Effects of reflection and social isolation on crayfish behaviour.

by

Joanna K. Drozdz

A Thesis
submitted to the Department of Biological Sciences
in partial fulfillment of the requirements
for the degree of
Master of Science

October, 2005
Brock University
St. Catharines, Ontario
Canada

© Joanna K. Drozdz, 2005
Abstract

Visual stimuli and socialization influence exploratory behaviour in crayfish. The predominant components of spontaneous exploratory behaviour were determined by observing the activity of solitary adult crayfish (*Procambarus clarkii*) in a glass aquarium containing fresh water and no objects. Five distinct behaviours were observed: rearing up (climbing on the wall), turning around, cornering (facing the corner), backward walking, and crossing (crossing the midline of the aquarium). The frequency of rearing up, cornering and turning around decreased when reflection from the glass wall was blocked with black cardboard, black paint or non-reflective transparent plastic. In a tank containing mirrors on one side and non-reflective plastic on the other, crayfish cornered, reared up, and turned around more in front of the mirrors. Socialization was necessary for crayfish to respond to the reflection. Crayfish that were housed in pairs for two weeks exhibited more rearing up, turning around and cornering in front of the mirrors than in the non-reflective side. Crayfish isolated for two weeks did not show these differences. Socialized crayfish also exhibited more rearing up, turning around and cornering than did isolated crayfish. Thus, crayfish respond to visual stimuli provided by a glass tank, but the responds depends on socialization.
Acknowledgments

I would like to thank my supervisor Dr. A. Joffre Mercier for guidance, patience and insight. I would like to thank Dr. G. Spencer and Dr. S. Brudzynski for their constructive criticism and valuable input during many committee meetings.

Chapters 2 and 3 of this thesis are being submitted to the Journal of Crustacean Biology.
Chapter 3

The influence of social isolation on behaviour in reflective and non-reflective environment 59

Abstract 60
Introduction 61
Materials and methods 63
Results 65
Discussion 71

Chapter 4

General conclusions 74
Perspectives 75

References 83
List of Tables

Table 2.1 Average total distance covered, speed and parallelism index 52

Table 3.1 Average of behavioural responses for socialized and isolated P. clarkii 68

List of Figures

Figure 1.1 Basic crayfish anatomy 11

Figure 2.1 Examples of rearing up and cornering in solitary P.clarkii in novel tank 39

Figure 2.2 Example of turning around in solitary P.clarkii in novel tank 40

Figure 2.3 Schematic diagram of mirror/matte tank 42

Figure 2.4 Schematic representation of experimental set up employed in Digiscan Activity Monitor 45

Figure 2.5 Behavioural patterns of P. clarkii in transparent and cardboard line tanks 47

Figure 2.6 Behavioural patterns of P. clarkii in transparent and matte tanks 49

Figure 2.7 Behavioural patterns of P. clarkii in mirrored and matte sides of a tank 51

Figure 2.8 Behavioural patterns of P. clarkii in mirrored and matte sides of a tank (Digiscan Activity Monitor) 53

Figure 3.1 Behavioural patterns of P. clarkii after two weeks in social isolation 66

Figure 3.2 Behavioural patterns of P. clarkii after two weeks of socialization 67

Figure 3.3 Time spent on each side of the tank (mirror vs. matte) 70
Chapter 1

Introduction

This thesis examines the effects of reflection and social isolation on crayfish behaviour. Invertebrates display surprisingly complex behaviours despite the relative simplicity of their nervous systems. There have been many behavioural studies utilizing invertebrates, particularly crustaceans. Crayfish, crabs and lobsters display such complex behaviours as aggression, mating, searching (exploration), feeding and molting. Each of these behaviours involves a series of coordinated activities that are ultimately controlled by the central nervous system. Sensory cues can elicit and shape such behaviours through their actions on the central nervous system. The goal of this thesis is to provide a better understanding of the role sensory inputs play in eliciting specific behaviours.

When introduced to a new environment most animals immediately display exploratory activity. This behaviour is guided continuously by inputs from chemosensory, mechanosensory and visual receptors. The primary purpose of this thesis is to study the role of visual inputs during exploration. The work focuses specifically on whether or not crayfish respond to their own mirror image when exposed to a novel environment. The work was inspired by my preliminary observations of crayfish exploring glass aquaria. Their behaviour suggested to me that crayfish can respond to their mirror images. There is extensive literature on responses of many animals to their reflection (see Literature Review), but only two such studies (Dunham and Tierney, 1986; Hazlett 1966c) had been performed with a crustacean (the hermit crab). I designed several experiments to demonstrate unequivocally that crayfish respond to their mirror image. I identified a series of behaviours that crayfish exhibit during exploration and sought to determine whether any were influenced by a reflective environment. The effects of reflection on other quantitative parameters of
exploration, such as average speed of progression, total distance traveled and degree of turning, were also determined.

Social isolation is known to influence exploratory behaviour in many animals (see Literature Review). This effect is thought to involve a lack of socially related sensory inputs, which can either enhance or reduce exploratory activity when the animal is returned to social conditions. It was of interest, therefore, to examine the effects of social isolation on the responses crayfish exhibit to mirrors when they explore a new environment. The strategy was to isolate crayfish for two weeks and then observe their behaviour in a test tank, half of which was lined with mirrors and half of which was lined with non-reflective plastic. Their responses were compared to those of crayfish maintained in pairs for the same duration.

These experiments investigated the question of whether or not reflection-dependent behaviours require continuous socialization. Overall, this thesis examines the extent to which behaviour is influenced by past social experience and current visual inputs.
Literature Review

1. Physical environment and exploration.

1.1. Animal exploration

Understanding the effect of different visual environments on crayfish behaviour requires an understanding of the way in which animals become familiar with novel environments. Welch (1965) stated that every environment exerts its own characteristic stimulation to which animals respond both psychologically and physiologically, in a “sensitive and continuous manner”. Such responses are components of any behavioural act and the range of their manifestations extends from elementary orientational responses to exploratory or searching activity.

The ability of animals to survey or search their environment provides several selective advantages that increase their chance of survival and reproduction. Exploratory behaviour is a prerequisite to feeding behaviour (O’Brien et al., 1986), shelter seeking (Boal et al. 2000) and mate finding (Uy et al., 2001). Exploratory behaviour can serve to acquaint animals with their surroundings by surveying the environment, often more than once. Kleerekoper et al. (1974) showed that goldfish (*Crassius aratus*) established a familiar area by gradually expanding the size of the area occupied in a tank. Meadow voles (*Microtus pennsylvanicus*) learn the topography of their environment by gathering information that becomes important later in avoiding predation (Ambrose, 1972).

Exploratory activity in crustaceans is commonly observed in novel environments (Cromarty et al., 1999; Rubenstein and Hazlett, 1973). In comparison to mammals, invertebrates display similar responses to neutral environments such as orienting reflexes and investigatory activity. In addition, measurements of heart activity suggest that crayfish
constantly sample their surroundings for information about predators, conspecific intruders, currents, shadows and food availability (Shuranova and Burmistrov, 1996).

Searching behaviour depends on physical characteristics of the environment (e.g. lighting and visual input) as well as the animal’s past experience (e.g. social isolation).

1.2. Strategies for exploration

When placed in a novel environment crayfish rapidly learn to discriminate between changing spatial configurations (Basil and Sandeman, 2000, Sandeman and Varju 1988; Varju and Sandeman, 1989). For example, Basil and Sandeman (2000) demonstrated that crayfish (Cherax destructor) detect differences in the spatial configuration of the area topography. Crayfish quickly responded not only to the presence or absence of partitions in the tank but also to changes in the position of the partitions.

Generally, animals begin exploration by walking around the perimeter, an activity known as thigmotaxis – one of the initial exploratory behaviours in novel environments common to many animals. Thigmotaxis is the tendency of an animal to remain in contact with vertical objects rather than traveling through open spaces. Thigmotaxis is a general tendency of many animals including earthworms (Lumbricus terrestris) (Doolittle, 1971), fresh water mussels (Limnoperna fortunei) (Uryu et al., 1996), Paramecium (Iwatsuki et al., 1995; Sikora et al. 1992) and rats (Meyer and Meyer, 1992; Treit et al., 1989). Also, lobsters (Homarus americanus) spend the vast majority of their locomotor activity exploring the outer edge of the area in which they are placed (Snyder et al., 2000).

Studies show that thigmotaxis is displayed in crayfish as young as 30-90 day old juveniles (Burba, 1999). Varju and Sandeman (1989) observed that adult crayfish (C. destructor) use both antennae and legs (for basic crayfish anatomy please refer to Figure 1.1) to keep contact with the wall of the tank as they explore it. Another species of crayfish,
Figure 1.1  Basic crayfish anatomy
*Orconectes virilis*, exhibit a slow exploratory behaviour while walking around in the tank during early evening hours (Rubenstein and Hazlett, 1973). When placed in an empty aquarium, blindfolded *C. destructor* explore by walking along the walls (Basil and Sandeman, 2000). Blind crayfish (*Orconectes australis packardi*) display thigmotaxis and only travel into the center of the tank indicating that they finally obtained familiarity with their new environment (Li and Cooper, 2001). *Procambarus clarkii* when placed in an experimental aquarium explores throughout the available space, while touching the walls with their chelae and antennae (Delgado-Morales et al., 2004). The fact that many animals exhibit thigmotaxis during exploration implies that tactile inputs are important.

1.3. Factors influencing exploration

1.3.1. Tactile cues

Animals use tactile cues extensively to explore their environment, and it is generally accepted that crayfish are well provided with mechanosensory and proprioceptive information from the antennal flagella. For example, when visual inputs were blocked by eliminating environmental illumination, increased use of tactile cues was observed in *Orconectes propinquus* (Smith and Dunham, 1990). These crayfish carried their chelipeds higher and held their chela open wider in the dark, resulting in a higher sampling rate of tactile information, as compared to animals supplied with visual cues from an illuminated environment. Tactile inputs, received through the extended chelipeds, probably served as a partial substitute for missing visual input due to darkness in this crayfish species. Similar conclusions were reached by blindfolding the animals. Basil and Sandeman (2000) showed that blindfolded crayfish walked along the walls of the arena and explored the tank using their antennae. In this experiment, crayfish exploration was impaired when the antennae were lesioned. Naturally blind cave crayfish also use their antennae extensively to explore
their environment (Li and Cooper, 2001). Sandeman and Varju (1988) showed that experimentally blinded crayfish (*C. destructor*) gain positional information through the flagellum of their second antennules. This experiment was designed in such a way that it eliminated visual cues and consequently showed that crayfish can adapt to living without sight by relying on tactile cues. Kraus-Epley and Moore (2002) have shown that crayfish (*Orconectes rusticus*) with antennules either completely or partially ablated are no longer able to locate an odor source, indicating an impairment of orientation capabilities. Rutherford et al. (1996) inferred that antennules in *O. rusticus* are used to pick up mechanoreceptive cues as well as chemoreceptive cues. Therefore, organs such as chelipeds, chela, antennules and antennae are used to obtain information through tactile and chemoreceptive cues.

### 1.3.2. Chemoreceptive cues

Chemoreception is used in locating odor sources and can provide information relevant to courtship, food source and social dominance in many animals including crustacean species.

Chemoreceptive cues can aid exploration and are used in locating odor sources in snails (Rohrkasse and Atema, 2000), foraging in cephalopod mollusks (Basil et al., 2000), and foraging in crustaceans such as lobsters (*H. americanus*) (Basil and Atema, 1994; Kozlowski et al., 2001). Lobsters, for example, are capable of navigation based on the characteristics of rapid odor signals carried by currents or urine-based pheromones spreading in the sea. It is even inferred that the ability to recognize individuals in male and female pairs of *H. americanus* is based on olfactory recollection (Atema, 1995). Kozlowski et al. (2003) showed that crayfish (*Orconectes rusticus*) oriented more successfully toward odors released continually compared to release in pulses. *O. rusticus* were also capable of
following food-odor stimuli in variety of habitats (i.e. sand, cobble) (Moore and Grills, 1999).

Detection of sex pheromones from a conspecific has been noted in various crustaceans (Dunham, 1988; Hazlett, 1985a,b; Tierney and Dunham, 1982; Tierney et al., 1984). Hazlett (1985b) showed that *O. virilis* males respond differently to water from a tank occupied only by females than to water taken from a tank containing only males. Tierney and Dunham (1982) showed that *O. virilis* and *O. propinquus* males and females only orient toward the chemicals of their own conspecific of the opposite sex. Additionally, it was established that outer antennular flagella mediates pheromone reception in *O. propinquus* (Tierney et al. 1984) and *P. clarkii* (Ameyaw-Akumfi, 1976).

Pheromone release also communicates information about the social status of another conspecific in crayfish interactions (Dunham, 1988; Schneider et al., 1999; Thorp and Ammerman, 1977). Thorp and Ammerman (1977) demonstrated that *Procambarus acutus acutus* and *P. clarkii* become more aggressive after receiving chemical information from a stressed conspecific than when exposed to water lacking this chemical cue. Bechler et al. (1988) inferred that in *P. clarkii* and *P. a. astacus* pheromonal communication can help recognize their own conspecific. Also, urine contains a dominance signal and is an important chemoreceptive cue in lobster (*H. americanus*) (Breithaupt and Atema, 1993) and many crayfish species (Breithaupt and Eger, 2002).

