THE ROLE OF THE ANTENNULE INNER RAMUS IN MEDIATING RESPONSE TO DISTANT CHEMICAL INFORMATION BY FEMALE CRAYFISH  
(PROCAMBARUS CLARKII)  

by  

Tuhin Giri  

A thesis submitted in conformity with the requirements for the degree of Master of Science  
Graduate Department of Zoology  
University of Toronto  

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THE ROLE OF THE ANTENNULE INNER RAMUS IN MEDIATING RESPONSE TO DISTANT CHEMICAL INFORMATION BY FEMALE CRAYFISH

*(PROCAMBARUS CLARKII)*

Tuhin Giri

Department of Zoology, University of Toronto

Master of Science, 1998

The bifurcated antennules of decapod crustaceans are believed to be the most important organs used in long-distance chemoreception. Specific antennular rami were ablated to determine if female red-swamp crayfish (*Procambarus clarkii*) used the inner rami to locate food and social stimuli. Rami were not required for the initiation of searching behaviours over short distances, but were required over long-distances. Although all animals in all treatment groups were able to detect a food stimulus over long distances, only one-half of the animals possessing only the inner rami were able to locate it. These successful animals walked slower, and needed a longer search duration than did controls or other treatment groups. Crayfish with only inner rami experienced no such disadvantage when locating the source of a social stimulus, and were similar to other treatment groups in latency to search, search duration, meander ratio and average walking speed. These results demonstrate that the inner rami are used by female *P. clarkii* in the localisation of social stimuli.
Acknowledgments

There have been quite a few people who have provided invaluable assistance over the past two years, and unfortunately this space cannot adequately convey my gratitude to them.

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Finally, I wish to thank my cat (to whom I dedicate this thesis), 21 goldfish, 2 axolotls, 423 Betta splendens, an innumerable number of Cambarus robustus, Cambarus bartonii, Fallicambarus fodiens, and over 200 Procambarus clarkii, for showing me how much was actually going on, if only I bothered to look.

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CHAPTER 1

General Introduction
Chapter 1: General Introduction

Chemical signals are used by a large number of organisms, inhabiting both terrestrial and aquatic environments, to identify objects of biological importance in the surrounding environment. Chemicals are known to be used in the detection and localisation of potential mates (e.g. Akers, 1989; Amey-Akumfi & Hazlett, 1975; Dunham, 1978; Svensson, 1996), in aggressive interactions with conspecifics and members of other species (Karavanich & Atema, 1991; Karavanich & Atema, 1993), and in the detection and localisation of food (e.g. Weissburg & Zimmer-Faust, 1994). Chemical stimuli are also used by various organisms to avoid predators and toxic substances (Atema, 1980). Animals which possess the appropriate receptors can therefore use chemicals as signals, with the information contained therein of benefit to the sender, the receiver, or both individuals (Atema, 1995).

Certain properties of chemical stimuli make them particularly good at carrying information. Although other stimuli, such as light or sound, may be faster and easier for an animal to locate (Dusenbery, 1992), chemical stimuli have several advantages which have helped make them the most commonly used of all stimuli (Atema, 1980; Zimmer-Faust et al., 1995). These stimuli, for example, are not dependent upon light levels and are thus usable under both light and dark conditions (Atema, 1980). Second, they are cheap to generate, and can thus be produced in large quantities (Dusenbery, 1992). Chemical stimuli can also be highly specific, depending upon the size of the molecular structure—larger stimulus molecules, such as proteins, have greater specificity (Atema, 1980; Atema, 1985). One consequence of this specificity is that an almost infinite
number of stimulus combinations can be created from mixtures of compounds, producing a unique chemical picture of the object or animal that is releasing it (Ache, 1988; Atema, 1985; Dusenbery, 1992).

The compounds which are used to create this chemical picture come from a number of sources. Amino acids, for example, are often used as chemical signals in aquatic environments because they are soluble in water and are also present in large quantities. In addition, amino acids are present in cells (Carr, 1988) and are released by aquatic animals through metabolic activity, and in breath, urine and faeces (Atema, 1995; Cowan, 1991; Gleeson, 1980). The specific profile created by mixtures of chemical compounds released by an organism can be detected by other animals, and used to discriminate between species, sexes, and stress levels (Atema, 1980).

There is, however, a significant disadvantage to the use of chemical stimuli. In both terrestrial and aquatic environments, turbulent current flows—such as wind in a terrestrial environment and water currents in an aquatic one—impede homogeneous distribution. Chemical plumes result, which are distributed in a non-uniform pattern, sometimes resulting in pockets of low odour concentrations near the source and much higher concentrations further away (Atema, 1985; Moore et al., 1991b).

Although turbulent flow may hinder orientation, it cannot be argued that the absence of all flow is desirable. Under stagnant conditions, odours are expected to diffuse slowly, creating a gradient with concentrations of odour exponentially decreasing with increasing distance from the source (Okubo, 1980). Weissburg & Zimmer-Faust (1993) observed that a blue crab, *Callinectes sapidus*, was unable to locate prey in the absence of
a current. Other animals have also been observed to require flow to successfully locate an odour source. Male gypsy moths, *Lymantria dispar*, respond to the presence of female sex pheromone by orienting into the wind (Baker, 1986; David et al., 1983a; David et al., 1983b; Svensson, 1996). Sharks are also believed to require flow to successfully locate an odour source (Hodgson & Mathewson, 1971).

