaviour and the response to the predatory stimulus was found (p = 0.00536; Spearman correlation, Table 5 and Fig. 12)

Table 6 Table showing correlation coefficients (top) p-values (middle) and sample size ‘n’ (bottom) for correlations between behavioural parameters analysed during the behavioural trials and aggressive behaviour. An asterisk (*) indicates statistically significant correlations.

Day 1	Wal & Rtr*	-0.404 0.0269 30
	Wal & Sh*	0.547 0.00185 30
	Hoff & Wal*	0.433 0.0170 30
Day 2	Lat & Wal*	-0.376 0.0404 30
	Rtr & Sh*	0.643 0.000289 27
	Wal & Rtr*	-0.463 0.0151 27
	Wal & Sh*	-0.428 0.0263 27
	Loff & Stim	-0.462 0.0535 17
	Hoff & Em	-0.117 0.520 27
	Def & Em	-0.366 0.0507 27
Day 3	Wal & Rtr*	-0.546 0.00281 28
	Wal & Sh*	-0.437 0.0202 28
	Stim & Sh	0.386 0.0827 21
	Em & Rtr	-0.337 0.0637 31

Day 8	Wal & Rtr*	-0.551 0.00252 28
	Wal & Sh*	-0.650 0.000171 28
	Sh & Rtr*	0.538 0.00326 28
	Rtr & Stim*	0.455 0.0381 21
	Loff & Stim*	0.586 0.00736 20

Def = Defensive behaviour; Loff = Low-offensive behaviour; Hoff = High-offensive behaviour; Lat = latency of emergence; Wal = time spent walking; Sh = time spent in shelter; Rtr = number of retreats; Em = duration of emergence; Stim = response to the predatory stimulus

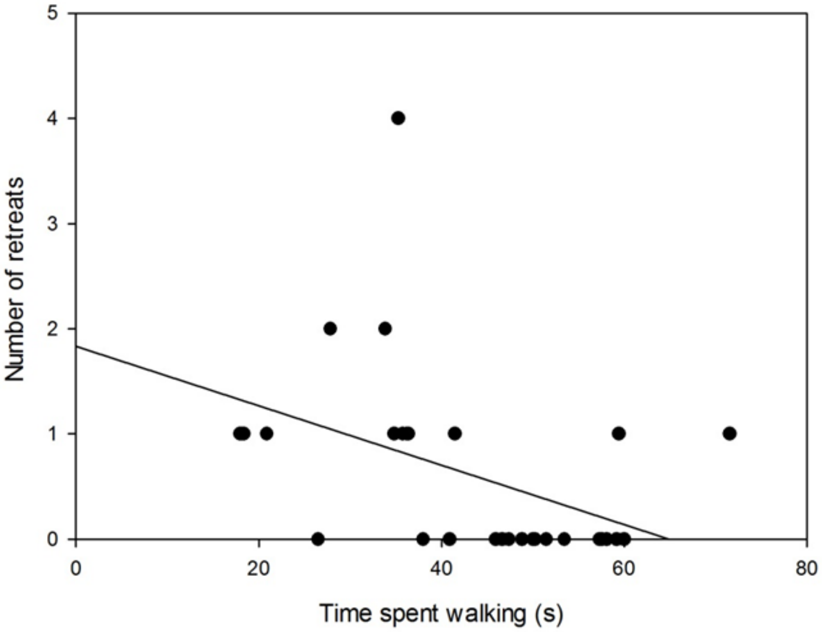

Fig. 6 Correlation between the time spent walking and the number of retreats displayed during the first behavioural trial (one day prior to the agonistic encounter). A regression line showing the correlation between the variables has been inserted for illustration.