### 1.3.3. Visual cues

When exploring a novel environment an animal typically obtains information about its conspecifics (e.g. for agonistic encounters or courtship), its prey and its predators using vision. Visual cues undeniably alter behaviour, and there are several examples of this in studies of various crustacean species.
1.3.3.1. Agonistic encounters

Vision is important for agonistic communication in many decapod species including fiddler crabs (Crane, 1966), mantis shrimp (Dingle, 1969), lobsters (Scrivener, 1971), crabs (Dunham and Tierney, 1983; Dunham et al., 1986; Wright, 1968) and various crayfish species.

Cryptic (camouflaged) hermit crabs (*Pagurus marshi*) have been valuable subjects in the study of agonistic behaviour because they depend on vision for shell exchanges with each other, manifested through complex ‘shell fights’. Visual communication through social signals during these interactions is crucial and frequent. Experimental removal of epibiota and detritus from the legs and shell of cryptic hermit crabs (*P. marshi*) increases communication efficiency during agonistic bouts with conspecifics (Dunham and Tierney, 1983). Also, mirrors proved to be an interesting and essential visual tool in studies of social interactions in these cryptic crabs. First, Hazlett (1966c) showed that cryptic hermit crabs (*P. marshi*) respond less readily to mirrors than non-cryptic species in which 80% of tested animals responded. Dunham and Tierney (1986) further supported this hypothesis with evidence that *P. marshi* individuals with less cryptic epibiotic cover respond sooner to their mirror image than do more cryptic individuals. The amount of adherent cover on the shell was inversely proportional to the time that it took the animal to produce its first social response in front of a mirror image.

Many crayfish species are also territorial and will protect their environment and repel intruders or competing conspecifics. As with other decapods, the initiation and the outcome of agonistic interactions in crayfish is widely believed to be partially dependent on the input from visual sensory organs. Bovbjerg (1953, 1956) demonstrated that vision was important during agonistic interactions and was used in the status hierarchy formation (i.e. submissive
and dominant animals) in the crayfish *O. virilis* and *Procambarsus alleni*. Also, in *O. rusticus* visually mediated behaviour was demonstrated by Bruski and Dunham (1987) to decrease in frequency as general visibility diminished. Crayfish have been described to respond to visual cues so profoundly that they can detect such details as specific postures during agonistic encounters (Rutherford et al., 1995) and antennule movements (Rutherford et al., 1996). Rubenstein and Hazlett (1973) also demonstrated that crayfish (*O. virilis*) presented with a model of a conspecific with certain postures and anatomic details assumed different postures accordingly. From the responses to various crayfish models it was concluded that spread postures, revealing the white areas, are more aggressive than the tight chelae postures that obscure whiteness. Meral spread (a behaviour in which the chelae are spread apart and raised) is a distinct visual cue for sighted crayfish. The comparison between social behaviours of sighted crayfish and those of blind crayfish (*O.a.packardi*) revealed that blind crayfish do not perform meral spread (Cooper et al., 2001). Delgado-Morales et al. (2004) provide evidence that once the social status has been established, in order to maintain it *P. clarkii* need to activate just one sensory modality (i.e. visual, tactile and olfactory) in order to gather information about conspecific status.

In appeasement displays, the use of motion of various structures, including antennae and chelate ambulators, was demonstrated in *O. virilts, P. clarkii* and *O. propiquus*. Rubensten and Hazlett (1974) reported a behaviour called ‘antennal wave’, which they inferred to be an indication of the acceptance of defeat during fighting in *P. clarkii* and other crayfish species. During such appeasement displays *O. virilis* was shown to detect the long and the short antennae (Ameyaw-Akumfi, 1979).
1.3.3.2. Prey and predators

A species of climbing crab, *Sesarma leptosoma*, has been used to examine how crabs use visual stimuli to distinguish between predators and prey. These animals react (by stopping in their normal migratory flow) to open claws of the perceived predator the crab *Epixanthus detatus* (Cannicci et al., 2002). Therefore, *S. leptosoma* show considerable ability to perceive shape and specifically to react appropriately to open claws of their predator. Fiddler crabs (*Uca pugilator*) display spatially sensitive visual recognition capabilities as they are able to identify their predators (i.e. flying birds) only when the image appears above their heads. If the image appears below the horizon it is identified as a conspecific, non-predator such as mate (Land et al., 1995).

Crayfish have developed the tail flip response to escape predators, and the frequency of occurrences of this behaviour seems to be dependent on visual inputs. Kellie et al. (2001) have shown that crayfish that live in complete darkness (*O. a. packardi*) are more likely to tail flip than sighted crayfish like *P.clarkii*. Therefore, sighted crayfish have an advantage of being able to use visual information to recognize predators and, hence, avoid the extensive use of tail flip response. Crayfish lacking vision must have more sensitive but less specific tactile sensory inputs, resulting in a low threshold for the tail flip response. Li and Cooper (2002) showed that *O. a. packardi* not only display more rapid tail flips, but also show fewer visual displays and postures (i.e. raised meral spread) as compared to sighted crayfish (*O. rusticus*) and lobsters (*H. americanus*).

1.3.3.3. Courtship

Visual communication is important for social communication during courtship in *P. clarkii* (Ameyaw-Akumfi, 1981; Dunham and Oh, 1996) and *Orconectes nais* (Pippet, 1977). *P. clarkii* females are capable of discriminating sex based exclusively on visual or
chemical information received from a conspecific. Furthermore, female *P. clarkii* are attracted to visual and chemical stimuli from males and avoid chemical and visual stimuli from females (Dunham and Oh, 1996).

1.3.3.4. Complex behaviours

The behaviour of animals in any given situation has been shown to depend on their previous visual experience. Presentation of a conspecific model in the form of a dried specimen affected how hermit crabs (*Calcinus tibicen*) responded subsequently to a live conspecific. The two most common aggressive displays exhibited by *C. tibicen* are cheliped extension and the ambulatory raise. These are stereotyped upward-outward movements of appendages. In cheliped extension model the animal has ambulatory legs in neutral position and chelipeds in an extended position. In ambulatory raise model chelipeds are in neutral position and ambulatory legs in raised position. The animals were initially presented with a cheliped extension model and ambulatory raise model. Three hours later all animals were placed together and it was observed that during aggressive interactions cheliped displays were much more frequently executed at the cheliped extension model. Ambulatory raises were more frequently executed at the ambulatory raise models. Therefore, it has been concluded that seeing a display (i.e. the cheliped extension or the ambulatory raise model) temporarily changes the probability of execution of that display in the future by hermit crab (Hazlett, 1966b).

Eisenstein et al. (2000) showed that vision plays an important role in formation of species specific habits in *P. clarkii*, suggesting that the optic ganglia are an integrative site for learning. When the eyes were removed the crayfish were unable to make an association between a specific area and food; whereas they were successful in doing so when the both sets of optic ganglia were intact.
Some colorful crustaceans such as mantis shrimp (*Odontodactylus scyllarus*) are known to perceive color, as they learn to choose different colors from arrays of greys (Marshall et al., 1996). Similar conclusions were reached from studies with crabs (*Leptograpsus variegatus, Gecarcinus lateralis*) based on retinal anatomy (Lall and Cronin, 1987; Leggett, 1979; Stowe, 1980).

### 1.3.3.5. Importance of light

The presence of light in the environment seems to be an important component of information that animals receive through visual input. Because light changes regularly with time, it provides information about the time of day and can entrain activities, producing circadian rhythm.

Many studies have shown that specific physiological changes take place as crustaceans experience different light intensities in their environment. For example, heart rate in lobster (*Astacus astacus*) responded positively to peaks in light intensity (Bojsen et al., 1998). Furthermore, both *P. clarkii* and *Procambarus digueti*, react to normal and extremely high irradiance photoperiods by decreasing their oxygen consumption and increasing hemolymph lactate concentration (Fanjul-Moles et al., 1998). In addition, Stephens (1955) found that molting and reproduction are regulated by photoperiods in crayfish.

Light is also generally acknowledged as the most important proximate cue controlling behavioural cycles in organisms. Sensitivity to light is widespread in living organisms, as light influences behaviour in both vertebrate and invertebrate animal species. For stream macroinvertebrates such as mayfly nymphs (*Stenonema modestum*), relative light change and light intensity have been proposed as proximate cues for initiating heightened locomotor activity and vertical location changes (that indicate an onset of drift) on the substrate (Schloss, 2002). Cnidarians are the simplest present-day invertebrates to have multicellular
light-detecting organs, called *ocelli*. Light affects the behavioural activities of cnidarians, including diel vertical migration (changing from day time to night time behaviour and vice versa), responses to rapid changes in light intensity and reproduction (Martin, 2002). Jensen et al. (1999a) demonstrated that light is an important factor in the formation of aggregates of daphnids (formation of swarms) irrespective of present food levels. Wolf spiders (*Lycosa tarentula*) use vision for navigational strategies during homing behaviour (Ortega-Escobar, 2002). In darkness, where spiders could not use the sun nor the polarized light pattern, 71.4% of spiders turned at random, when light was present, however, each spider turned at a constant angle, which would allow them to walk to a point near their burrow (Ortega-Escobar, 2002). Many fish are believed to rely on environmental light level for feeding. Young marine and freshwater fish species become dependent on light when feeding (Dabrowski and Jewson, 1984). It was shown by Neverman and Wurtsbaugh (1992) that bear lake sculpin also feed differently at different light intensities. Feeding at optimal light intensity (resembling one present at the bottom of the lake during the day) was nine times faster than in the dark.

Attraction and withdrawal responses are important adaptive mechanisms in crustaceans. These responses may be considered as initial reactions that give rise to more sophisticated behaviour patterns such as searching for food, reproduction and other social interactions. It is interesting then that high light intensity gives crustaceans a basic signal to withdraw, protecting from predators. For example, in both species of crayfish *P. clarkii* and *P. digueti*, extremely high light intensity seems to diminish locomotor activity (Fanjul-Moles et al., 1998). However, Welsh (1934) indicated that the frequency of leg movement in the crayfish *O. virilis* and *P. clarkii* is directly proportional to the intensity of illumination. Pei et al. (1996) also suggested that crayfish alertness increases when they venture into an illuminated part of the environment, where they are more visible to predators. Increased
illumination with either natural or artificial light also led to increased locomotor activity in *A. astacus* (Bojsen et al., 1998), but to restricted *P. clarkii* (Fernandez-de-Miguel, 1992a) and lobsters (*Nephrops norvegicus*) to their burrows (Archiega et al., 1975). In addition, a study by Bojsen et al. (1988) suggested that high levels of light promote escape behaviour in *P. clarkii* to avoid exposure to predators.

Removal of one eye caused an increase in leg activity on the side of the normal eye upon illumination, demonstrating negatively phototactic locomotor behaviour (Kropp and Enzmann, 1993). When a light stimulus was present in front of *P. clarkii*, the tendency was to walk backward or on a curved forward path in order to avoid the stimulus (Yamane et al., 2002). Even in blind crayfish (*O. a. packardi*) white light was demonstrated to alter interest in social interactions (Li and Cooper, 2001).

These experiments demonstrate the importance of light on physiological and behavioural processes in many crustacean species. Light alters the animal’s level of attraction or withdrawal and modifies locomotor and social behaviours.

1.3.3.6. **Physiology of vision – crayfish eye**

There is considerable amount of information in the literature regarding physiological aspects of crustacean vision. The crayfish eye is an important photoreceptive organ that is of importance in determining the behaviour of the crayfish. In 1879, Thomas Huxley defined a crayfish eye to be “an apparatus, which, in part sorts out the rays of light and serves as the medium by which the luminous vibrations are converted into molecular nerve changes”. The visual organs of crustaceans can include simple eyes (ocelli) and compound eyes. Ocelli are essentially designed to gather illumination, but their rudimentary structure does not support form vision. Decapod crustaceans have compound eyes, which can contain up to several thousand subunits or ommatidia, each with its own facet or lens. Photoreceptors or retinula
cells are found in each ommatidium. It was suggested that light and darkness can affect the structure and performance of the crustacean retinula cells (Meyer-Rochow and Reid, 1994). The light-sensing structure of the ommatidium is the rhabdom, which is composed of microvilli oriented perpendicular to the light path. The axons of the retinula cells from individual ommatidia converge to form a single columnar structure, the lamina cartridge, located in optic lobe. The optic lobe is located in the eyestalk and is composed of four optic neuropils that are arranged in series, beginning with a structure called the lamina ganglionaris, which lies just beneath the retinula cell layer. The successively deeper structures of the optic lobe include the medulla externa, the medulla interna and the medulla terminalis. The axons that emerge from the optic lobe project to the supraesophageal ganglion (brain) via the optic peduncle (optic nerve). Evidence showed that the optic lobe is intimately involved with locomotor behaviour. Severing the optic peduncle of crayfish proximal to the medulla terminalis resulted in enhanced general activity and apparent loss of locomotor activity (Schallek, 1942).