Turbulent flow is thus an advantage and a disadvantage to animals attempting to localise the source of an odour. Nevertheless, the information contained within a chemical plume's movements, coupled with the direction of the current flow, can provide an animal with information regarding the distance and direction to the odour source (Gomez et al., 1994; Moore & Atema, 1988). To successfully locate a stimulus, an animal must extract information from the plume by sampling at various points in space and time (Atema, 1995), and also through filtering by the receptor (Moore & Atema, 1988).

By sampling the chemical environment, an animal can identify particular compounds and concentrations of compounds. This is of importance to animals which rely on chemoreception, as the chemical picture and the information contained within it can be used only if an animal can discern it from a background of other chemical stimuli found in the surrounding environment. Discrimination is possible if a compound is particularly rare, or if it appears in unusually high concentrations (Atema, 1995; Dusenbery, 1992).

Chemical stimuli are of particular importance to decapod crustaceans, which are able to detect a large number of compounds (Rittschof, 1992). Chemical compounds are produced and detected by crustaceans during aggressive interactions (Breithaupt &
Decapod crustaceans possess a large number of chemoreceptors (Table 1.1), located on every cephalothoracic appendage (Derby, 1982). Chemoreceptors can be divided into those used for “smell” and those used for “taste” (Atema, 1980; Devine & Atema, 1982). Although many compounds can be detected by both smell and taste receptors, there are structural differences between the two (Atema, 1980), and the two receptor types project to different parts of the crustacean brain (Mellon et al., 1992; Schmidt & Ache, 1992). Both smell and taste receptors use bipolar neurons, but the overall structure of the receptors is very different. Receptors used for smell are usually contained within fine hairs (e.g. the aesthetasc hairs located on the outer rami of the antennules of the crayfish Orconectes propinquus (Tierney et al., 1986), whereas those used for taste are housed within much sturdier, thick walled hairs (such as the setae found on the chelated periopods (walking legs) of the lobster Homarus americanus (Derby, 1989), and the crayfish Austropotamobius torrentium (Altner et al., 1983).

The differences in receptor “housing” may be an adaptation to the environment in which the receptors operate. Antennules are moved rapidly through water, an action which is analogous to “sniffing” by vertebrates (Schmitt & Ache, 1979). This movement
<table>
<thead>
<tr>
<th>References</th>
<th>Function</th>
<th>Appendage Function of the cephalothoracic appendages of decapod crustaceans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shepherd, 1974; Vong &amp; Aher, 1992</td>
<td>Enhances chemosensory of antennule through water</td>
<td>-</td>
</tr>
<tr>
<td>McKeel, 1973; Schmid &amp; Aher, 1972; Schmid &amp; Aher, 1974; Hodgson, 1965; Hodgson, 1967</td>
<td>Function</td>
<td>-</td>
</tr>
<tr>
<td>Puzessary et al., 1977; Puzessary &amp; Childress, 1971; Childress et al., 1972; Cowan, 1994; Dunham et al., 1994; Puzessary et al., 1972; Puzessary &amp; Childress, 1971</td>
<td>Olfaction, Rapid movement</td>
<td>-</td>
</tr>
<tr>
<td>Deveau &amp; Aher, 1976; Aher &amp; Aher, 1979; Aher &amp; Aher, 1980; Spruner, 1988; Puzessary et al., 1977; Puzessary &amp; Childress, 1971</td>
<td>Enhances chemosensory of antennule through water</td>
<td>-</td>
</tr>
<tr>
<td>Puzessary &amp; Childress, 1971; Childress et al., 1972; Bernier et al., 1980; Aher &amp; Aher, 1979</td>
<td>Function</td>
<td>-</td>
</tr>
<tr>
<td>Puzessary et al., 1972; Aher &amp; Aher, 1979; Aher &amp; Aher, 1980; Spruner, 1988</td>
<td>Function</td>
<td>-</td>
</tr>
<tr>
<td>Puzessary et al., 1977; Puzessary &amp; Childress, 1971</td>
<td>Olfaction, Rapid movement</td>
<td>-</td>
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<td>Puzessary et al., 1977; Puzessary &amp; Childress, 1971</td>
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<td>Puzessary et al., 1977; Puzessary &amp; Childress, 1971</td>
<td>Olfaction, Rapid movement</td>
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<tr>
<td>Appendage</td>
<td>Function</td>
<td>References</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Antennae</td>
<td>Known to possess mechanoreceptors, but are also highly chemosensitive.</td>
<td>(Altner &amp; Prillinger, 1980; Bruski &amp; Dunham, 1987; Bruski &amp; Dunham, 1990; Sandeman, 1989; Smith &amp; Dunham, 1996; Solon &amp; Cobb, 1980; Tautz et al., 1981; Tazaki, 1977; Voigt &amp; Atema, 1992; Wilkens et al., 1996; Zeil et al., 1985)</td>
</tr>
<tr>
<td>Chelipeds</td>
<td>Used in feeding, social interactions and anti-predator defence.</td>
<td>(Bruski &amp; Dunham, 1987; Smith &amp; Dunham, 1990; Solon &amp; Kass-Simon, 1981)</td>
</tr>
<tr>
<td>Maxillipeds</td>
<td>Used for feeding, and grooming. Appear to determine whether a substance will be ingested.</td>
<td>(Corotto et al., 1992; Factor, 1978; Lavalli &amp; Factor, 1995; Voigt &amp; Atema, 1992)</td>
</tr>
</tbody>
</table>
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enhances the response of olfactory receptors to changes in the concentration of detected stimuli. Although crayfish can be found with damaged or missing antennules (Dunham et al., 1997), they are not often exposed to sustained mechanical abuse. As a result, aesthetasc hairs found on the antennules do not require much more protection than the guard hairs surrounding them (Gleeson et al., 1993b; Hallberg et al., 1992; Heimann, 1984). The periopods, however, are used in several stages of feeding, such as grabbing food, digging in substrate, and transport of food to the mouth (Lavalli & Factor, 1995). Because of these activities, receptors located on the periopods are more like to suffer abrasion and subsequent damage. The sturdy setae found on the periopods are thus likely used to prevent or minimise injury to these receptors.