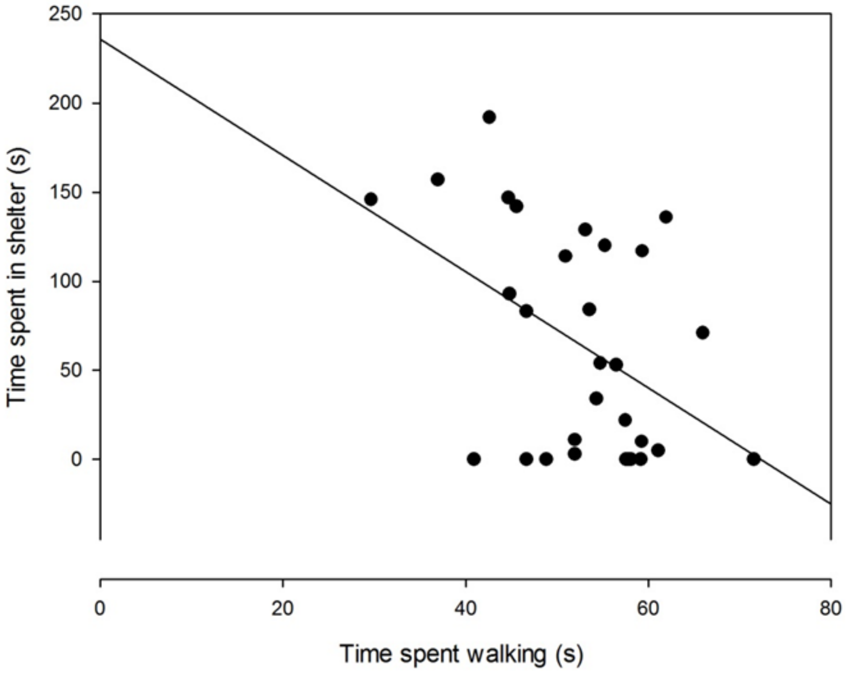

Fig. 7 Correlation between the time spent walking and the time spent in the shelter during the first behavioural trial (one day prior to the agonistic encounter). A regression line showing the correlation between the variables has been inserted for illustration.

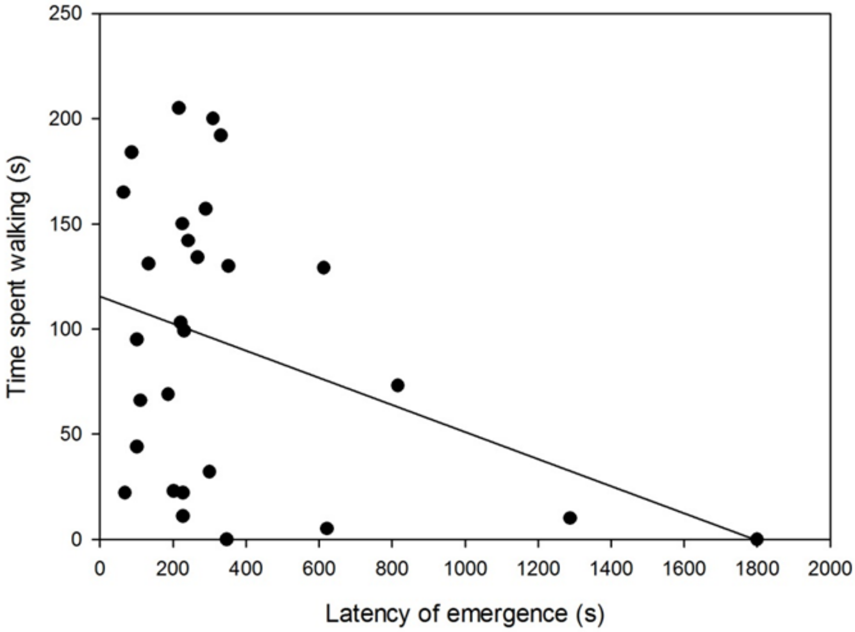

Fig. 8 Correlation between the latency of emergence and the time spent walking during the second behavioural trial (immediately after the agonistic encounter). A regression line showing the correlation between the variables has been inserted for illustration. Data were transformed (log + 1) for the analysis but raw data used for illustration.