Many of the cells in the optic peduncle have unique identities. Among these are interneurons known as sustaining fibers (SFs). In crayfish there are 14 SFs, each of which has a unique excitatory receptive field and a characteristic axonal location in the cross section of the optic peduncle. The somata of the SFs are localized in the internal medulla of the optic lobe (Kirk et al., 1982), and their axons run through the optic peduncle to the cerebral ganglion, where they establish connections with descending visual interneurons (Wood and Glantz, 1980) and local interneurons (Okada and Yamaguchi, 1988). Each of the 14 neurons of the group can be distinguished by its defined sensory field in the retina (Wiersma and Yamaguchi, 1966). SFs discharge in a phasic-tonic manner at a frequency proportional to the light intensity and with a duration equal to that of the light stimulus (Glantz, 1972; Wiersma and Yamaguchi, 1966).
Light also has an effect on endocrine structures that are responsible for the control of behaviour and metabolic functions. In addition to the optic lobe and optic peduncle, the eyestalk also contains a major endocrine organ, the sinus gland, which plays an important role in diurnal and nocturnal locomotor activity (Durand, 1956; Schallek, 1942). This sinus gland receives inputs from the medulla terminalis and functions in photoperiodic responses of crayfish (Welsh, 1941). Glantz et al. (1983) showed that SFs axon collaterals also synapse on cells of the X-organ among other optomotor neurons and interneurons in the brain and optic lobe. The X-organ is a neuroendocrine structure associated with the medulla terminalis, and illumination elicits synaptic potentials and impulses in X-organ cells. This suggests that illumination may modulate the release of hormones like light-adapting hormone, which, in turn, modulates visual sensitivity. Another neurosecretory structure that seems to mediate complex responses of various organs to changing environmental conditions is the medulla neurosecretory organ (MNO), whose cells are spontaneously active in the dark and are inhibited by retinal illumination. Kirk et al. (1983) demonstrated that the MNO strongly inhibits impulse activity in the ipsilateral retina during illumination of the compound eye, suggesting that these cells may be involved in the control of some aspects of dark adaptation and/or circadian changes in visual sensitivity in the crayfish, *Pacifastacus leniusculus*.

Arthropod eyes are called compound eyes because they are made up of repeating units, the ommatidia, each of which functions as a separate visual receptor. The mechanism of migratory shielding pigment involves increasing the aperture of the ommatidia in the dark by movement of screening pigments out of the light path within the eye, and exposure of a reflecting tapetum at the back of the eye or around the retinula cells. Fernandez-de-Miguel and Arechiga (1992b) have shown that these screening pigments are influenced by selection of attraction or withdrawal responses. Neurohormonal effects also modulate this process (Larimer and Smith, 1980; Welsh, 1941). Rhabdom is a transparent rod in the center of each
ommatidium in the compound eye of an arthropod. Visual sensitivity necessary for crustacean dark/light adaptation is modulated via migratory shielding pigments and changes in the ability of the rhabdom to act as an efficient light guide (Meyer-Rochow, 2001).

1.3.3.7. Mirrors

Naturally occurring surfaces, such as quiet pools of water and some shiny rocks, probably provide reflections of light, which may affect animals with adequately developed visual receptors. Mirrors provide fairly accurate visual reproductions of reflective objects. Consequently, they can be considered a useful tool for studying animal social behaviour as they seem to be potent positive reinforcers.

Social behaviours are exhibited in response to mirror images by animals such as African grey parrots (Pepperberg et al., 1995), bottlenose dolphins (Marten and Psarakos 1996), California sea lions (Schusterman et al., 1967) and many other animal species (Gallup, 1968). Presentation of a mirror to Siamese fighting fish (Betta splendens) produced an increase in well recognized social behaviour elements, even when the preference for the live conspecific over the mirror presentation was considered (Craft et al., 2003). Moreover, the waning of this activity was inferred to be extremely slow in B. splendens (Clayton and Hinde, 1968). Aggression is a frequently noted component of behaviours elicited by mirrors. Mirrors were used to elicit aggressive behaviour in B. splendens (Thompson, 1963) and to classically condition their aggressive display (Thompson and Sturm, 1965). Chicks (Gallus domesticus) placed in front of mirrors peeped less, indicating that they were less fearful when mirrors where present (Montevecchi et al., 1973). Among crustaceans, hermit crabs (Pagurus marshi) have been shown to respond to their own mirror image by exhibiting social displays (Dunham and Tierney, 1986). In addition, Hazlett (1966c) reported that non-cryptic
hermit crabs (epibiotic cover removed) (P. marshii) showed greater responses than cryptic hermit crabs in front of mirrors.

The available data suggest that the behaviour of many animal species to mirrors is unmistakably social and at many times aggressive in nature.

2. Social isolation and exploration

2.1. Mirrors and social isolation

According to Welch (1965), physical factors of the environment are important, but social interaction is the principal factor determining the level of environmental stimulation. An enriched environment has been demonstrated many times to explain animals’ physiological and behavioural changes. The importance of social interaction is often demonstrated in studies involving social isolation, where one animal is housed for several hours or days in its usual condition except that no conspecifics are present. Such isolation alters stress responses, aggression behaviour and activity levels.

Mirrors have been used in social isolation studies as an environmental enrichment tool in order to reduce the consequences of social frustration and as a testing tool in studies dealing with isolated or socialized subjects. For example, the mirrors have been used in stables to reduce stress in horses (e.g. weaving behaviour) (McAfee, 2002). Kaufman and Hinde (1961) used mirrors to measure the effect of social isolation in chicks. The authors found that the mean number of distress calls given by isolated chicks increases in front of mirrors, whereas it decreases for socialized chicks. Social isolation seems to induce aggressive behaviour in front of mirrors. Halperin et al. (1998) applied mirrors for behavioural priming of isolated fighting fish B. splendens before their interaction with a live conspecific and showed that fish that were not primed were not initially hyper-aggressive. Hinkel and Maier (1974) used mirror presentation to measure the components of fighting
null
behaviour (e.g. gill erection) in *B. splendens*, after varied periods of isolation, which were applied following an initial mirror presentation. The animals showed greater recovery of strength of display following longer periods of social isolation with no mirror. Davis et al. (1974) demonstrated that showing mirrors to male paradise fish (*Macropodus opercularis*), after seven days of isolation, resulted in an increase in aggression. Franck et al. (1985) used mirrors as a test tool for the effects of isolation on swordtail fish (*Xiphophorus helleri*). Isolation was shown to reduce attack readiness in front of a conspecific as well as in front of a mirror.

Therefore, mirrors can serve as an environmental enrichment tool and can activate or enhance existing social behaviours during or after social isolation of an animal.

2.2. Effects of social isolation

Measurement of reactivity in novel environments have been used in several experiments examining the effect of individual housing or social isolation on different animal species. Generally, social isolation results in changes in the behavioural reactivity toward novel environments, although there are large differences between species and between studies for the same species. However, many studies have given conflicting results. The following paragraphs concentrate on the various changes induced by social isolation on locomotory and social interactions, specifically aggression.

2.2.1. Decreased general activity

Some animals exhibit decreased locomotor activity and decreased social interaction when returned to field conditions or re-introduced to conspecifics following social isolation.
Such observations have been reported for rats (Francolin-Silva and Almeida, 2004; Shukin et al., 2003) and chickens (Gallup et al., 1971; Gallup and Suarez, 1980). Piglets and calves also showed markedly lower reactivity in a novel environment and decreased locomotory behaviour after isolation (Herskin and Jensen, 2000; Jensen et al., 1999b). In addition, Boe and Faerevik (2003) showed that calves showed more fear behaviour after social isolation. Franck et al. (1985) inferred that social isolation lasting weeks in swordtail fish (*X.helleri*) resulted in a decrease of attack readiness in both mirror tests and standard-opponent tests. Isolated cichlid fish were also found to become less aggressive the longer they were kept apart (Heiligenberg, 1964).

### 2.2.2. Increased general activity

Lorenz (1966) proposed the hypothesis that depriving an animal of the opportunity to express its aggression towards conspecifics results in increasing aggressiveness. Increases in aggressiveness in isolated animals can be interpreted as an indication that the threshold for social stimuli is lowered by social deprivation and that the incentive for encounters is correspondingly increased. Hazlett (1968) inferred, from his studies on habitat complexity with hermit crabs (*Pagarus bernhardus*), that experience within a group is important in decreasing an individual’s level of aggression. Once the individual is isolated, aggressiveness is thought to increase. Rats, mice, fighting fish and crustaceans have been most extensively used as animal models for studying the effects of social isolation, although similar tendencies have been observed in other animal species such as chicks (Rajecki et al., 1977) and even crickets (Nagao and Yamamato, 1999). Social isolation can lead to increased locomotor activity in chicks and excessive aggression in both chicks and crickets (Nagao and Yamamato, 1999; Rajecki et al., 1977).
Increased reactivity to a novel environment after social isolation has been most notably described using the rat model. After social isolation, rats generally exhibited increased hyperactivity and locomotor activity when placed in a novel environment (Gentsch et al., 1982; Wilkinson et al., 1994; Sahakian et al., 1975). Rats also spent more time in social interactions (Cheeta et al., 2001; Douglas et al., 2004) and showed increased willingness to make social contacts (i.e. social play or play fighting) (Ikemoto and Panksepp, 1992; Douglas et al., 2004) after being socially isolated. However, these effects of isolation were shown to be age-contingent. Rats that were isolated at a younger age (25-30 days of age) showed an increase in exploratory behaviour, whereas rats isolated at an older age (60-70 days of age) showed a decrease in exploratory behaviour (Pisula et al., 1992). A similar tendency was noted by Wahlstrand et al. (1983), where early isolation (at 14-41 days of age) was shown to have a significant impact on agonistic behaviour as compared to isolation applied later in life (at 41-68 days of age).

Mice display similar responses to social isolation as rats. Valzelli (1969) used mice to suggest a method to induce aggressive behaviour in laboratory animals. As the isolation time increased, so did aggressive behaviour in mice, and the two factors were directly proportional. Aggressive behaviour in mice after social isolation was also later observed by Guo et al. (2004). Also, increased locomotor behaviour was observed when mice were exposed to a novel environment (Essman, 1968; Guo et al., 2004; Rilke et al. 1998a) and allowed to explore (Bartolomucci et al., 2003). As the time in isolation increased, so did the general excitability of the mice (i.e. hyperkinesia) (Banerjee, 1971). Isolation also altered the behaviour of the mice during a social stress situation, in which they showed more escape behaviour (i.e. jumping and fleeing) as compared to socialized mice, which were more passive (Avitsur et al., 2003).
After complete isolation from other fish, male fighting fish (*B. splendens*) showed increased locomotion and displayed a significantly higher probability of winning aggressive encounters without any difference in the incidence of agonistic behaviours (Ichihasi et al., 2004). In cichlid fish (*Oreochromis niloticus*), the onset of latency of aggressive behaviours markedly increased after isolation (Barki and Volpato, 1998). Similarly, as the time in isolation increased for coral reef fish (*Microspathodon chrysurus*), their response to a predator increased. Also, their color pattern decreased, signifying increased excitement, but kinetic activity (recorded by the number of 90 degree turns) decreased. After 4 days of isolation, *M. chrysurus* showed aggressive behaviour to model fish, whereas only an increase in excitement level was observed in response to a ball (Rasa, 1971). Isolation has induced aggressiveness in other fish species including convict cichlid (*Cichlasoma nigrofaciatum*) (Gallagher et al., 1972), Indian paradise fish (*Macropodus cupanus*) (Pal, 1968), and paradise fish *Macropodus opercularis* (Davis, 1975; Ward, 1967).

Crustaceans such as shrimp (Dingle, 1969), lobster (Huber and Kravitz, 1995) and crayfish *O. virilis* (Heckenlively, 1970; Rubenstein and Hazlett, 1973), *A. astacus* (Goessmann et al., 2000) and *P. clarkii* (Copp, 1986) readily display aggressive encounters. Dunham et al. (1972) reported that lobsters (*H. americanus*) with social or visual contact for 36 days showed less aggression when subsequently tested against a target lobster, than lobsters that were housed in individual compartments and had no visual contact with one another. However, no difference was observed between socialized and isolated lobsters with regard to how much time they spent exploring their tank. Hoffman et al. (1975) also showed that isolated lobsters (*H. americanus*), which were in visual contact with other animals or were communally housed, were less aggressive. Similar results were reported by Hazlett (1966a) where aggression (the number of aggressive encounters or fights initiated) and locomotion increased significantly in hermit crabs (*C. tibicen*), after 2-7 days in isolation.
Another species of hermit crab (Pagurus samuelis) showed increased aggressiveness (with direct fight) after isolation, whereas locomotion, after 30 days of isolation, decreased (Courchesne and Barlow, 1971).

Interestingly, there are no known studies investigating the effect of social isolation on social interactions or activity in crayfish.