Studies have demonstrated that in decapod crustaceans, a large number of appendages possess chemoreceptors. Behavioural studies, however, have not always been conducted to determine the functional roles that a particular appendage may play. The antennae, for example, are known to possess mechanoreceptors (Altner & Prillinger, 1980; Tautz et al., 1981). Behavioural studies have indicated that these organs are used to locate objects through “touch” (Tautz et al., 1981; Tazaki, 1977; Wilkens et al., 1996; Zeil et al., 1985), and are also used during social interactions (Bruski & Dunham, 1987; Bruski & Dunham, 1990; Smith & Dunham, 1996; Solon & Cobb, 1980). However, Voigt & Atema (1992) showed in a physiological study that the antennae, in addition to being mechanoreceptive organs, are also highly chemosensitive. Behavioural studies have yet to be performed to determine what role they may play in the localisation of chemical stimuli.
Chapter 1: General Introduction

The peripods of decapod crustaceans are used in a variety of feeding activities, such as searching through substrate, grabbing and tearing food, and the transport of food to the maxillipeds and mouthparts (Derby & Atema, 1982b). They are known to be chemosensitive (Derby & Atema, 1982a; Derby & Atema, 1982c; Johnson et al., 1984; Voigt & Atema, 1992), with receptors tuned to specific chemicals (Bayha et al., 1993; Derby & Atema, 1982a; Derby & Atema, 1982c; Johnson & Atema, 1983; Johnson et al., 1984). Mixtures of chemicals are required, however, before specific behaviour patterns, such as dactyl clasping, are initiated (Borroni et al., 1986). The maxillipeds are also chemosensitive (Corotto et al., 1992) and appear to determine whether or not a substance will be ingested (Lavalli & Factor, 1995).

The sensory organs which have received the most attention thus far have been the antennules. These are considered the most important organs used in distant chemical communication (Ache, 1975; Devine & Atema, 1982; Hamner & Hamner, 1977; Hazlett, 1971; McLeeese, 1973a; McLeeese, 1973b; Moore et al., 1991b; Pearson & Olla, 1977), and physiological studies have indicated their high degree of sensitivity to specific chemicals (Corotto et al., 1992; Fuzessery et al., 1978; Johnson & Atema, 1983; Johnson et al., 1989; Levandowsky & Hodgson, 1965; Merrill et al., 1994; Shepheard, 1974; Thompson & Ache, 1980; Tierney et al., 1988; Trapido-Rosenthal et al., 1990; Voigt & Atema, 1992). Each antennule is bifurcated, with one outer (lateral) and one inner (medial) ramus. The two rami differ morphologically, with aesthetasc hairs present on the outer ramus but not on the more slender inner ramus (Tierney, 1985; Tierney & Dunham, 1982; Tierney et al., 1986). Other sensilla found on both rami are believed to serve both a
mechanosensory and chemosensory function (Derby, 1989). The chemosensory function
of the aesthetasc hairs is enhanced through the flicking of the antennules, which increases
their exposure to the surrounding chemical environment (Schmitt & Ache, 1979).

It has been recognized that crustaceans rely on their antennules for long-distance
chemoreception. Moore et al. (1991b), for example, found that the antennules were used
in the detection and localisation of chemical stimuli at distances greater than 22 cm from
the stimulus source. However, the roles played by the two types of rami have been
controversial. Although a number of ablation studies have been performed to date, flaws
in experimental design may have masked the role played by each type of ramus in the
detection and localisation of chemical stimuli. Table 1.2 summarizes the results of these
experiments.