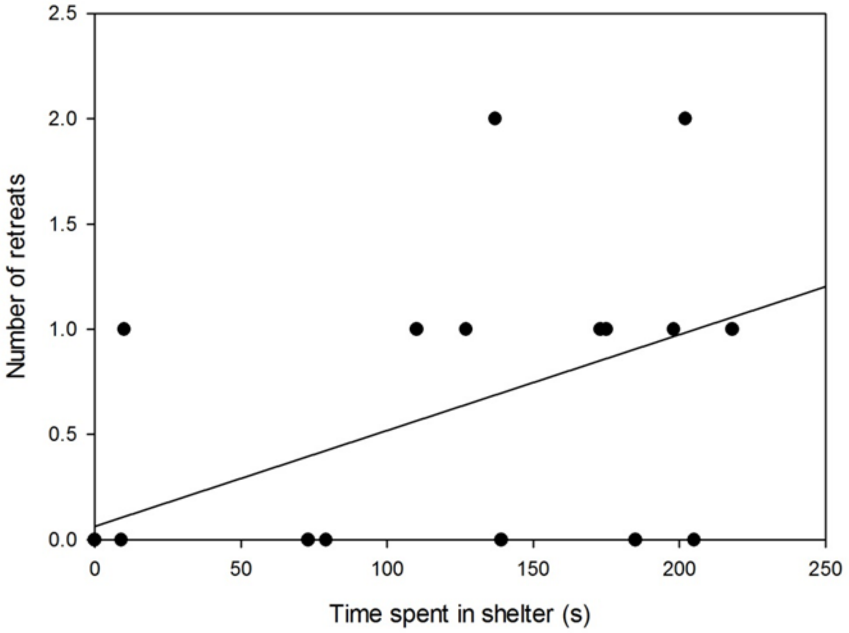

Fig. 9 Correlation between the time spent in the shelter and the number of retreats displayed during the second behavioural trial (immediately after the agonistic encounter) A regression line showing the correlation between the variables has been inserted for illustration.

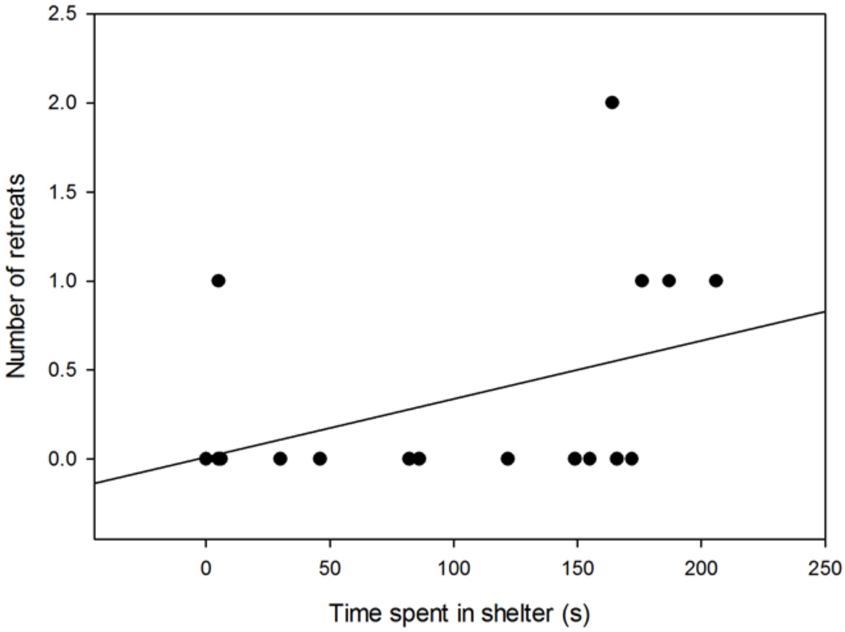

Fig. 10 Correlation between the time spent in the shelter and the number of retreats displayed during the final behavioural trial (five days after the agonistic encounter) A regression line showing the correlation between the variables has been inserted for illustration.