2.3. Long versus short term isolation

To account for such divergent effects of social isolation described above in various animal species, Gallup and Suarez (1980) proposed a model, in which the initial behaviour of an animal in a novel environment is a compromise between attempts to reinstate contact with a conspecific and to avoid detection by predators by minimizing visibility. The outcome of the compromise appears to depend on the amount of time spent in isolation. The Suarez and Gallup model was further supported by experiments on rats and mice (Suarez and Gallup, 1981) and on guinea pigs (Suarez and Gallup, 1982).

Adolescent rats were isolated for either 8 days (long term) or 24 hours (short term), and it was reported that long-term isolation resulted in greater social investigation and contact behaviour (Varlinskaya et al., 1999). After a short isolation time the angelfish (Pterophyllum scalare) had a tendency to try to reestablish contact with conspecifics, and after a longer isolation time the tendency shifted towards trying to minimize predator detection (Gomez-Laplaza and Morgan, 1991: 2000). Isolation for 10 days resulted in lower activity levels in a normal environment than in fish isolated for a shorter period of time (3-4 days). Fish isolated for 3-4 days showed a lack of adaptation to the solitary condition and, therefore, displayed a higher level of activity. Ferno (1977) reported that cichlid fish (Haplochromis burtoni) showed an increased number of attacks after a short social isolation
(15 minutes – 12 days) whereas a longer isolation reversed this effect and the attack rate was decreased. Similar observations were made by Cromarty et al. (1999) on studies with lobster (*H. americanus*). These animals exhibited a decreased escape response (i.e. tail flipping), increased locomotor behaviour and increased aggressiveness after being isolated from visual inputs for 24 hours as compared to 1 hour. Thus, the tendency for aggression versus predator avoidance behaviour seems to depend on the duration of social isolation.

Gentsch et al. (1981) made an effort to distinguish between two types of activities that are dependent on the time spent in isolation: spontaneous and reactive activity. Male rats during the first day of isolation displayed lots of reactive activity, which was defined as a response to a novel situation, in this case an activity platform. After the 24 hours, rats displayed more spontaneous activity in an open field test, but after 9 weeks of isolation that spontaneous activity diminished and again more reactive activity was observed (Gentsch et al., 1981). The amount of time in isolation affects not only the intensity of observed behaviours but also their type.

### 2.4. Physiological changes

Behavioural and environmental factors have been shown to have profound effects on brain architecture and neurochemistry in a variety of species, and these changes were shown to be functionally significant in animals such as pigs (Sachser, 1986) and the rhesus monkey *Macaca mulatta* (Lewis et al., 1990; Martin et al., 1991). However, such modifications in the central nervous system have been most extensively studied in rats (Bennett et al., 1969; Diamond et al., 1964, 1966; Forgays and Forgays, 1952; Hymovitch, 1952; Rosenzweig, 1969). Increased activity of the dopaminergic system was shown to occur in isolated rats (Sahakian et al., 1975). Isovich et al. (2001), however, showed that a dopamine transporter,
an important component that regulates dopaminergic neurotransmission by reuptake of the transmitter in to presynaptic terminals, is not affected by isolation in rats. Increased corticosterone levels were also observed in isolated rats (Bartolomucci et al., 2003) as well as in female prairie voles (Microtus ochrogaster) (Kim and Kirkpatrick, 1996).

Essman et al. (1968) reported that in mice, approximately 24 days of isolation increased serotonin concentrations and serotonin metabolism in the brain. However, the opposite results were reported by Rilke et al. (1998b). Welch and Welch (1965) have accounted for behavioural differences resulting from differential housing on the basis of elevated brain norepinephrine levels in individually housed mice.

Social isolation also seems to alter the neuroendocrine system and the probability to display sexual and aggressive behaviours in many animal species. Rats reared in social isolation exhibited a decreased volume of the medial amygdala, which resulted in deficits in adult sexual behaviour (Cooke et al., 2000). Male leopard geckos, previously housed with female geckos, became more active in a novel environment and more territorial than males housed in isolation. It was suggested that this social experience elevated circulating concentrations of androgens in male gecko (Sakata et al., 2002).

In the crayfish C. destructor, housing in an impoverished environment decreased cell proliferation and cell survival (Sandeman and Sandeman, 2000). In socially isolated crayfish, serotonin was shown to persistently enhance the response to sensory stimuli of the lateral giant (LG) tail flip command neuron (Yeh et al., 1996).
Chapter 2

The effect of a reflective environment on exploratory behaviour in crayfish

J.K. Drozdz, A.J. Mercier and S.M. Brudzynski
Abstract

The study examined effects of visual cues on spontaneous exploratory behaviour in intact crayfish *Procambarus clarkii*. Exploratory activity is guided continuously by input from chemosensory, mechanosensory and visual receptors. Extensive literature suggests that visual cues seem to play an important role during exploration and that many animals respond to their own mirror images (see Literature Review). It is unknown, however, if crayfish display the same tendency.

The predominant components of spontaneous exploratory behaviour were determined by observing the activity of solitary adult crayfish for 20 minutes in a glass aquarium containing fresh water and no objects. Five distinct behaviours were observed: rearing up (climbing on the wall), turning around, cornering (facing the corner), backward walking, and crossing (crossing the midline of the aquarium). The frequency of rearing up, cornering and turning around decreased when reflection from the glass wall was blocked with black cardboard or non-reflective plastic. In a tank containing mirrors on one side and non-reflective plastic on the other, crayfish cornered, reared up and turned around more frequently in front of the mirrors than on the non-reflective side. Thus, the glass walls of an empty tank provided reflective visual stimuli, which influenced spontaneous exploratory behaviour of crayfish.

Results of this part of the study provided better insight into the role of visual cues in crayfish behaviour and contributed to a more detailed description of the exploratory behaviour in this species.
Introduction

The ability of animals to explore their environment increases their chance of survival and reproduction by facilitating feeding, shelter seeking and mate searching. Exploratory behaviour can serve to acquaint animals with their surroundings by surveying the environment, often more than once. For example, goldfish (*C. auratus*) explore a new area repeatedly, establishing familiarity and gradually expanding the size of the space they explore (Kleerekoper et al., 1974). Meadow voles (*M. pennsylvanicus*) learn the topography of their environment by gathering information that becomes important later in avoiding predation (Ambrose, 1972).

Animals rely heavily on sensory feedback during exploratory behaviour. When exploring a new enclosure, many animals initially demonstrate a preference to remain in contact with the walls or other vertical objects, staying in the periphery of open spaces and in corners. Such thigmotactic behaviour is displayed during exploration by rats (Treit and Fundytus, 1989), lobsters (Snyder et al., 2000), blind crayfish (Li and Cooper, 2001), blindfolded crayfish (Basil and Sandeman, 2000) and sighted crayfish (Burba, 1988) indicating the importance of tactile cues. Chemoreceptive cues can also aid exploration and are used in locating odor sources in snails (Rohrkasse and Atema, 2000), lobsters (Basil and Atema, 1994; Kozlowski et al., 2001) and crayfish (Kozlowski et al., 2003; Moore and Grills, 1999). In crayfish, detection of sex pheromones of another conspecific was noted (Hazlett, 1985a,b; Tierney and Dunham, 1982; Tierney et al., 1984). Additionally, the social condition (or status) of another conspecific was shown to be communicated in crayfish through phermonal release (Bechler et al., 1988; Schneider et al., 1999; Thorp and Ammerman, 1977) or urine (Breithaupt and Eger, 2002).

Although several studies have demonstrated that visual cues influence behaviour in crustaceans, few have examined the role of visual cues in guiding exploration. Increased
illumination with either natural or artificial light increased locomotor activity in crayfish (Bojsen et al., 1998). However, it also restricted crayfish and lobsters to their burrows (Archiega et al., 1975; Fernandez-de-Miguel et al., 1992a). These observations suggest that crayfish and lobsters prefer dim light during exploratory activity. Some studies with fiddler crabs have used a mirror or various objects or artificial crabs to examine the role of visual images in eliciting responses to conspecifics (Dunham and Tierney, 1986; Land et al., 1995). The main finding of these studies was that crabs use visual cues to recognize and respond to conspecifics with either courting or aggressive behaviour. The goal of these studies, however, was to examine social interactions rather than exploration.

No studies to date have investigated how reflective images from the walls of a holding tank might influence exploratory behaviour in crustaceans. This is an important issue because crustaceans are often maintained and studied in glass aquaria that possess some reflective properties. Such an environment could provide visual cues that may be interpreted as a conspecific and could potentially influence exploratory behaviour. The primary purpose of this investigation was to determine whether or not the reflective environment of a standard glass aquarium would influence exploratory behaviours in crayfish. In order to answer this question, it was first necessary to classify the predominant elements of crayfish exploratory behaviour when the animals are placed in a novel environment, a freshwater aquarium with no objects. Subsequently, the aquarium was altered in several ways to eliminate light reflection, and effects of these alterations on the various behaviours were determined. The effects of reflection on other quantitative parameters of exploration, such as average speed of progression, total distance traveled and degree of turning, were also investigated. Results of this study will provide better insight into the role of visual cues in crayfish behaviour and will contribute to a more detailed description of the exploratory behaviour in this species.
Materials and methods

Animals

Naïve male and female red swamp crayfish, *Procambarus clarkii*, were used for all parts of this investigation and were obtained from Atchafalaya Biological Supply (Raceland, LA, U.S.A.). The crayfish were adults and had carapace lengths of approximately 40 mm and an average weight of 22.82±3.27 g. All animals used in experiments were healthy, in intermolt stage and physically intact, possessing a complete set of legs including chelae. For identification and visualization purposes, all crayfish were numbered on the thorax using bright red or pink nail polish. Following numbering, crayfish were allowed to rest in their housing tanks for a minimum of 24 hours.

The crayfish were housed in glass tanks measuring 52 cm L x 25 cm W x 30 cm H in a water depth of 8 cm. Each tank contained two cylindrical tubes (cut from plastic pipe) measuring 10-15 cm in length and 5 cm in diameter, which served as shelters for crayfish. The water was filtered, aerated and changed weekly. Water changes never occurred on the day of experimentation. Animals were kept on a 12:12 hours light: dark cycle and at a water temperature ranging between 19-22°C. The crayfish were fed bi-weekly with commercially available sinking fish pellets and were always fed after testing was complete. Animals were never disturbed or stressed. Crayfish were housed in these conditions in same-sex pairs for a minimum of 14 days before testing.

All crayfish were subjected to both control and experimental conditions. Crayfish that died or molted before completion of both trials were eliminated from the study.

Behavioural observations

The predominant elements of adult male and female crayfish behaviour were determined by live observation and confirmed from videotaped activity. Videotaping was performed
using a Sony Handicam® video camera. The camera was positioned directly above the testing tank. Specific behaviours were identified based on the consistency of the movement among animals and the frequency with which each activity occurred. Behaviours were identified as follows.

1. *Rearing up* (Lundberg, 2004) occurred when crayfish stood on their 4th and 5th pereopods while lifting the ventral surface of the thorax against the vertical wall of the tank (Figure 2.1A).

2. *Cornering*: the crayfish stood, with all legs on the ground, for a minimum of 5 s facing a corner of the aquarium with the chelae extended or bent (Figure 2.1B).

3. *Turning around* occurred when crayfish were walking along the aquarium wall and changed direction 90°-180° (Figure 2.2).

4. *Reverse walking*: (Lundberg, 2004) walking backwards, usually with the tail-fan curled under the abdomen. Always occurred along the aquarium walls.

5. *Crossing* entailed moving away from the aquarium wall and walking into the open space and across to the opposite wall.

6. *Middle stance* behaviour occurred when the crayfish stood motionless in the middle area of the tank, with all of its legs on the ground, orienting towards any wall for a minimum of 5 s.

7. *Curled tail* behaviour occurred when the animal stood motionless for a minimum of 5 s with the tail curled under the abdomen, regardless of location in the tank.
Figure 2.1 Examples of rearing up (A side view) and cornering (B top view) behaviours of solitary crayfish in novel tank.
Figure 2.2. Example of turning around behaviour of solitary crayfish in novel tank (top view).
Four experiments were used to determine whether or not crayfish behaviour is influenced by reflection. In the first experiment, 29 crayfish were first observed in a glass tank in which the reflection was prevented by lining the inner walls with black Bristol cardboard; these crayfish were observed again 3-6 days later in a standard glass aquarium without cardboard lining. The second experiment involved observing 19 crayfish in the same glass tank with and without cardboard lining, but in the reversed sequence of treatments to provide a control for the order effect. The third experiment involved observing 26 crayfish in a standard glass tank first followed by observation in a tank lined with matte-finished transparent plastic. The plastic eliminated reflection but allowed light to pass through. In each of these three experiments a paired design was used in which the same animals were observed in both conditions. The fourth experiment involved observing 23 crayfish in a tank, that was half reflective and half non-reflective. This was done by installing mirrors, inside of the tank, on one half of the tank walls and lining the other half with matte finish plastic. A black line was drawn across the middle of the tank floor to help the observer distinguish the borderline between the reflective and non-reflective areas (Figure 2.3).