Ameyaw-Akumfi (1977) ablated the entire antennular structure of the crayfish
Procambarus clarkii to examine the functional integration of the different sense organs in
feeding behaviour. Ablation did not appear to affect the ability of the crayfish to
perceive, locate or ingest the stimulus. It was concluded from this result that the
antennules were not the main chemosensory organs used in food detection. However, the
food used in this experiment was placed only 18-20 cm away from the animal being
tested. Moore et al. (1991b) have suggested that chemoreceptors located on the walking
legs of H. americanus are involved in short-distance chemoreception. It is possible that a
similar situation occurred with the species used by Ameyaw-Akumfi (1977), and that
stimulation of the walking legs could have allowed the crayfish to successfully locate the
stimulus source.
Table 1.2. Summary of results found in previous research examining differential antennule function.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
<th>Stimulus Used</th>
<th>Outer Ramus</th>
<th>Inner Ramus</th>
<th>Methodological Issues</th>
</tr>
</thead>
<tbody>
<tr>
<td>McLeese (1973)</td>
<td>Homarus americanus</td>
<td>food</td>
<td>Didn’t find a differential function for inner and outer rami. Both ablate types were able to respond to a food extract. Total ablates were unable to locate extract.</td>
<td>2 animals were repeatedly used, with the result that the effect of learning cannot be eliminated.</td>
<td></td>
</tr>
<tr>
<td>Devine &amp; Atema (1982)</td>
<td>Homarus americanus</td>
<td>food</td>
<td>Ablation of outer ramus altered directional choices, and search path length</td>
<td>Ablation had no effect on any reported behaviour. Only antennular structures on the right side were removed. As a result, tested animals always had at least one inner and one outer ramus</td>
<td></td>
</tr>
<tr>
<td>Reeder &amp; Ache (1980)</td>
<td>Panulirus argus</td>
<td>food</td>
<td>Outer ablates did not detect food odour, nor did they orient towards or locate the food source</td>
<td>Ablation had no effect on latency to search, search duration, number of turns, or success in locating the source</td>
<td></td>
</tr>
<tr>
<td>Ameyaw-Akumfi (1977)</td>
<td>Procambarus clarkii</td>
<td>food</td>
<td>Ablated entire antennule. Observed that total ablation did not affect the ability of crayfish to detect food odours</td>
<td>Food was presented from 18-20 cm away. Moore et al. (1991) have suggested that chemoreceptors on the walking legs are increasingly involved at distances less than 22 cm from the stimulus source</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>Species</td>
<td>Stimulus Used</td>
<td>Outer Ramus</td>
<td>Inner Ramus</td>
<td>Methodological Issues</td>
</tr>
<tr>
<td>---------------------------</td>
<td>----------------------</td>
<td>---------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Derby &amp; Atema (1982)</td>
<td>Homarus americanus</td>
<td>food</td>
<td>Entire antennule was covered with glue. Found no effect on the performance of feeding behaviour after contact with food was made.</td>
<td></td>
<td>Did not examine the time needed to detect or find prey items.</td>
</tr>
<tr>
<td>Dunham et al. (1997)</td>
<td>Cambarus bartonii</td>
<td>food</td>
<td>Outer Ablates did not perform sustained substrate searching, but increased movement of the antennules</td>
<td>No effect on performance</td>
<td></td>
</tr>
<tr>
<td>Gleeson (1980)</td>
<td>Callinectes sapidus</td>
<td>conspecific</td>
<td>Outer rami were needed for the performance of courtship behaviours</td>
<td>Ablation of the inner rami did not change courtship behaviour</td>
<td></td>
</tr>
<tr>
<td>Ameyaw-Akumfi &amp; Hazlett (1975)</td>
<td>Procambarus clarkii</td>
<td>conspecific</td>
<td>Ablation of the outer rami not reported</td>
<td>Inner rami seem to be used for detection. Ablation precludes response</td>
<td>No ablation of outer rami.</td>
</tr>
<tr>
<td>Tierney et al. (1984)</td>
<td>Orconectes propinquus</td>
<td>conspecific</td>
<td>Outer rami appear to be responsible for the localisation of odour source</td>
<td>Ablation of inner rami had no effect on the animals’ ability to locate stimulus source.</td>
<td>No separation of detection and localisation.</td>
</tr>
<tr>
<td>Oh &amp; Dunham (1991)</td>
<td>Procambarus clarkii</td>
<td>conspecific</td>
<td>Both rami (but not necessarily together) are required to discriminate “self” from “stranger”</td>
<td></td>
<td>No separation of detection and localisation.</td>
</tr>
<tr>
<td>Dunham &amp; Oh (1992)</td>
<td>Procambarus clarkii</td>
<td>conspecific</td>
<td>Outer rami appear to be used in sex discrimination</td>
<td>Inner rami appear to be used for localisation of social stimuli</td>
<td></td>
</tr>
</tbody>
</table>
In experiments with *H. americanus*, Derby & Atema (1982b) examined the feeding behaviour on live mussels both before and after the antennules were covered with glue. They observed very little effect on the performance of feeding behaviour, once contact with the food was made. As in Ameyaw-Akumfi (1977), their study did not separately investigate the two rami, nor did it examine the time required to detect or locate the mussel prey. Separate study of the two types of rami, coupled with more detailed measures of searching and feeding behaviour, might have demonstrated a differential function of the rami.

The two types of rami were separately studied by Reeder & Ache (1980). Input from the outer (lateral) ramus of the spiny lobster *Panulirus argus* was needed for the initiation of searching behaviour. Animals missing this ramus did not detect, nor orient towards the food odour. Ablation of the inner rami had no effect on latency to search, search duration, the number of turns performed, or ultimate success in locating the stimulus source.

Devine & Atema (1982) examined searching behaviour in the lobster *H. americanus*. Lobsters with one outer ramus removed were observed to make incorrect direction choices twice as often as control animals, and also increased the length of the search paths used to locate the source. However, only antennule structures on the right side were ablated. As a result, test animals always possessed at least one inner and one outer ramus.

McLeese (1973a) did not find a differential function for the inner and outer rami of *H. americanus*, as both ablate types were able to locate the food source within a 2 minute
time limit. Lobsters missing all rami were unable to locate the fish extract used. Although a large number of trials were used in this experiment, two animals in each treatment were re-used repeatedly. As a result, the possibility that the animals learned the location of the food source cannot be eliminated. In fact, the response times reported range from minimums of about 10 s to maximums of around 110 s. This large range could be attributed to the effects of learning, with lobsters consistently improving their performance as they learned the location of the food extract.