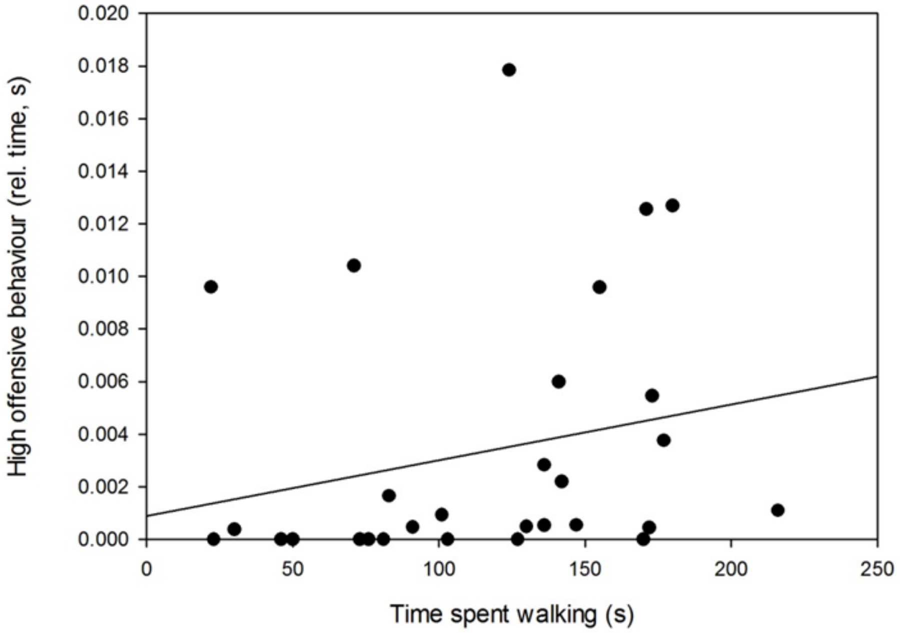

Fig. 11 Correlation between the time spent walking during the first behavioural trial (one day prior to the agonistic encounter) and relative amount of time spent in high-offensive behaviour during the agonistic encounter. A regression line showing the correlation between the variables has been inserted for illustration.

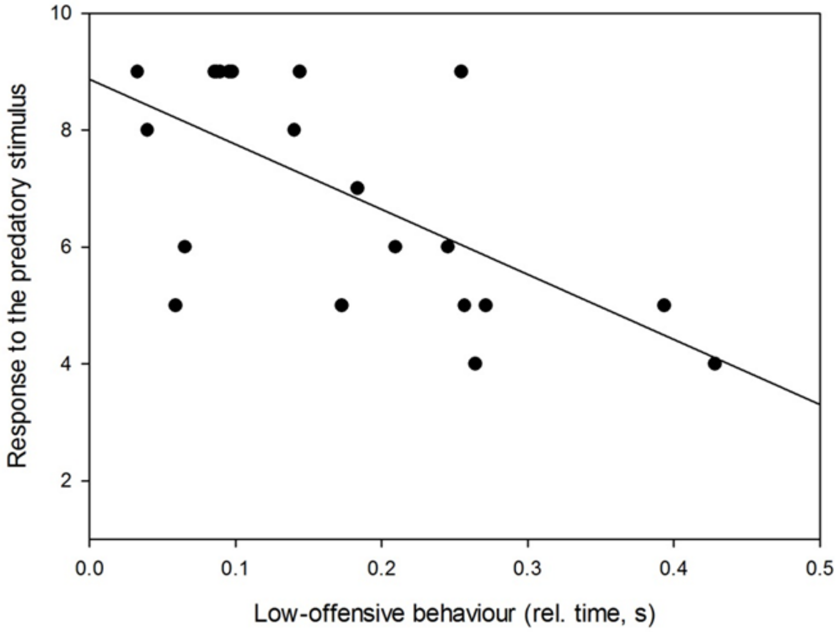

Fig. 12 Correlation between the relative amount of time spent in low offensive behaviour during the agonistic encounter and the response to the predatory stimulus in the final behavioural trial (six days after the agonistic encounter). A regression line showing the correlation between the variables has been inserted for illustration. Data were transformed (arcsin) for the analysis but raw data used for illustration.

For a full summary of all statistical results for all correlations see tables 1-4 of the appendix.

5 Discussion

The results of this study show that dominant and subordinate individuals differed significantly in one aspect of their aggressive behaviour, with dominants showing less defensive behaviour than subordinates (Fig. 3). They also differed in the consistency of their response to the predatory stimulus, with subordinates showing a high degree of consistency in their response - but dominants showing no consistency. In addition, the results revealed several correlations between aggressive and non-aggressive behaviours, thereby indicating the existence of behavioural syndromes.