Three to five crayfish were tested daily, five days per week between 12:00 and 16:00 hours. Each crayfish was transferred by hand directly from the housing tank into the middle of the test tank, which was novel to the animal. Behavioural observations began after ripples in the surface of the water, caused by the experimenter’s hand, had subsided (approximately 15 s). When the reflective tank was used, the experimenter observed the crayfish behaviour by facing the tank, which was positioned at eye level. When the cardboard lined or matte
Figure 2.3 Schematic diagram of mirror/matte tank with the dividing line (dashed line).
tank was used, the experimenter observed the animal in an additional mirror positioned above the tank at an angle of approximately 30° from horizontal. Animals were carefully observed and behaviour recorded for 20 minutes.

In experiments with the half reflective, half non-reflective tank, the time the crayfish spent on the matte side was determined by subtracting the time spent on the mirrored side from the total time spent in the testing tank: \( T_{\text{matte}} = T_{\text{total}} - T_{\text{mirror}} \). The occupancy of either side (mirrored or matte) was noted once the chelae and head crossed the dividing line. The experimenter was positioned directly above the test tank.

**Parameters of exploration measured in the Digiscan Activity Monitor**

In a new experiment, crayfish activity was recorded using a Digiscan Activity Monitor (Acuscan, Columbus, Ohio, USA.) equipped with an acrylic tank \((L \times W \times H = 42 \text{ cm} \times 42 \text{ cm} \times 31 \text{ cm})\) and with 16 beams of infrared light forming an 8x8 grid on the x and y horizontal axes. The acrylic tank contained water 8 cm deep. Signals generated by the interruption of infrared light beams by the movement of the animal were fed via the Digiscan Analyzer to an IBM-compatible 486 computer. The analyzer, operated by the software PLOTONE (Acuscan, Ohio, U.S.A.), provided data on the position of the central point of the crayfish body (see below) in x and y coordinates every 0.02 s for 20 min. The animal’s position was monitored continuously, and movement was calculated by the computer program, based on the sequence in which light beams were broken and re-activated, with an effective accuracy of 0.32 cm.

The following parameters were measured:

1) total distance covered by crayfish per 20 minutes (in meters);

2) average speed of progression (in cm/s);
3) parallelism index, which indicated how parallel each movement was in comparison to the previous movement (every 0.02 s, Brudzynski and Krol, 1997). Parallelism index was calculated by taking the cosine of the angle \( \alpha \) between the current and the previous directions of movement and averaging for all angles. It is expressed as \( P = \frac{1}{N} \sum \cos \alpha \) (Brudzynski and Krol, 1997). The parallelism index was inversely related to the number of turns and the magnitude of the turning angles.

Water interfered with the infrared light beams. To track the position of crayfish in water, it was necessary to mount a probe on the back of the crayfish and lower the tank so that the light beams were above water level. A piece of hollow plastic (Q-Tip®), 1.2 cm long, was glued to the crayfish thorax (central point of the body) using Epoxy® glue and Krazy Glue®. A removable probe made of Styrofoam® with an oval shape (approximately 4x2 cm) and weighing 1.0 g was constructed, and a second piece of Q-Tip® was permanently attached to the probe. At the time of testing, the probe was attached to the crayfish by the means of removable toothpick allowing for adjustment of the height of the probe according to crayfish height (Figure 2.4).

Computerized data acquisition commenced as soon as crayfish started to walk. The probe interrupted the infrared beams in the air and the signals were fed via the Digiscan Analyzer to the computer. Simultaneously, the predominant elements of crayfish exploratory behaviour were observed and recorded by the experimenter during each 20-minute time testing session.

**Statistical tests**

Time spent on mirrored and non-reflective sides of a tank (Figure 2.7B) were compared using a Student’s t-test for correlated samples. All other statistical comparisons were made using a Wilcoxon Signed Rank test for correlated samples. SPSS statistical package was used.
Figure 2.4 Schematic representation of experimental set up employed in Digiscan Activity Monitor. The position and mounting of the Styrofoam probe on the body of crayfish is shown on the left hand side. A draft of the Digiscan Activity Monitor is illustrated on the right-hand side. The dotted lines with arrowheads depict the infrared light beam and its position above the water surface (wavy line) and in relation to the probe mounted on the crayfish (gray oval).
Results

General description of crayfish behaviour

When placed in a glass aquarium containing no obstacles and observed for 20 minutes, solitary crayfish typically walked around the perimeter of the tank, staying close to the walls. In some cases, they turned around either spontaneously or when they encountered a wall and walked back and forth along a single wall. Sometimes, while still staying close to the walls, crayfish walked backwards, usually with the tail fan curled under the abdomen. Toward the end of the 20-minute observation period, some crayfish would discontinue walking along the sides and start walking across the tank. The speed of walking and time spent being completely inactive varied from one crayfish to another. In addition to walking, several distinct behaviours were commonly observed. The five most frequent and most consistent were rearing up, turning around, cornering, reverse walking and crossing. In some cases, crayfish charged at the corner after cornering. Reverse walking (Lundberg, 2004) and crossing were the least frequent behaviours.

Influence of a reflective environment on exploratory behaviours

Several experiments were designed to determine whether any of the observed behaviours were induced by the reflection from the glass walls of the aquarium. In the first such experiment, crayfish were placed in a transparent tank that either contained or did not contain a black cardboard lining to remove the reflection. Twenty-nine animals were tested in the non-reflective (cardboard-lined) condition first and were tested 3-6 days later in the reflective condition (Figure 2.5 A). For another 19 animals this order of testing was reversed (Figure 2.5 B). The animals performed significantly more rearing up, turning around and cornering in the reflective condition, regardless of the order of presentation.
Figure 2.5 Behavioural patterns of P. clarkii in transparent (reflective) and cardboard-lined (non-reflective) tanks. A. Crayfish (n = 29) were tested first in the non-reflective tank and were tested again 3-6 days later in the reflective tank. B. A separate set of crayfish (n = 19) was tested first in the reflective tank and then again 3-6 days later in the non-reflective tank. The behavioural patterns are listed on the x-axis. The frequency of rearing up, turning and cornering during 20-minute sessions was significantly higher in the reflective tank regardless of the order of presentation. The frequency of crossing was higher in the cardboard-lined tank, but only when it was presented first. Error bars represent standard deviations. Statistical comparisons were made using a Wilcoxon Signed Rank test for Correlated Samples (Ferguson, 1971) (*P<0.05, *** P<0.001).
(*) P<0.05, *** P<0.001). No significant differences were observed when comparing the frequency of reverse walking. Crossing behaviour was seen more frequently in the cardboard-lined tank (P<0.05), but only when animals were tested in the non-reflective condition first (Figure 2.5A). Thus, the most consistent effects of reflection appeared to be increases in the frequency of rearing up, turning around and cornering. Very similar results were obtained when reflection was reduced by painting the outside of the aquarium with black enamel rather than inserting cardboard (data not shown). This approach, however, reduced reflection by decreasing the amount of light rather than blocking the reflection itself.

Light intensity inside the tank was noticeably reduced when it was lined with black cardboard. To circumvent possible effects of changing light intensity, behaviours were compared between glass tanks with or without transparent, matte finish plastic, which permitted light passage while minimizing reflection. In this experiment, the order of presentation was counter-balanced, with 50% of the animals placed in the reflective tank first and 50% placed in the non-reflective tank first. The crayfish displayed significantly more rearing up (Wilcoxon Signed Rank test, P<0.05), turning around (P<0.001) and cornering (P<0.01) in the reflective environment (Figure 2.6). However, there were no significant differences in the frequency of crossing or reverse walking between the two conditions.

To further examine the influence of a reflective environment on behaviour, mirrors were installed in one half of a glass tank, and the other half was lined with transparent, matte-finish plastic. In this experiment two other behaviours, referred to as middle stance and curled tail, were observed. The presence of middle stance and curled tail behaviours
Figure 2.6 Exploratory behavioural patterns of *P. clarkii* in a transparent tank (reflective condition) and in a tank lined with plastic matte to prevent reflection (non-reflective condition). Rearing up, turning around and cornering occurred more often in the reflective tank during a 20-minute session (Wilcoxon test, * P<0.05, ** P < 0.01, *** P< 0.001, n = 26). The frequency of reverse walking and crossing behaviour was not significantly different between the two conditions. The order of testing was randomized. Error bars represent standard deviations of the mean.
exclusively in the mirror/matte tank suggests that these behaviours were elicited by the strong reflection produced by the mirrors. Rearing up, turning and cornering behaviours occurred more frequently in the mirrored half of the tank than in the non-reflective half (Figure 2.7A; lifting: P<0.01, turning around: P<0.01, cornering: P<0.001). Curled tail behaviour also occurred more frequently in the mirrored area of the tank (P<0.01). There were no significant differences for middle stance behaviour or reverse walking between mirrored and non-mirrored halves of the tank (P>0.05). Crayfish also spent significantly more time in the mirrored area than in the matte area (Figure 2.7B).

Influence of reflective environment on exploration in activity monitor

Crayfish locomotor activity was recorded using a Digiscan Activity Monitor, which provided measures of the total distance traveled, average speed of locomotion and parallelism index (Table 2.1). There was no significant difference between the average speeds in a transparent (reflective) tank compared to a matte (non-reflective) tank (P=0.427). There was also no significant difference between the parallelism indices calculated for these two conditions (P=0.713). There was a trend suggesting a greater total distance traveled in the reflective tank than in the non-reflective tank; the effect was marginally non-significant (P=0.069). The predominant patterns of exploratory behaviour were also observed and recorded while the crayfish were in the Digiscan Activity Monitor. Figure 2.8 illustrates the results from observing crayfish with probes in transparent (reflective) and matte (non-reflective) tanks. Rearing up (P<0.05), turning around (P<0.001) and cornering (P<0.001) all occurred more frequently in the reflective tank than in the non-reflective tank.
Figure 2.7 Behavioural patterns of *P. clarkii* observed on mirrored and matte sides of a glass tank during 20-minute observation sessions. A. The frequency of rearing up, turning around, cornering and curled tail behaviour was significantly higher in the mirrored half of the tank. The frequency of reverse walking and middle stance behaviour was not significantly different between the two sides of the tank. (* P<0.05, ** P<0.01, *** P<0.001, n = 23; Wilcoxon Signed Rank test for Correlated Samples). B. Crayfish spent more time in the mirrored half of the tank (mirror area) than in the non-reflective half (matte area). Error bars represent standard deviations. One sample t-test statistics were used.
Table 2.1 The effects of reflective environment (transparent tank) on average total distance, speed and parallelism index (degree of turning). Data were obtained using a Digiscan Activity Monitor while crayfish performed exploratory activities in reflective (transparent) and non-reflective (matte) tanks during 20-minute sessions. (Wilcoxon Signed Rank test for Correlated Samples, n = 25). Errors indicate standard deviations of the mean.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Reflective (transparent tank)</th>
<th>Non-reflective (matte tank)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average total distance (m)</td>
<td>14.56 ± 8.85</td>
<td>11.36 ± 5.48</td>
<td>0.069</td>
</tr>
<tr>
<td>Average speed (cm/s)</td>
<td>18.54 ± 2.81</td>
<td>18.06 ± 4.47</td>
<td>0.427</td>
</tr>
<tr>
<td>Average parallelism index</td>
<td>0.39 ± 0.11</td>
<td>0.39 ± 0.12</td>
<td>0.713</td>
</tr>
</tbody>
</table>
Figure 2.8 Behavioural patterns of *P. clarkii* in transparent (reflective) and matte (non-reflective) tanks placed in a Digiscan Activity Monitor for 20-minute sessions. The frequency of rearing up, turning and cornering was significantly higher in the reflective environment, but there was no significant difference for the frequency of reverse walking or crossing (*P* < 0.05, ***P* < 0.001, n = 25; Wilcoxon Signed Rank test for Correlated Samples). The order of testing was randomized. Error bars represent standard deviations.
Discussion

Crayfish reared up, turned around and cornered more in a reflective aquarium than in a non-reflective aquarium, regardless of whether reflection was decreased or blocked with paper or plastic matte. When placed in a mirror/matte-lined tank, crayfish spent significantly more time on the mirrored side of the tank, but also reared up, cornered and turned around more frequently than they did in the matte area. It can be concluded, therefore, that a reflective environment, and not the amount of light, influences crayfish exploratory behaviour in an empty, novel tank. Cornering, turning around and rearing up behaviours are not necessarily elicited by reflection, since they occur in non-reflective aquaria, but their frequency increases in a reflective environment.