Complete ablation treatments were carried out by Dunham et al. (1997), in a study using the crayfish *Cambarus Bartonii*. They observed that crayfish with the outer rami ablated increased the number of antennule movements performed, but did not perform sustained substrate digging. Total Ablates (animals missing all rami) also failed to perform sustained substrate digging behaviours. No effect on measured behaviours was observed following the ablation of the inner rami. Their study could not examine long-distance localisation, because of the small testing tank used.

Gleeson (1980) observed that blue crabs *Callinectes sapidus* which had the outer rami ablated did not perform courtship behaviours, demonstrating the importance of this type of ramus in the reproductive behaviour of this species. Animals with the inner rami ablated did not alter their behaviour appreciably.

Ablation experiments were performed to establish sites of chemosensory organs in *P. Clarkii* (Ameyaw-Akumfi & Hazlett, 1975). Animals with intact rami responded appropriately to social stimuli, but were no longer responsive once the inner rami were removed. A similar result was reported for crayfish with no rami. Results from this
experiment indicate that the inner ramus is the site of reception for social stimuli. However, since outer rami were not ablated, it is unclear whether a differential function can be attributed to each type of ramus.

The outer rami of the crayfish *Orconectes propinquus* appear to mediate pheromone reception in that species (Tierney et al., 1984). Ablation of the inner rami had no effect on behavioural responses. The behavioural criteria used in that study, however, did not differentiate between detection and localisation. It isn’t clear if animals possessing only inner rami were able to detect a conspecific but unable to successfully locate it, or if they would have been able to localise the odour source, had detection been carried out by some other chemosensitive organ.

Oh & Dunham (1991) ablated specific combinations of rami in *P. clarkii*. Intact animals, with all 4 rami present, were able to distinguish between “self” and “stranger” conditioned water. Crayfish with one inner ramus and one outer ramus were also able to make this distinction, but animals possessing only one type of ramus were not. Localisation and attention paid to the source were not separated in this study.

The role of the antennules in sex discrimination was examined by Dunham & Oh (1992). Outer rami were used by both males and females in sex discrimination, while the inner rami were used for localisation. These results contradict those of Ameyaw-Akumfi & Hazlett (1975), who found that the inner rami were the site of reception, and of Tierney & Dunham (1984), who reported that the outer rami of the crayfish *O. propinquus* were responsible for localisation.
The role played by the different types of rami in mediating response to chemical information is still unclear, as methodological flaws may have masked the primary function and use of each olfactory organ. The purpose of the present study was to elucidate the role played by the inner rami in the localisation of distant chemical odours by the red swamp crayfish, *Procambarus clarkii*. Chapter 2 examines the responses of female crayfish to a food stimulus. Chapter 3 examines responses to a social stimulus. Chapter 4 integrates the results of the two experiments.
Use of the inner rami in the localisation of food odours by female red swamp crayfish, *Procambarus clarkii.*
Antennules are used by decapod crustaceans in long-distance chemoreception. Specific combinations of rami were ablated to determine if the inner (medial) rami are used by female red swamp crayfish, *Procambarus clarkii*, to locate food. Rami were not required for the initiation of searching behaviours over short distances, but crayfish with all rami ablated were unable to perform long-distance chemoreception. Only half of the crayfish missing the outer (lateral) rami were able to successfully locate a food source, indicating the importance of these rami in detection and localisation. A closer examination revealed that the performance of successful animals within this group was comparable on most measures to non-ablated crayfish, as well as those missing the inner rami, or one outer and one inner ramus. The absence of the outer rami resulted in slower walking speed. Although the inner rami can be used for long-distance chemoreception and the localisation of food stimuli, they do not appear to be the primary organ used in this process.
Chemical cues are used by a large number of organisms, both terrestrial and aquatic, when searching for resources (Atema, 1988; Bell & Carde, 1984; Rittschof, 1990; Svensson, 1996). The ability to detect chemical stimuli is therefore quite common, and organs specialized for this function are found in many animal groups (Price & Ache, 1977; Schmitt & Ache, 1979). Decapod crustaceans rely heavily on chemical information, and are able to detect practically every class of biological molecule (Rittschof, 1992). The American lobster, *Homarus americanus*, may initiate and continue a search for food based on chemical cues alone (Devine & Atema, 1982). Mellon et al. (1992) report that 30 to 40% of crayfish brain volume is devoted to the processing of olfactory input.

The flexible antennules of crayfish and other decapod crustaceans are considered the most important organs used in distant chemical communication (Ache, 1975; Devine & Atema, 1982; Hamner & Hamner, 1977; Hazlett, 1971; McLeese, 1973a; McLeese, 1973b; Moore et al., 1991b; Pearson & Olla, 1977). Each antennule is bifurcated, with one outer (lateral) and one inner (medial) ramus. The two rami differ morphologically, with aesthetasc hairs being found on the outer ramus but not on the more slender inner ramus (Tierney, 1985; Tierney & Dunham, 1982). Other sensilla found on both rami are believed to serve both a mechanosensory and chemosensory function (Derby, 1989). The chemosensory function of the aesthetasc hairs is enhanced through the flicking of the
antennules, which increases their exposure to the surrounding chemical environment (Schmitt & Ache, 1979).

The functional role played by the two types of ramus is controversial, with conflicting results obtained in different studies. Ameyaw-Akumfi (1977) observed that the ablation of all rami did not affect the ability of red swamp crayfish, *Procambarus clarkii*, to perceive food odours. McLeese (1973b) used a series of selective ablation experiments to examine the function of the antennules in *H. americanus*. Animals which were intact, or had either the inner or outer rami ablated, were able to locate the source of a fish extract. Lobsters which had all their rami removed however, were not as successful. This suggested that the two rami did not differ in function, but that antennules were required for the animal to locate a food source.