Most notably, there was a correlation between the amount of low-offensive behaviour displayed during the agonistic encounter and the response to the predatory stimulus during the behavioural trials. Individuals which showed more low-offensive behaviour showed a weaker response to the predatory stimulus. This was statistically significant only on day eight (Fig. 12, Table 6) although on day two a trend towards this correlation was apparent. Furthermore, individuals which showed more high offensive behaviour during the agonistic encounter had also spent more time walking on the day prior to the encounter (Fig. 11, Table 6).

The fact that dominant and subordinate individuals did not differ significantly in their aggressive behaviour, save in the amount of defensive behaviour displayed, suggests that individuals were relatively evenly matched in their aggression and the outcome of the fight was determined by factors other than aggressive tendency. As illustrated in the introduction the outcome of an agonistic encounter is potentially influenced by a number of different factors (Goessmann *et al.* 2000). In this study it is likely that claw size was one of the factors determining the outcome of the fight, since claw size differed significantly between dominants and subordinates. The outcome of an agonistic encounter is decided when one contestant withdraws from the opponent. Research has shown that the winner of a bout continues to display aggression towards the loser (subordinate), such that the loser continues to retreat from the presence of the opponent (Huber et al 2001). Hence it is no surprise that subordinates show more defensive behaviour than dominants. This being the case one would however expect to see significant differences in low-offensive behaviour as well. It is possible though that the amount of aggression needed to deter the subordinate individual from any further aggression and thereby assert social status is minimal. This may be particularly true if there is a difference in physical attributes between contestants, as was the case in this study. The fact that in this study it appeared that contestants appeared to spend a considerable amount of time not being aggressive, which seems to confirm this idea.

The fact that at least one aspect of behaviour was consistent over time, does indicate the potential for personality existing in this study. It is unclear why dominant individuals should show a lack of consistency though it is possible that this is simply due to unintentional disturbance during the experimental procedure. It is intriguing, that dominants and subordinates differed in the consistency of their response to the aversive stimulus, but seemingly not in the strength of their response. The response to a predatory stimulus is an indication of boldness, which is a measure of the willingness to take risks (Sinn *et al.* 2008). As highlighted in section 2.1, boldness and aggressiveness are often positively correlated with each other Huntingford (1976). Since one would expect aggressive individuals are more likely to win an agonistic encounter and become dominant one would expect dominant individuals to also show a weaker (i.e. bolder) response to the predatory stimulus than subordinates. However the above results have already established that dominant and subordinates did not differ significantly in their aggressive behaviour. It is therefore perhaps no surprise that no significant differences were found between dominants and subordinates in regards to the strength of response to the predatory stimulus.

Nevertheless, the fact that individuals which showed more low offensive behaviour tended to show a weaker response to the predatory stimulus confirm the existence of a boldness-aggressiveness syndrome in this study. Thus it confirms the finding the boldness-aggressiveness syndrome is common across several taxa including crustaceans (Pintor *et al.* 2008, Reaney & Blackwell 2007, Mowles *et al.* 2012). Correlations were also found between boldness and activity: Individuals which emerged from the shelter earlier occasionally showed more activity. Likewise individuals which were more active showed a weaker response to the predatory stimulus. The fact that boldness, aggression and activity all appear to form a behavioural syndrome is not surprising. The linkage of these traits is due to the fact that together they reflect the proactive and reactive coping styles described in the introduction. Since proactive individuals respond actively to challenges, it is no surprise that they tend to be more bold and aggressive as well as more active. Reactive individuals show the opposite trends. Previous studies found that dominant individuals tended to show a more proactive behavioural response whilst subordinate individuals tended to show a reactive response (Overli *et al.* 2004, Pottinger

& Carrick 2001). Since dominants and subordinates did not differ in their low-offensive behaviour, the results of this study do not appear to support these findings. However, individuals who spent more time walking on the day prior to the agonistic encounter also displayed more high-offensive behaviour during the agonistic encounter, which suggests that aggression can be predicted by previous behaviour. Since low-offensive behaviour was significantly correlated with defensive behaviour and defensive behaviour differed significantly between dominants and subordinates, there is a possibility that social status may be predictable prior to an agonistic encounter. Once again this would corroborate with previous studies (Dahlbohm *et al.* 2011).