Behavioural patterns of exploratory behaviour

Turning around (Bowerman and Larimer, 1974; Breithaupt et al., 1995; Burba 1988; Copp and Jamon, 2001; Cukerzis, 1983; Yamane and Takahata, 2002), cornering (Basil and Sandeman, 2000; Li and Cooper, 2001; Snyder and Peeke, 2001), crossing (Basil and Sandeman, 2000; Burba, 1988; Li and Cooper, 2001) and reverse walking (Andrews, 1909; Bowerman and Larimer, 1974; Bruski and Dunham, 1987; Cukerzis, 1983; Edwards, 1984; Kovac, 1974 a,b; Lundberg, 2004; Simon and Edwards, 1990; Yamane and Takahata, 2002) have been noted or briefly described in behavioural experiments with several crustacean species. Rearing up or ‘climbing’ has been described as a type of exploration (Lundberg, 2004), an active behaviour pattern (Stein and Magnuson, 1976) and also as a sign of agitation (Hazlett, 1985b) in different species of crayfish. The results presented here indicate that all these behaviours are components of exploratory behaviour exhibited when *P. clarkii* are placed in a novel tank containing no obstacles.
Tail curling has been reported as a component of agonistic postures elicited by injection of serotonin into crayfish, lobsters and squat lobsters (Antonsen and Paul, 1977; Livingstone and Kravitz, 1980; Tierney and Mangiamele, 2001). Such postures are judged to be part of agonistic behaviour when accompanied by meral spread (the chelae are spread apart and raised) and by elevation of the walking legs, which heightens the overall stance (Bruski and Dunham, 1987; Karavanich and Atema, 1998; Tierney et al., 2000). Neither meral spread nor heightened posture was observed in the present experiments. Thus, it is possible that the curled tail behaviour observed in the present study might not represent aggressive posturing. It may, instead, represent a social display. Result presented in Figure 2.7A suggests that this behaviour is enhanced by reflection.

The appearance of curled tail and middle stance behaviours in the tank with mirrors but not in the standard glass aquarium suggests that visual cues are different between these two conditions. This is not surprising because mirrors provide greater detail in a reflected image than transparent glass (at least to human perception). However, the number of occurrences of middle stance behaviour was not different on mirrored and non-reflective sides of the aquarium (Figure 2.7A), suggesting that middle stance behaviour is not affected by reflection. Since the compound eyes of crustaceans can provide nearly 360° vision (Bovbjerg, 1956; Shaw and Stowe, 1982), we cannot exclude the possibility that crayfish facing the non-reflective wall exhibit middle stance behaviour in response to the reflection from the mirrored wall behind them. The same argument can be made for the small number of occurrences of curled tail behaviour when facing the non-reflective wall.

Although several studies have demonstrated that animals respond to their mirror image as if it were a conspecific, it is not clear whether crayfish did so in the present study. Mirrors elicit social behaviours, such as courtship displays or aggression, in such diverse species as non-human primates (Gallup, 1968; Harris and Edwards, 2004; Neel, 1996), dogs
(Siwak et al., 2001), chicks (Montevecchi and Noel, 1978; Tolman, 1965), parrots (Pepperberg et al., 1995), sea lions (Schuster et al., 1967), fighting fish (Craft et al., 2003; Thompson and Sturm, 1965a,b; Thompson, 1963) and hermit crabs (Dunham and Tierney, 1986). Since mirror images elicit social behaviours in so many species, it is reasonable to propose that the behavioural patterns enhanced by reflection in the present study are components of social behaviour.

Crayfish usually respond to conspecifics in an aggressive manner. When two crayfish are placed together in one tank, they typically face each other, approach and fight until a dominance hierarchy is established (Bovbjerg, 1953; Bruski and Dunham, 1987; Huber et al., 1997; Tierney et al., 2000). Agonistic episodes include many behavioural components, such as aggressive posturing, antenna tapping, meral spreading, lunging, pushing, striking and tail flipping. Visual cues are not necessary to elicit agonistic encounters, since such encounters occur in blind crayfish species (Kellie et al., 2001; Li and Cooper, 2002) and when crayfish are blindfolded (Breithaupt and Eger, 2000). However, visual cues play a role during agonistic encounters. Some components of agonistic behaviour, such as lunging and following, appear to be visually mediated because their frequency decreases in darkness, while other components, such as antenna tapping, chela striking and pushing are mainly tactile in nature and occur more frequently in darkness (Bruski and Dunham, 1987).

Most of the known aggressive behaviours (e.g., aggressive posturing, meral spreading, striking, pushing and tail flipping) were not observed in the present experiments. There were a few cases in which cornering behaviour was followed by charging the corner, suggesting lunging and contact. Reflection increased the occurrence of rearing up, crossing and turning behaviours. Rearing up against a reflection might represent an attempt to make contact. Crossing might represent an attempt to approach a reflection on the opposite wall or
to avoid a reflection on the adjacent wall. Likewise, turning around might represent an attempt to approach a reflection on the adjacent wall or to avoid a reflection on the opposite wall. However, there is no definitive evidence that the behaviours reported here are components of agonistic encounters. The present data show only that crayfish behaviour during exploration is altered by the presence of a reflection. If rearing up, turning around and crossing are components of agonistic encounters, they are probably subtle components of aggression. More obvious components, such as threatening displays, posturing and meral spreading, probably require sensory cues not provided by the mirror image.

**Behaviour in the activity monitor**

A reflective environment did not significantly alter the total distance traveled, but a 22% increase in the distance traveled in the reflective environment approached the significance level. The average speed and parallelism index were not affected by reflection. These data suggest that a reflective environment might have some influence on the magnitude of locomotor activity, but did not affect the speed of progression or the geometry of exploration. It seems surprising that reflection, which increased the occurrence of turning around behaviour, did not decrease parallelism index. However, the relationship between the number of occurrences of turning around behaviour and parallelism index is not linear (Brudzynski and Krol, 1997), and the number of episodes with straight-line progression remained very high. The data indicate that the cumulative effect of all turns is not significantly different between reflective and non-reflective environments and suggest a similar pattern of exploration in both cases.

The presence of the position-indicating probe above the thorax did not prevent the reflection from enhancing the number of occurrences of cornering, turning around and rearing up. This suggests that the probe had no substantial effect on exploratory behaviours.
Overall, the results indicate that parallelism index measurements do not describe subtle spatial aspects of exploratory behaviour that could be influenced by the experimental conditions. Interestingly, mean parallelism index values reported here for crayfish (0.39 ± 0.12) are similar to those reported for rats in similar activity cages (0.3 ± 0.02; Brudzynski and Krol, 1997), suggesting similar exploratory patterns, particularly pertaining to straight line progression along the walls. The comparison of parallelism indices between crayfish and rats may also suggest that the square geometry of the recording cage may have a predominant effect on the final value of the parallelism index regardless of the species. If this is the case, then crayfish and rats, despite their distant locations in the evolutionary tree, may perform a similar thigmotactic behaviour and allocate most of the exploratory time to following walls. This behaviour is probably the most successful strategy of maximizing survival and was independently developed to a similar degree by vertebrate and invertebrate species.

In summary, the current investigation presented additional evidence for the importance of the crayfish visual system during exploratory behaviour. Several predominant elements of crayfish exploratory behaviour in a novel environment have been identified. These behaviours were affected by reflection, while other parameters such as speed of locomotion, distance and parallelism index were not. Rearing up, turning around, cornering, middle stance and curled tailed behaviours were predominantly influenced by visual cues.
Chapter 3

The influence of social isolation on behaviour in reflective and non-reflective environments

J.K. Drozdz, A.J. Mercier and S. Brudzynski
Abstract

This study examined effects of social isolation on the appearance of reflection-dependent behaviour in intact crayfish *Procambarus clarkii*. Social isolation is thought to involve a lack of socially related sensory inputs, which can influence exploratory activity (see Literature Review) and responses crayfish exhibit to mirrors.

Twenty-four crayfish were housed in separate glass tanks for two weeks, and twenty-four other crayfish were housed as pairs in glass tanks for two weeks. Following the two-week period, each crayfish was placed separately into an experimental tank for twenty minutes. Half of the experimental tank was lined with mirrors, and the other half was lined with matte-finished, non-reflective plastic. Rearing up, turning around, cornering, backward walking, middle-stance and curling the tail was observed and the frequency of these behaviours was recorded. The time spent in front of the mirror was also measured using a stopwatch. Crayfish housed separately showed no difference in rearing up, turning around, cornering, backward walking or curling the tail between the mirror and non-mirrored portions of the tank. They did show significantly more middle-stance in front of the mirror. Crayfish housed in pairs showed significantly more of all six behaviours in front of the mirror than in the non-mirrored portions of the tank and they spent significantly more time in front of the mirror than in the non-mirrored part of the tank.
Introduction

Recent experiments (chapter 2) indicate that visual cues provided by a reflective environment enhance several behaviours recorded during exploratory activity in crayfish (*Procambarus clarkii*). Specifically, crayfish lift themselves against the side of a tank (rearing up), turn around or position themselves in a corner more frequently in a tank with reflective walls than with non-reflective walls. It seems likely that at least some of these behaviours represent components of social interactions since many animals exhibit social behaviours in response to their own mirror image (e.g. Craft et al., 2003; Gallup, 1968; Pepperberg et al., 1995; Schusterman et al., 1967; Thompson, 1963). Amongst the crustaceans, hermit crabs have been shown to respond to their own mirror image by exhibiting social displays (Dunham and Tierney, 1986).

Social behaviour depends greatly on continued social interactions (Corkum and Cronin, 2004; Welch, 1965). The importance of social interaction is often demonstrated in studies involving social isolation, where one animal is housed for several hours or days in its usual condition except that no conspecifics are present. Such isolation alters many behaviours, such as aggression (Dunham, 1972; Hoffman et al., 1975; Hazlett, 1966a), sexual performance (Cooke et al., 2000), locomotor activity (Guo et al., 2004; Hazlett, 1966a; Rilke et al., 1998a) and exploration (Francolin-Silva and Almeida, 2004; Gallup and Suarez, 1980; Herskin and Jensen, 2000; Jensen et al., 1999b; Suarez & Gallup, 1980).

Some animals exhibit decreased locomotor activity and decreased social interaction when returned to field conditions or re-introduced to conspecifics following social isolation. Such observations have been reported for rats (Franklin-Silva and Almeida, 2004), chickens (Gallup et al., 1971; Gallup and Suarez, 1980b), ducks (Suarez and Gallup, 1980), and angelfish (Franck et al., 1985). In other animals, however, social isolation has been reported to increase locomotor activity and aggression. Such observations have been made for paradise
fish (Davis, 1975), Siamese fighting fish (Ichihashi et al., 2004), mice (Guo et al., 2004; Rilkie et al., 1998, 1998) hermit crabs (Hazlett, 1966a) and lobsters (Dunham, 1972; Hoffman et al., 1975).

The purpose of the present investigation was to determine whether social isolation influences reflection-enhanced behaviours in crayfish. The strategy was to isolate crayfish for two weeks (this amount of time was based on previous literature) and then observe their behaviour in a test tank, half of which was lined with mirrors and half of which was lined with non-reflective plastic. Responses were compared to those of crayfish maintained in pairs for the same duration.
Materials and methods

Animals

Forty eight naïve male and female red swamp crayfish, *P. clarkii*, were used. For additional information regarding animals used please see chapter 2.

Twenty-four crayfish were socialized for two weeks prior to testing by housing them in same sex pairs in twelve tanks. Another twenty-four crayfish were isolated for two weeks by being housing separately in twenty-four same size tanks. For additional information regarding housing and care of the animals please see chapter 2.

Behavioural observations

Specific behaviours were identified based on the consistency of the movement among animals and the frequency at which each activity occurred. All behavioural patterns observed during exploratory activity were identified as described previously (chapter 2).

In the present study, crayfish exhibited six predominant behaviours in the mirror/matte-lined test tank. These behaviours were rearing up (lifting against the wall), cornering (sitting in the corner), turning around, reverse walking, middle stance (standing in the middle of the tank facing one of the walls) and curled tail (curling the tail under the abdomen). For complete details of behavioural descriptions see chapter 2.

Testing took place 4 days per week between 12:00 – 16:00 hours and always before feeding if crayfish were fed on that day. Typically, three to five crayfish were tested daily. On the day of testing each crayfish was carefully transported to a separate experimental room in a white bucket with water and was left undisturbed for approximately 30 minutes. Each crayfish was then carefully placed in the middle of a test tank, containing mirrors lining one half and non-reflective plastic lining the other half (Figure 2.3). A black line was drawn along the midline of the tank floor to help the observer distinguish the reflective and non-
reflective areas. Immediately after the animal made its first move, the behaviour and time spent on the mirrored and matte sides of the tank were continuously recorded for 20 minutes with a stopwatch ($T_{\text{total}}$). The time the crayfish spent on the matte side of the tank was determined by subtracting the time spent on the mirrored side from the total time spent in the testing tank: $T_{\text{matte}} = T_{\text{total}} - T_{\text{mirror}}$. A change of occupancy to either side (mirrored or matte) was defined to occur when the animal crossed the dividing line with its chelae and head. The experimenter was positioned directly above the test tank and the crayfish. Animals were carefully observed and behaviours recorded (frequency of occurrences) for 20 minutes.
Results

Animals isolated for two weeks prior to testing performed approximately the same amount of rearing up, turning around, cornering, backward movement and curled tail behaviour on the mirrored side of the tank as on the non-mirrored (matte) side (Figure 3.1). There were no significant differences between the number of occurrences of any of these behaviours on the mirrored and matte sides. The only behaviour showing any significant difference in isolated crayfish was middle stance behaviour, which was performed more frequently in the mirrored area than the matte area (p <0.05). Of the six behaviours exhibited by isolated crayfish, the most commonly observed in both mirrored and matte areas was cornering.