Other studies, however, have found that the antennules do differ in function. Devine and Atema (1982) observed that lobsters which had one of their outer rami ablated altered their search path when looking for a food source. Ablation of an inner ramus did not appear to have any effect. A similar result was obtained by Reeder and Ache (1980) using spiny lobsters, *Panilurus argus*. Ablation of the outer rami affected the ability of the animals to locate a food source, but no effect was observed following ablation of the inner rami.

Cross-species differences may account for the different results obtained in the various studies. Flaws in experimental design, however, may have also masked the role of the inner rami, leading to the suggestion that they are not used in distant chemoreception,
despite being very chemosensitive (Tierney et al., 1988). Moore (1991b) reports that receptors found on the walking legs of \textit{H. americanus} may initiate search behaviours at distances less than 22 cm from the source. Small testing tanks can therefore influence the results obtained, as they may not have be large enough to properly test the abilities of Crustacea to locate the source of an odour over greater distances (e.g. Ameyaw-Akumfi, 1977; McLeese, 1973a).

The role played by the inner rami during the localisation of food sources is thus still unclear. This study was conducted to determine if the inner rami were used by female red swamp crayfish, \textit{Procamburus clarkii}, to mediate their response to food odours over long distances.

**METHODS**

**Housing**

Adult female \textit{Procamburus clarkii} measuring approximately 5 cm in carapace length were obtained from a wholesaler. Only animals with intact antennules, antennae, and walking legs were used, as these structures are known to have some chemosensory function (Table 1.1). Similarly, any animals which developed eggs or moulted during the course of the experiment were excluded, since these conditions might affect the responses to be measured.
Fig. 2.1. (A) Fish breeding containers used to house crayfish. Each tank held one crayfish, and measured 16.5 (length) x 12.5 (width) x 13 cm deep. Containers were constructed of a cloth mesh wrapped around a plastic frame. (B) Three containers were placed within one 10L aquarium (41 (l) x 20.5 (w) x 26 cm deep), allowing each crayfish social stimulation while avoiding aggressive interactions.
Crayfish were individually housed within fish breeding containers measuring 16.5 (length) x 12.5 (width) x 13 cm deep, with the four walls and bottom composed of a cloth mesh wrapped around a plastic frame. These containers were placed within 10L aquaria, measuring 41 (l) x 20.5 (w) x 26 cm deep, in sets of three (Fig. 2.1). This ensured that each crayfish would receive some social stimulation while avoiding aggressive fights which could lead to damage being inflicted on various sensory organs. Aeration of the water was accomplished by the diffusion of compressed air through airstones. Light was provided by fluorescent tubes located above each tank, as well as a 300W incandescent ceiling lamp. Air temperature was maintained at 19°C ± 1°C. A reverse light cycle of 12 hours dark:12 hours light was used, with lights turned on at 2130h. Animals were fed a piece of haddock fillet every second day, but were not fed for 24 hours prior to testing. Haddock was used for regular feedings, as well as during testing. Derby and Atema (1981) have reported that the lobster *H. americanus* will more readily initiate searching behaviour when presented with a prey odour if they have previously been exclusively fed the same prey item on a regular basis.

Each tank was inspected daily and any uneaten food removed. Three-quarters of the water in each tank was replaced once per week. All crayfish were held under these conditions for three weeks before they were used in any experiments.
Fig. 2.2. Specific combinations of antennular rami were ablated: (A) Non-Ablate, with all rami intact; (B) Inner Ablate, with the inner rami ablated; (C) Outer Ablate, with the outer rami ablated; (D) Cross Ablate, with one inner ramus and one outer ramus ablated; and (E) Total Ablates, with all rami ablated.
Selection and Ablation

Animals were selected at random and placed into sets of 5 animals each. One week prior to their testing, each individual within a set was assigned to one of five treatment groups: (i) a Non-Ablate group, with no antennular rami ablated (N=15), (ii) an Inner Ablate group, with the ablation of both inner rami (N=15), (iii) an Outer Ablate group, with both outer rami ablated (N=15), (iv) a Cross Ablate group, with one inner ramus (either left or right, determined randomly) and one contralateral outer ramus ablated (N=14); or (v) a Total Ablate group, with all four rami ablated (N=15). Figure 2.2 illustrates the different ablate types. All animals were anaesthetised using a 20 minute immersion in ice water, and then had specific rami quickly ablated. Control animals which didn't have any rami ablated were still exposed to the ice water treatment. Following this ablation procedure, crayfish were returned to their individual mesh containers.

Testing Procedures

Each set of crayfish was tested over two consecutive days. Responses toward different solutions were observed on the first day (Experiment A), and the ability of the animals to localise the source of a chemical stimulus was tested on the second day (Experiment B).

Seven days after ablation treatments, the five crayfish to be tested were placed within individual 10L testing tanks and left for 24 hours. Movement within these tanks was limited to the front third (41 x 9 cm wide) through the use of a clear Plexiglas wall. In
addition, a platform angled upwards at 45° from the bottom of the tank to this wall was
used to provide a floor upon which the crayfish could move (Fig. 2.3). A substrate was
created by attaching a layer of black gravel to the Plexiglas platform with aquarium-grade
silicon. Since the crayfish were more active during the dark period of the light cycle than
during the light period, testing was performed under low light conditions at this time.
Since red light did not appear to have a detrimental effect on crayfish activity, low light
levels were provided by a 60W red light bulb located above each testing tank.