The lack of further significant differences in other aspects of behaviour which were investigated, such as latency of emergence (the other measure of boldness used in this study) is somewhat surprising, given that previous studies found differences in these or similar aspects of behaviour (Colleter & Brown 2011, Verbeek 1996, Fox *et al.* 2009). Thus, Colleter & Brown (2011) found that in rainbow fish, high ranked males emerged earlier from a shelter than lower ranked ones. High ranked individuals were also more active. Some of the difference is likely to be due to differences in methodology. Whereas boldness was investigated in isolation, activity was measured in the home aquarium in the presence of other conspecifics. Since individuals which became dominant in the agonistic encounter most likely already had a high rank in their home aquarium it is no surprise that their activity would have been a reflection of their rank and it is no surprise that higher ranked individuals showed more activity in the presence of conspecifics than lower ranked individuals. Other studies also confirm the importance of social context for the expression of personality traits. Herberholz *et al.* (2003) examined burrowing behaviour of dominant and subordinate crayfish before, during and after an agonistic encounter and found that the two groups showed statistically significant differences only during the agonistic encounter. Once removed from the presence of the opponent, differences still persisted but were no longer significant. Similarly, Song *et al.* (2006) found that subordinates differed in their response to a tactile stimulus only in the presence of the opponent.

In this study, non-aggressive behaviours were not investigated in the presence of the opponent, rather dominant and subordinates were investigated in separate tanks, which may be part of the reason for a lack in significant differences. Though there were no significant differences in boldness or activity between subordinates the fact that the latency of emergence was significantly higher for both dominants and subordinates following the agonistic encounter suggests that agonistic experience did have a negative effect on boldness. However, it is possible that the observed differences in emergence latency between the first behavioural trial and the subsequent related behavioural trials might not have been solely an effect of the agonistic experience, but in part a result of various unintended disturbances occurring during the experimental procedure.

It is clear that the results go some way in answering the questions set out at the beginning of this research. However there are still some outstanding questions:

One important question that this study sought to address was whether the behavioural tendencies of dominants and subordinates were consistent over time. Since consistency is a key criterion for personality, its existence would indicate that dominant and subordinate individuals showed different personalities (as opposed to just different behavioural syndromes). As highlighted in the beginning of the discussion, consistency in response to the predatory stimulus was found among subordinates. Yet it was not found in any other aspect of behaviour. Research suggests that some behavioural traits are more repeatable than others (Bell *et al.* 2009). The response to the predatory stimulus may be one of them. It is effectively a startle response, and as such may be considered analogous to similar responses found in other species such as hermit crabs. When threatened, hermit crabs withdraw into their shell obstructing the aperture with their major cheliped (Briffa *et al.* 2008). The duration of such a startle response (i.e. the time until re-emergence) has been demonstrated to be repeatable across different situations in these animals (Briffa *et al.* 2008). One possible reason for this is that startle responses appear to be fairly simple behaviours and may therefore not show much plasticity within individuals as other behaviours.

The expression of the other behaviours that were investigated may be much more susceptible to environmental influences and the degree of plasticity in these behaviours may be considerably higher. During the experimental procedure specimens were exposed to several potential disturbances which could have influenced crayfish behaviour and therefore confounded the possibility of consistency being expressed in these behaviours. Particular factors which could have influenced crayfish behaviour include the transportation and handling of the specimens. Thus, prior to the start of a small number of specific experimental stages, tail flipping was noticed indicating a possible disturbance. On occasions where disturbance was deemed to be severe, specimens were given a longer acclimation time to recover. Further environmental challenges such as fluctuations in water temperature and unintentional variations in experimental regime could also have affected the behaviour of the specimens.

In other studies specimens were generally not handled between successive trials. Thus Vainikka *et al.* (2011) when testing for consistency of shelter use in crayfish in the presence and absence of predatory stimuli (both physical and olfactory) introduced specimens into the experimental tanks after a control period and measured their behaviour over successive days during which there was no disturbance apart from intentional disturbance which was meant to mimic a predator. Similarly, Verbeek et al (1994) did not move subjects from the experimental cage when testing the repeatability of the response to a novel object in great tits. It seems likely that with fewer disturbances in between behavioural trials and an improved methodology more positive results with regards to consistency in latency of emergence and activity could be found.