In contrast to isolated animals, crayfish housed in pairs for 2 weeks prior to testing showed significant differences in the number of occurrences of each of the six exploratory behaviours on mirrored and non-mirrored portions of the tank (P<0.05 in each case; Figure 3.2). Thus, socialized crayfish responded to the reflective environment, but isolated crayfish did not.

A comparison of Figures 3.1 and 3.2 suggests that the frequency of at least some exploratory behaviours on the mirrored side was higher in socialized crayfish than in isolated crayfish, in particular, for turning around, cornering and curled tail behaviours. To determine whether such trends were statistically significant, the number of occurrences of each behaviour on the mirrored side was compared between socialized and isolated crayfish using a Mann-Whitney U-Test for independent samples (Table 3.1, mirror condition). On the mirrored side, socialized crayfish turned around (P<0.0001), cornered (P=0.001) and curled the tail (P=0.0011) significantly more than isolated crayfish. There were no significant differences for rearing up, middle stance or reverse walking behaviours between socialized and isolated crayfish in the mirrored area. There were no significant differences between
Figure 3.1 Exploratory behavioural patterns of *P. clarkii* on mirrored and matte sides of a tank after 2 weeks of social isolation. Middle stance occurred more often on the mirrored side of the tank during a 20-minute session (* indicates P < 0.05, N = 24) Statistical comparisons were made using a Wilcoxon Signed Rank test for Correlated Samples (Ferguson, 1971). Error bars represent standard deviations.
Figure 3.2 Exploratory behavioural patterns *P. clarkii* on mirrored and matte sides of a tank after 2 weeks of socialization. Rearing up, turning around, cornering, reverse walking, curled tail and middle stance occurred more often on the mirrored side of the tank during a 20-minute session (Wilcoxon test, *P* < 0.05, *N* = 24). Error bars represent standard deviations.
<table>
<thead>
<tr>
<th></th>
<th>MIRROR</th>
<th></th>
<th></th>
<th>MIRROR</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>socialized</td>
<td>isolated</td>
<td>P-value *</td>
<td>socialized</td>
<td>isolated</td>
<td>P-value *</td>
<td></td>
</tr>
<tr>
<td>Rearing up</td>
<td>3.29 ± 3.03</td>
<td>2.67 ±2.37</td>
<td>n.s.</td>
<td>1.33 ± 1.40</td>
<td>2.50 ± 2.93</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Turning around</td>
<td>6.08 ± 2.92</td>
<td>2.42 ±1.56</td>
<td>&lt;0.0001</td>
<td>2.54 ± 1.61</td>
<td>3.29 ± 2.11</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Cornering</td>
<td>10.46 ± 4.40</td>
<td>5.83 ± 2.44</td>
<td>0.001</td>
<td>4.87 ± 2.79</td>
<td>6.08 ± 3.12</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Reverse walking</td>
<td>1.00 ± 1.61</td>
<td>0.62 ±1.47</td>
<td>n.s.</td>
<td>0.17 ± 0.38</td>
<td>0.33 ± 0.56</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Curled tail</td>
<td>3.92 ± 2.95</td>
<td>1.50 ± 1.93</td>
<td>0.0011</td>
<td>1.45 ± 1.84</td>
<td>1.67 ± 1.49</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Middle stance</td>
<td>1.08 ± 1.10</td>
<td>0.79 ±1.02</td>
<td>n.s.</td>
<td>0</td>
<td>0.08 ± 0.28</td>
<td>n.s.</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1  Average number of behavioural responses calculated for previously socialized and isolated *P. clarkii* from mirrored and matte area of the tank (Mann-Whitney test, N=24). Error bars represent standard deviations
* n.s. – no significance (P>0.05)
<table>
<thead>
<tr>
<th>INDEX</th>
<th>PREPIED</th>
<th>FINISHED</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>50</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>100</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

The table above shows the comparison between prepied and finished measurements. It indicates that there is a significant difference between the two values.
socialized and isolated crayfish for any of the behaviours in the non-reflective area (Table 3.1, matte condition).

Total time spent on mirrored and non-reflective sides of the tank was also determined (Figure 3.3). Socialized animals spent significantly more time in the mirrored area than in the matte area (P<0.05). Isolated animals showed no significant difference between time spent on mirrored and non-mirrored sides. On average, socialized crayfish spent 12.64 ± 1.89 minutes (out of a total of 20) in the mirrored area, whereas isolated animals (P<0.05) spent 8.66 ± 2.31 minutes in the mirrored area (Figure 3.3).
Figure 3.3 Socialized animals spent significantly more time in the mirror area than matte area (* P<0.05, N=24, student t-test for correlated samples). Error bars represent standard deviation.
Discussion

The current investigation demonstrates that social isolation influences the appearance of reflection-enhanced behaviours in *P.clarkii*. Socialized crayfish exhibited higher frequencies of rearing up, turning around, cornering, reverse walking and curled tail behaviours when exploring the mirrored half of a test tank than when exploring the non-reflective half. In isolated crayfish, however, the frequencies of these behaviours were not different between mirrored and non-reflective conditions. In addition, socialized crayfish spent significantly more time in the reflective area, whereas isolated crayfish did not (Figure 3.3). Thus, my observations clearly indicate that socialized crayfish respond to mirrors in ways that isolated crayfish do not. We can not completely rule out the possibility that isolated crayfish respond to mirrors in ways that were not detected by our methods, but we observed no differences in behaviours of isolated crayfish in reflective and non-reflective environments.

When the data were analyzed with the respect to the frequency of behavioural occurrences that took place only on the mirrored side of the test tank, turning around, cornering and curled tail behaviour were significantly higher for socialized animals than for isolated animals (Table 2). There were no significant differences between socialized and isolated crayfish with respect to rearing up, reverse walking and middle stance behaviours. Thus, socialization appears to enhance the production of three of the six behaviours observed in front of the mirror. This observation supports the notion that socialization enhances the responses of crayfish to visual cues associated with their own mirror image. An alternative explanation is that social isolation alters the behavioural state by suppressing the expression of reflection-enhanced behaviours. My observations are not consistent with such a notion of behavioural suppression. Specifically, there were no differences in the frequency of any
behaviours on the non-reflective side when comparing isolated animals with socialized animals (Table 2). Thus, isolation *per se* does not appear to suppress these behaviours.

It was shown that social isolation enhances activity and aggression in crustaceans (Courchesne and Barlow, 1971; Dunham, 1972; Hoffman et al., 1975; Hazlet, 1966). However, in the present study there is no evidence that responses of crayfish to mirrors represent aggressive displays or constitute aggressive behaviour. In fact, most of the components of aggressive displays in crayfish, such as meral spreading and elevated posture (Bruski and Dunham, 1987; Tierney et al., 2000) were not observed in the present experiments. Social isolation did not appear to increase activity in the present study, based on the frequencies of the behaviours observed. This may be related to the duration of the isolation period. Hazlett (1966a) reported that isolating hermit crabs for 2-3 days increased activity but isolation for 5 days or longer did not.

In many animal species social isolation enhances aggressive displays and aggressive encounters with conspecifics (Davis, 1975; Ichihashi et al., 2004; Rasa, 1971; Valzelli, 1969, 1973). Such observations have been made for crustaceans, specifically for hermit crabs (Courchesne and Barlow, 1971; Hazlett, 1966a) and lobsters (Dunham et al., 1972; Hoffman et al. 1975). Lobsters, *H. americanus*, isolated for 36 days (Dunham et al., 1972) and for up to 132 days (Hoffman et al., 1975) exhibited enhanced aggressive behaviour. Hermit crabs (*P. samuelis*) were socially isolated for 3, 8, 12 and 30 days and it was shown that their aggressive activity seemed to have stabilized after 12 day isolation period (Courchesne and Barlow, 1971). Another species of hermit crab (*C. tibicen*) socially isolated for only 2-7 days, displayed the highest peak of aggressive behaviour during the 3rd day of isolation. After the 5th and 7th day of isolation crabs' aggression level still continued to increase but not as significantly as after the 3rd day (Hazlett, 1966a).
If responses to reflection represent components of or preludes to aggressive encounters, one would expect the frequency of such behaviours to increase after social isolation. In the present study, however, the frequency of reflection-enhanced behaviours decreased with social isolation. This would suggest that reflection-enhanced behaviours are not components of aggressive behaviour. On the other hand, following social isolation crayfish may be less likely to initiate any behaviour including aggressive behaviour, thus, the reflective image they see may lack components necessary to be perceived as a threat. Moreover, the mirror image is only a visual cue, and other sensory cues, such as tactile and chemoreceptive inputs, may be essential for initiating aggressive displays.

To account for differences that social isolation might have on animal behaviour in general, Gallup and Suraez (1980) proposed a model, that suggests that the initial behaviour of a previously isolated animal in a novel environment is a compromise between the need to reinstate social contact with a conspecifics and the need to avoid predators. The choice or the intensity of behaviour that will be displayed depends on the amount of time spent in isolation. Gomez-Laplaza and Morgan (1991, 2000) demonstrated that after a short isolation time the angelfish (*P. scalare*) has a tendency to try to reestablish contact with conspecifics, and after a longer isolation time this tendency shifts towards trying to minimize predator detection. In the current study isolation of *P. clarkii* for 14 days had a profound effect on their behaviour in a mirrored environment. These socially isolated crayfish did not display a higher frequency for identified behaviours (Table 3.1). In fact, even in front of mirrors, which are known to enhance aggressive behaviours and other social displays, social isolation did not increase these behaviours (Figure 3.1). Therefore, in the current study the lower frequency of behaviours displayed by isolated crayfish compared to socialized animals, might reflect an attempt to minimize detection. Studies are underway to determine whether reflection-enhanced behaviours in crayfish are related to aggression.
The text in the image is not legible due to the quality of the scan or the nature of the content. It appears to be a page from a book or a document with text that is not clearly visible. Without clearer visibility, it is not possible to provide a natural text representation of the document.
Chapter 4

General conclusions

Chapters 2 and 3 provided added insight into factors (i.e. reflective stimuli and social isolation) affecting exploratory behaviour of crayfish in a novel environment.

Studies presented in chapter 2 demonstrate that crayfish (*P. clarkii*) reared up, turned around and cornered more in a reflective aquarium than in a non-reflective aquarium, regardless of whether the reflection was blocked with paper or plastic matte. It was concluded that a reflective environment, and not the amount of light, influences crayfish exploratory and general behaviour in an empty, novel tank by increasing the frequency of occurrence of these observed behaviours. Although many animals, including hermit crabs do respond to mirror images as though they were conspecifics (Craft et al., 2003; Dunham and Tierney, 1986; Gallup, 1968; Hazlett, 1966c; Montevecchi et al., 1973; Thompson, 1963; Thompson and Sturm, 1965), it is not clear whether crayfish did so in the present study. It is, however, reasonable to propose that the behavioural patterns enhanced by reflection in the present study are components of social behaviour. There is no definitive evidence that the reported behaviours are components of agonistic behaviour. Many components of the stereotypical aggressive displays of crayfish were missing from the behaviours exhibited in front of the reflection (e.g. aggressive posturing, meral spreading, striking, pushing and tail flipping). Therefore, it is suggested that if behaviours such as rearing up and turning around are components of agonistic encounters, they are probably subtle components of aggression. Mirror image alone probably does not provide adequate sensory cues for more obvious components of aggressive behaviour, such as threatening displays, posturing and meral spreading.
Chapter 3 demonstrated that social isolation influences the appearance of these reflection-enhanced behaviours in *P. clarkii*. Socialized crayfish exhibited higher frequencies of rearing up, turning around, cornering, reverse walking and curled tail behaviours when exploring the mirrored half of a test tank than when exploring the non-reflective half. In socially isolated crayfish, however, the frequencies of these behaviours were not different between mirrored and non-reflective conditions. In addition, socialized crayfish spent significantly more time in the reflective area, whereas isolated crayfish did not. After social isolation, many animals exhibit a decrease in general activity and aggression (Franck et al., 1985; Gallup et al., 1970; Heiligenberg, 1964). This thesis suggests that social isolation may suppress the reflection-enhanced behaviours and socialization may permit reflection-enhanced behaviours to occur more frequently.

The general conclusion of this thesis is that reflective visual stimuli influence crayfish spontaneous exploratory behaviour and that the ability of crayfish to respond to a reflective environment is affected by their previous social experience. Further experimentation will be required to better understand these two phenomena and the factors on which they depend.

**Perspectives**

*Long term housing conditions*

In this thesis it was demonstrated that exposure to a novel reflective environment for 20 minutes affects crayfish behaviour, specifically rearing up against the aquarium wall, cornering and turning around when walking. Little attention has been paid to housing conditions of experimental animals during many behavioural studies. Therefore, the conclusions described in this thesis should be of some interest and importance when planning long-term housing of experimental animals. Housing mice, chicks, rats, fish and crayfish in potentially reflective environments, such as glass tanks and cages (Essman, 1968; Rajecki et
al., 1977; Gentsch et al., 1981; Davis et al., 1974; Hinkel and Maier, 1974; Heckenlively, 1970) may affect the frequency of specific behaviours or considerably alter behaviour in subsequent experimentation. Further experiments could be undertaken to establish the difference between reflective and non-reflective long term housing environments and their effect on exploratory behaviour. Such studies would help establish the optimal housing conditions for crustaceans used in behavioural studies.