Experiment A

Experiment A examined the responses of a crayfish following the ejection of
chemical solutions (a control solution or one made from fish filtrate) over the antennular
region of the test animal. The responses to a change in the chemical environment
immediately surrounding the test animal were videotaped and later scored.

Preparation of Solutions

A fish solution was created by homogenising 20g of haddock fillet with 20mL of
distilled water. Vacuum filtration was used to collect the filtrate. Fresh solutions were
made each testing day.

A control solution was created using 18mL of water which was gently scooped
out of the rear portion of the tank used for testing the crayfish, and in which the crayfish
had resided for 24 hours. Control solutions were collected just prior to testing.
Fig. 2.3. Testing tank used in Experiment A. A Plexiglas wall confined test animals to the front third of a 10L aquarium (41 x 9 cm wide). A platform angled 45° from the bottom of the tank to this wall provided a floor upon which crayfish could move. Black gravel was siliconed onto the platform.
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Testing

A 60W red lamp positioned directly above the testing tank was turned on 40 minutes prior to testing. The crayfish being tested was videotaped for an initial 7 minutes. A 14.6 cm disposable Pasteur capillary pipette was then used to eject 2.0 ± 0.1mL of the control solution over the antennules and maxillipeds of the crayfish, at a rate of 0.5 ± 0.1 mL/s. The pipette was inserted gently into the water in order to reduce water disturbance and held approximately 18 cm away (Fig. 2.4). The crayfish’s responses were videotaped for the next 7 minutes (see Behaviours Examined below). The fish filtrate was the next stimulus presented, immediately after the control, and in the same manner as the control. Behaviours were again videotaped for 7 minutes. The control solution was then immediately presented again, and behaviours videotaped for a final 7 minutes. Following testing, the crayfish was returned to its mesh container. Behaviours and movements were observed to return to original levels by the end of each 7-minute observation period.

Behaviours Examined

Antennular movements are likely used to facilitate the chemosensory function of the antennules (Gleeson et al., 1993b; Moore et al., 1991a; Schmitt & Ache, 1979). These movements can thus be used as an indication of the animal’s ability to detect a change in its surrounding chemical environment (Ciruna et al., 1995). At rest, antennules are held at an angle of approximately 45° above the horizontal plane. Since antennules can move
**Fig. 2.4.** Control and food stimuli were ejected over the antennular region of test crayfish using a disposable pipette. Stimulus is coloured in this picture to demonstrate fluid movement.
independently or in tandem (Berg et al., 1992), antennule flicking can involve either or both antennules. The following antennule movements were examined (Fig. 2.5; definitions are from Dunham et al., 1997):

(i) Small Amplitude Flicks (SAF) are the rapid, fluid and phasic depression and elevation of either or both antennules, and involve both the inner and outer rami. The depression of the antennule does not extend more than 45° below the horizontal.

(ii) Large Amplitude Flicks (LAF) are similar to SAF, but are slower, and extend to a maximum angle of 90° below the horizontal.

(iii) Large Amplitude Depressions (LAD) are depressions of the antennule to a maximum of 90°, as in LAF, but the antennule is held in this position before elevation.

(iv) Antennular Twitches are a very rapid depression and elevation of the outer ramus, to a maximum of 15° below the horizontal. The paired inner ramus is not involved.

The time spent in substrate digging behaviour was also measured. Substrate digging is one of the major movement patterns used by decapod crustaceans in feeding (Dunham et al. 1997). In the present study, substrate digging was defined as the manipulation of the gravel substrate by the dactylate periopods (the first two pairs of walking legs), including contact between dactylate periopods and maxillipeds or mandibles (the latter behaviour was defined as Leg-to-Mouth Movements by Dunham et al. (1997))
Fig. 2.5. Antennular movements analysed. (A) Small Amplitude Flicks; (B) Large Amplitude Flicks; (C) Large Amplitude Depressions; (D) Twitches.
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Experiment B

The ability of the different treatment groups to localise the source of a food odour was examined in Experiment B.

Testing apparatus

Testing was performed in a large rectangular flow-through Plexiglas tank (100 cm length x 60 cm width x 30 cm high). Black gravel lined the bottom of the tank, to a depth of 1 cm, and aged municipal supply water from two head tanks was used to fill the testing tank to a depth of 10 cm. A current was created by siphoning aged municipal supply water from another three head tanks. Water entered the tank via a single entry point at one end of the tank (the “inflow” end) and was drained through a tube located at the opposite end (the “outflow” end). Mean (± s.e.) current velocity in the centre of the tank was 1.7 ± 0.1 cm/s. Plastic grates at both ends of the tank served as collimators, and also confined the test animal to a smaller area of the tank (80 cm length x 60 cm width); this area was videotaped using a camera located above the tank (Fig. 2.6).

A food stimulus was placed into one of two plastic cylinders siliconed onto a clear plastic board. Cylinders were made of black plastic mesh, and were each 4 cm in diameter, and 10 cm in height. The cylinders were 8 cm apart, as measured from the centre of each one (Fig. 2.7). Twenty grams of shredded haddock fillet was placed into one of the two cylinders (selected randomly) just prior to testing.
Testing

Crayfish were tested in the same order as in Experiment A. The mesh container containing the test animal was placed at the “outflow” end of the tank, directly opposite the current inflow. The water current was then created by siphoning water from a head tank. Following this, the cylinder apparatus containing the 20 g of shredded haddock was placed approximately 12 cm from the inflow.