In conclusion, the results do seem to provide some support for the hypothesis that dominant and subordinate individuals differ in their behavioural tendencies. They demonstrate dominants and subordinates differed significantly in their defensive behaviour, indicating that social status acquisition is the result of agonistic experience. The two groups also differed to some extent in their non-aggressive behaviour which suggests that differences in behavioural consistency might exist between them. Correlations between aggressive behaviour and non-aggressive behaviours suggest the existence of a behavioural syndrome. Yet further research is needed to investigate the lack of more striking differences between dominants and subordinates, as well as the lack in consistency of behaviours.

The research field of behavioural tendencies is still growing and the role that social factors play in their development and expression is still not well understood. The agonistic behaviour of crayfish provides significant scope for investigating, in particular, the relationship between behavioural tendencies and social rank. It is hoped that this study can provide a framework for future studies to build on.

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7 Appendix

Table 1 Matrix showing correlation coefficients (top) p-values (middle) and sample size ‘n’ (bottom)for correlations between behavioural parameters analysed during the behavioural trial on day one (prior to the agonistic encounter) and aggressive behaviours displayed during the agonistic encounter. An asterisk (*) indicates statistically significant correlations.

	Loff	Hoff	Lat	Wal	Sh	Rtr	Em	Stim
Def	-0.242 0.195 30	-0.379 0.0387* 30	0.0103 0.955 30	-0.0072 0.968 30	-0.119 0.526 30	-0.0304 0.871 30	-0.0114 0.951 30	-0.140 0.510 24
Loff		0.395 0.0309* 30	0.144 0.445 30	0.116 0.543 30	-0.0492 0.794 30	-0.303 0.102 30	-0.249 0.236 30	-0.0167 0.929 24
Hoff			-0.0205 0.875 30	0.433 0.0170* 30	-0.300 0.106 30	-0.274 0.141 30	-0.0934 0.621 30	-0.0993 0.641 24
Lat				-0.246 0.188 30	0.196 0.295 30	0.0906 0.619 30	0.0503 0.783 32	0.330 0.106 25
Wal					-0.404 0.0269 30	-0.547 0.0019* 30	-0.130 0.488 30	-0.264 0.209 24
Sh						0.552 0.0017* 30	-0.0164 0.931 30	0.341 0.102 24
Rtr							0.117 0.536 30	0.355 0.0877 24
Em								0.259 0.208 24

Def = Defensive behaviour; Loff = low offensive behaviour; Hoff = high offensive behaviour Lat = latency of emergence; Wal = time spent walking; Sh = time spent in shelter; Rtr = number of retreats; Em = duration of emergence; Stim = response to the predatory stimulus

Table 2 Matrix showing correlation coefficients (top) p-values (middle) and sample size 'n' (bottom)for correlations between behavioural parameters analysed during the behavioural trial on day two (immediately after the agonistic encounter) and aggressive behaviours displayed during the agonistic encounter. An asterisk (*) indicates statistically significant correlations.

	Loff	Hoff	Lat	Wal	Sh	Rtr	Em	Stim
Def	-0.242 0.195 30	-0.379 0.0387 30	0.134 0.478 30	-0.0041 0.981 28	0.0828 0.689 25	-0.140 0.483 27	-0.356 0.0679 27	0.0217 0.928 17
Loff		0.395 0.0309* 30	-0.212 0.258 30	0.187 0.337 28	-0.180 0.385 25	0.260 0.189 27	0.0752 0.707 27	-0.462 0.0535 17
Hoff			0.106 0.574 30	-0.0983 0.615 28	-0.0778 0.709 25	-0.117 0.557 27	0.355 0.0689 27	0.246 0.336 17
Lat				-0.376 0.0404* 30	0.196 0.325 27	0.129 0.501 29	-0.0127 0.946 29	-0.0285 0.908 18
Wal					-0.428 0.0263 27	-0.463 0.0151* 27	-0.0936 0.640 27	-0.134 0.592 18
Sh						0.643 0.0003* 27	-0.0266 0.892 27	0.323 0.186 18
Rtr							-0.142 0.459 29	-0.0189 0.934 18
Em								0.245 0.321 18
Stim								

Def = Defensive behaviour; Loff = low offensive behaviour; Hoff = high offensive behaviour Lat = latency of emergence; Wal = time spent walking; Sh = time spent in shelter; Rtr = number of retreats; Em = duration of emergence; Stim = response to the predatory stimulus.