*Completely mirrored tank*

Reflection from the walls of experimental or housing tanks seems to have never been considered in previous studies. However, mirrors have often been an interesting tool in behavioural studies since they have been shown to elicit social behaviours in many animals including crustaceans (Dunham and Tierney, 1986; Hazlett, 1966c). The behaviours enhanced by mirrors in the present study and in other studies are considered to be components of social behaviours, including agonistic encounters. Mirrors are well known to provide greater detail in a reflected image than just a smooth piece of glass. Therefore, it was not surprising that in the current study, when crayfish were placed in a half-mirrored tank two additional, previously unseen behaviours were noted, curled tail and middle stance.

Curled tail behaviour is seen when crayfish assume aggressive postures during agonistic encounters (Antonsen and Paul, 1977; Livingstone and Kravitz, 1980; Tierney and Mangiamele, 2001). Middle stance is a ‘freezing response’ as it is characterized by immobility and inactivity in the middle of the tank. In *P. clarkii* a submissive posture consists of the animal holding its body flat against the substrate and holding the chelae forward (Huner and Barr, 1984). Such responses can be a consequence of acute stress or part of predator avoidance (Bovbjerg, 1953), thus middle stance is suggested to be an important component of submissive posture in crayfish.
The frequency of curled tail and middle stance behaviours was very low, and since these behaviours were not accompanied by other behavioural elements that are displayed during agonistic displays (i.e. meral spread and heightened posture), they can not be classified as aggressive postures. Also, the frequency of middle stance behaviour was not significantly different on mirrored and non-reflective sides of the aquarium (Figure 2.7A). Because compound eyes of crustaceans can provide nearly 360° vision (Bovbjerg, 1956; Shaw and Stowe, 1982), the possibility that the crayfish are responding to a reflection behind them on the mirror or non-reflective wall can not be eliminated. This possibility might be tested by comparing behaviours in a tank completely lined with mirrors with behaviours in a tank with no mirrors. A completely mirrored tank would be expected to increase the frequency of middle stance and tail curling and may also increase the frequency of the other behaviours.

**Sensory modalities.**

The experiments described in this thesis provided no definitive evidence that the observed behaviours are aggressive in nature. It seems that in order to elicit a full-blown aggressive response a crayfish may need more sensory input (i.e. tactile, chemoreceptive, mechanoreceptive inputs) (Bovbjerg, 1953; Bruski and Dunham, 1987; Huber et al., 1997; Tierney et al., 2000). *P.clarkii* are aquatic animals that heavily rely on chemoreceptive cues that spread easily through the water. However, in the current investigation where only visual cues were modified, a solitary crayfish seems to exhibit only a preface to aggression at most, and this may be displayed in the form of agitation or stress. To test the idea that only certain components of aggressive encounters are elicited by crayfish when only visual inputs are stimulated, visual cues could be combined with chemoreceptive, tactile or
mechanoreceptive cues. Exposing crayfish to additional sensory cues may enhance or completely modify the observed behaviours.

Visual cues are not necessary to elicit agonistic encounters in crayfish, since such encounters occur in blind crayfish species (Kellie et al., 2001; Li and Cooper, 2002) and blindfolded crayfish (Breithaupt and Eger, 2000). Testing blind, cave crayfish in an empty, novel, object-free tank may reveal additional information about the importance of visual inputs for the behaviours described in the present study. Behaviours such as rearing up, cornering and turning around are enhanced by reflective surfaces but are also present in non-reflective environments. It would be interesting to know if these behaviours are exhibited by naturally blind or blindfolded crayfish. If these behaviours are truly enhanced by reflection, their frequency should not increase when blind crayfish are exposed to a reflective environment.

Serotonin

Huber et al. (1997) showed that injection of serotonin into the haemolymph of a subordinate *A. astacus* reduced the likelihood of retreat and increased the duration of fighting. Similar results were obtained with other species of crayfish (Panksepp et al., 2002; Tierney et al., 2004; Tierney and Mangiamele, 2001). Similar trends were observed by Peeke et al. (2000) with juvenile lobsters (*H. americanus*), where higher doses of serotonin inhibited locomotor behaviour and prevented the lobster from securing or retaining possession of a shelter. Yeh et al. (1996) found that crayfish social status and social experience determine the effect of serotonergic modulation on the lateral giant motor neuron that mediates one form of escape behaviour.

Application of exogenous serotonin could be used to determine whether or not the behaviours observed in the current investigation also depend on serotonin. It would be
interesting to know if these behaviours are regulated by the same neurotransmitters as aggressive behaviours.

**Illumination levels**

In crustaceans it is reported that the diminished locomotor activity and withdrawal responses (Fanjul-Moles et al., 1998; Fernandez-de-Miguel and Arechiga, 1992a), as well as increased reactivity (Welsh, 1934; Pei et al., 1996; Bojsen et al., 1998) can be characteristic behavioural changes to increased illumination. Therefore, it would be interesting to investigate the effect of extremely high or low illumination level *per se* on observed behaviours (i.e. rearing up, turning around and cornering). The resulting data would shed additional information on how *P. clarkii* reacts in such extreme light conditions and if the observed behaviours would be affected by it.

In the current study exploratory patterns exhibited by *P. clarkii* consisted mainly of straight-line progression along the walls. This is in accordance with other literature describing thigmotactic behaviour of crayfish (Burba, 1988; Delgado-Morales et al., 2004; Rubenstein and Hazlett, 1973; Varju and Sandeman, 1989). This pattern of exploration was not changed by reflection, as indicated by measurements of parallelism index. Therefore, thigmotaxis seems to be a strongly embedded, instinctive behavioural response to novel environments and may represent a successful strategy for maximizing survival for many animal species. It is not known whether such robust thigmotactic behaviour can be altered with extreme illumination of the environment.

**Time in social isolation**

According to Gallup and Suraez (1980) the initial behaviour of a previously isolated animal in a novel environment is a compromise between the need to reinstate social contact
null
with another conspecific and the need to avoid predators. After relatively short social isolation, animals are seeking social reintegration by trying to reestablish contact with another conspecific, whereas after relatively long social isolation animals are trying to minimize their detection by predators by becoming immobilized and less active. Data from the current investigation show that social isolation prevents an increase in the frequency of specific behaviours in a reflective environment compared to a non-reflective environment. If the observed behaviours represent components of social behaviour, then according to Gallup and Suarez’s model, crayfish removed from social isolation are not initiating contact with reflection anymore, thus possibly minimize their activity in order to be less visible to potential predators. Such conclusions would also suggest that relatively long-term social isolation has been applied to the subjects, rather than short-term isolation. However, to validate such conclusions, additional experiments should be undertaken, in which the time spent in social isolation is varied. In the current investigation P. clarkii was socially isolated for two weeks. Additional experiments should use relatively longer and/or shorter amounts of time for the duration of social isolation.

Dominance and submission

Encounters with others enrich an animal’s life, usually resulting in accumulation of various and complex stimuli and experiences. In the current study, socialized crayfish came directly from an environment where a specific social hierarchy had been established through agonistic interactions. After being housed in pairs, crayfish have become dominant or submissive in their social status. Social isolation, on the other hand, seems to decrease the development of social responses and slows the establishment of a social hierarchy (Boe and Faerewik, 2003). The hierarchies determine who gets first access to food, shelter and mates, and they are formed through physical fights in crayfish (Bovbjerg, 1953,1956; Copp,
Crayfish kept in pairs thus learned the dominant or submissive behavioural ‘rituals’ that characterize each status. When placed in a half-mirrored, half-matte environment these crayfish should exhibit established and rehearsed social behaviours, characterized by either dominant or submissive status previously acquired while being housed with other conspecifics. Conversely, socially isolated crayfish should have less experience in anticipating unexpected stimuli and selecting an appropriate response and, thus, should appear less socially ‘adept’. Hoffman et al. (1975) suggested that in lobster the exposure to a conspecific leads to a reduction in aggression. This reduction may result from establishment of dominance hierarchies within the communal tanks that would lead to decreased aggressiveness in subordinate animals.

Several predictions can be made from the rationale described above. Dominant crayfish, taken from a social environment (i.e. housed in pairs), may be more likely to investigate the mirror image with aggressive intentions. Submissive crayfish may tend to avoid the mirror image in order to avoid contact with a dominant conspecific. Socially isolated crayfish, which are dishabituated (unused) to the presence of a conspecific, may become indifferent to a conspecific image. Therefore, socialized crayfish may react to their own reflected images in accordance with their social status previously acquired through housing (i.e. cornering by dominant crayfish suggesting lunging and contact, turning or crossing by submissive crayfish suggesting avoidance of the reflection). The possibility that dominant and submissive crayfish may react differently in front of mirrors was not considered in the current investigation. No attempt was made to determine the social status of each crayfish in socialized pairs. The process of hierarchy formation and its maintenance in *P. clarkii* may continue to take place in front of mirrors after being housed in pairs, and this element of crayfish behaviour should be further investigated.
Further perspectives

Renner (1990) states that the study of non-task behaviours has been inappropriately neglected, and that spontaneous activity can reveal much about the nature of animal behaviour. Exploration is a robust phenomenon with intrinsic value to animals. It is necessary to find food, mates and shelter. An additional hypothesis proposes that animals gain information by exploring, resulting in storage of data to be used in satisfying future needs instead of immediate needs (Toates, 1983; Welker, 1961).

Freshwater crayfish offer advantages over many other animals due to their high level of social interaction in both the field and the laboratory. Crayfish have a rather wide behavioural repertoire, and their relatively simple nervous system allows one to test various hypotheses to explain both simple and relatively complex animal behaviours. *P. clarkii* has been successfully used to study Pavlovian-based avoidance learning where light illumination was used a conditioned stimulus (Kawai et al., 2004).


Basil, J, D Sandeman, 2000, Crayfish (*Cherax destructor*) use tactile cues to detect and learn topographical changes in their environment. Ethology, 106, 247-259.

Bennett, EL, M R Rosenzweig, M C Diamond, 1969, Rat brain: effects on environmental enrichment on wet dry weights. Science, 163, 825-826.


Bowerman, RF, J L Larimer, 1974, Command fibers in the circumesophageal connectives of crayfish II phasic fibers. Journal of Experimental Biology, 60, 119-139.


Breithaupt, T, P Eger, 2002, Urine makes the difference: chemical communication in fighting crayfish made visible. Journal of Experimental Biology, 205, 1221-1231.


Copp, NH, 1986, Dominance hierarchies in the crayfish Procambarus clarkii (Girard, 1852) and the question of learned individual recognition (Decapoda, Asticdea). Crustaceana, 51(1), 9-24.


Hazlett, BA, 1966a, Factors affecting the aggressive behaviour of the hermit crab (Calcinus tibicen). Z. Tierpsychol., 6, 655-671.


Hinkel, TJ, R Maier, 1974, Isolation and aggression in Siamese fighting fish (Betta splendens). Psychological Reports, 34, 1323-1326.


Huner, JV, J E Barr, 1984, Red swamp crawfish: biology and exploitation. Louisiana Sea Grant College Program, Center for Wetland resources, Louisiana State University, Baton Rouge, Louisiana.


Jensen, KH, O T Kleiven, P J Jakobsen, 1999a, How important is light in the aggregation behaviour of Daphnia pulex (Cladocera: Crustacea)?. Hydrobiologia, 411, 13-18.


Kovac, M, 1974b, Abdominal movements during backward walking in crayfish. II. The neuronal basis. Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology, 95, 79-94.


Leggett, LMW, 1979, A retinal substrate for colour discrimination in crabs. Journal of Comparative Physiology, 133, 159-166.


Okada, Y, T Yamaguchi, 1988, Nonspiking giant interneurones in the crayfish brain: morphological and physiological characteristics of the neurons postsynaptic to visual interneurons. Journal of Comparative Physiology A: Sensory, Neural and Behavioral Physiology, 162, 705-714.


Peeke, HVS, G S Blank, M H Figler, E S Chang, 2000, Effects of exogenous serotonin a motor behaviour and shelter competition in juvenile lobsters (*Homarus americanus*). Journal of Comparative Physiology A: Sensory, Neural and Behavioral Physiology, 186(6), 575-582.


Siwak, CT, P D Dwight, N W Milgram, 2001, Effect of age and level of cognitive function on spontaneous and exploratory behaviours in the beagle dog. Learning and Memory, 8, 317-325.


Thompson, T, T Sturm, 1965b, Classical conditioning of aggressive display in Siamese fighting fish. Journal of the Experimental Analysis of Behavior, 8(6), 397-403.


Tolman, CW, 1965, Feeding behaviour of domestic chicks in the presence of their own mirror images. Canadian Psychologist, 6, 227.


Welk, CAG, T Yamaguchi, 1966, The neuronal components of the optic nerve of the crayfish as studied by single unit analysis. Journal of Comparative Neurology, 128, 333-358.