Table 3 Matrix showing correlation coefficients (top) p-values (middle) and sample size ‘n’ (bottom)for correlations between behavioural parameters analysed during the behavioural trial for correlations between behavioural parameters analysed during the behavioural trial on day three (one day after the agonistic encounter) and aggressive behaviour displayed during the agonistic encounter. An asterisk (*) indicates statistically significant correlations.

	Loff	Hoff	Lat	Wal	Sh	Rtr	Em	Stim
Def	-0.242 0.195 30	-0.379 0.0387* 30	0.0974 0.606 30	-0.0487 0.806 27	-0.180 0.367 27	-0.0151 0.934 30	0.0132 0.944 30	-0.0464 0.832 22
Loff		0.395 0.0309* 30	0.0149 0.936 30	0.307 0.113 27	-0.104 0.601 27	-0.130 0.490 30	-0.0666 0.724 30	-0.345 0.114 22
Hoff			0.0267 0.886 30	0.0776 0.698 27	-0.0834 0.675 27	-0.256 0.169 30	0.275 0.140 30	0.0556 0.801 22
Lat				0.0594 0.760 28	0.0722 0.712 28	-0.147 0.428 31	0.228 0.215 31	0.118 0.588 23
Wal					-0.437 0.0202* 28	-0.546 0.0028* 28	0.367 0.0546 28	-0.500 0.0210* 21
Sh						0.559 0.0021* 28	-0.186 0.341 28	0.386 0.0827 21
Rtr							-0.337 0.0637 31	0.207 0.338 23
Em								0.0418 0.848 23
Stim								

Def = Defensive behaviour; Loff = low offensive behaviour; Hoff = high offensive behaviour Lat = latency of emergence; Wal = time spent walking; Sh = time spent in shelter; Rtr = number of retreats; Em = duration of emergence; Stim = response to the predatory stimulus

Table 4 Matrix showing correlation coefficients (top) and p-values (bottom) for correlations between behavioural parameters analysed during the behavioural trial on day eight (six days after the agonistic encounter) and aggressive behaviours displayed during the agonistic encounter. An asterisk (*) indicates statistically significant correlations.

	Loff	Hoff	Lat	Wal	Sh	Rtr	Em	Stim
Def	-0.242 0.195 30	-0.379 0.0387* 30	0.0566 0.764 30	0.141 0.461 29	-0.107 0.590 27	-0.246 0.205 28	-0.178 0.362 28	0.171 0.468 20
Loff		0.395 0.0309* 30	-0.0142 0.938 29	0.169 0.378 27	-0.0417 0.835 27	-0.147 0.453 28	-0.0167 0.930 28	0.580 0.0074* 20
Hoff			0.00306 0.987 30	0.139 0.469 29	-0.0599 0.764 27	0.204 0.294 29	0.245 0.206 28	0.304 0.189 20
Lat				-0.297 0.110 30	0.0450 0.818 28	0.101 0.601 29	0.0250 0.895 29	0.226 0.320 20
Wal					-0.650 0.0002* 28	-0.551 0.0025* 28	0.164 0.399 28	-0.0493 0.831 20
Sh						0.538 0.0033* 28	0.0235 0.904 28	0.179 0.444 20
Rtr							-0.0986 0.608 29	0.455 0.0381 21
Em								-0.315 0.160 21
Stim								

Def = Defensive behaviour; Loff = low offensive behaviour; Hoff = high offensive behaviour Lat = latency of emergence; Wal = time spent walking; Sh = time spent in shelter; Rtr = number of retreats; Em = duration of emergence; Stim = response to the predatory stimulus

Table 5 Differences in claw size between dominant and subordinate individuals ($p = 0.002$, $t = 3.806$, $df = 13$; paired t-test). Two pairs were excluded from the analysis since measurements for at least one of each pair were not available

	Dominants	Subordinates
Claw size (mean)	43.071	39.188
SEM	0.820	1.001
Sample size (n)	14	16