Two mL of a fish solution, created in the same manner as in Experiment A, was ejected over the antennular region of the test crayfish. This ejection was used to “prime” the crayfish being tested, motivating the animal to perform feeding behaviours.

Thirty seconds after priming, the mesh container was gently tipped over and crayfish allowed to enter the large testing tank (Fig. 2.8). The entire testing area was then videotaped for the next 30 minutes, or until the crayfish located the food, if that occurred sooner. Once the testing session was terminated, both the crayfish and food were removed. The tank was then drained, thoroughly rinsed, and re-filled.

Analysis

Each crayfish was videotaped until it either located the food source, or until 30 minutes had passed. The specific behaviours used in analyses were observed and recorded directly from video playback.

Successful animals were defined as those crayfish which dug through the gravel substrate, located the food source, and began feeding. The latency between the time a
Fig. 2.6. Flow-through tank used in Experiment B. Although total tank dimensions were 100 cm x 60 cm x 30 cm high, a moveable plastic grate (A) confined test crayfish to a smaller area (80 cm x 60 cm). Water entered the tank at a single entry point (B), and was drained through a tube located at the other end (C; tube not pictured here). Two tubes found at the outflow end (D) were used for drainage.
Fig. 2.7. Cylinders used to present stimulus in Experiment B. Cylinders were 4 cm in diameter, 10 cm in height, and held 8 cm apart.
Fig. 2.8. Positioning of objects in testing tank. The mesh container and test crayfish (A) were placed at the outflow end, directly opposite the inflow end. The cylinder apparatus and food stimulus was positioned 12 cm away from the inflow.
crayfish left the mesh container and the time at which search behaviours (substrate digging) were first performed was recorded for each animal. Also recorded for each individual was the location where substrate digging first occurred. The most direct distance between this point and the location of the food source was traced onto a transparent plastic sheet. In addition, the pathway used by successful animals was traced from video playback onto the same sheet (Fig. 2.9). Since crayfish can walk sideways and backwards without a change in their overall body orientation, a crayfish’s rostrum was used as a reference point when tracing paths. Using only the rostrum as a reference point allowed the distance travelled to be measured independently from the direction in which a crayfish may have been facing. The distances travelled by individual crayfish were measured from the traces using SigmaScan™ (1995).

A “meander” ratio was created for successful animals by dividing the actual distance travelled by the most direct distance (Zach & Falls, 1976). Also measured was search duration (time elapsed from the moment at which searching behaviours were first performed to the successful localisation of the food source) and the animal’s mean walking speed.

Statistics and data transformations were calculated using either the statistical package SigmaStat™ (1995), or through procedures outlined by Siegel & Castellan (1988).
Fig. 2.9. Sample tracing of crayfish search movements. (A) time at which substrate digging was initiated; (B) path travelled by test crayfish; (C) time food source was reached; (D) most direct distance between point of initiation and location of food source.
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RESULTS

Experiment A

All crayfish in all 5 treatment groups performed searching digging behaviours during the two minutes immediately following the ejection of the fish filtrate over the antennular region. The time spent digging during this period was greater than that for the prestimulus period (prior to the ejection of the first control solution), as well as for the two control periods (Dunn’s Test, \( P<0.05 \)).

Figure 2.10 illustrates the mean cumulative duration spent in digging behaviours by each treatment group during the two minutes subsequent to the ejection of the fish filtrate. A highly significant overall effect of treatment on the cumulative digging duration was observed (Kruskal-Wallis, \( H=14.527, \text{df}=4, P=0.006 \)). Pairwise comparisons showed that Cross Ablates were significantly different than Total Ablates (Dunn’s Test, \( Q=3.621, P<0.05 \)). No other significant differences were detected.

In a study of antennule movements in *Cambarus bartonii*, Dunham et al. (1997) reported that gross antennule movements increased from a baseline measure after the presentation of a sucrose solution, and then decreased following the ejection of a control solution. This pattern was observed for the Cross and Outer Ablates in the present experiment. There was an overall difference observed for Cross Ablates (Page Test, \( z_l=384.5, k=4, N=14, P<0.01 \)), with a larger number of movements performed after the fish ejection than during the prestimulus period (Dunn’s Method, \( Q=3.601, P<0.05 \)).
Fig. 2.10. Comparison of time spent in digging behaviours during the 2 min subsequent to the ejection of fish filtrate. Bars represent median values; measures of variance represent 25% and 75% quantiles.
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Median Duration of Substrate Search (s)

- Cross Ablate
- Inner Ablate
- Non-Ablate
- Outer Ablate
- Total Ablate

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There were also more movements after the second control than during the prestimulus session (Dunn's Method, Q=3.217, P<0.05). The total number of movements performed following the ejection of the initial control solution was not significantly different from the number performed after the first or second control ejections.

Outer Ablates were also significantly different overall (Page Test, z_L=414.5, N=15, k=4, P≤0.05), with movements performed after the ejection of the fish solution significantly greater than those for the prestimulus (Dunn's Method, Q=4.932, P<0.05), and Control 1 periods (Dunn's Method, Q=4.822, P<0.05), but not the Control 2 period (Dunn's Method, Q=2.442, P>0.05).

Although antennule movements by the Inner Ablates increased following the presentation of the first control solution, and remained high (Page Test on all movements, z_L=413, k=4, N=15, P<0.05). Movements made after ejections of the first control, fish and second control solutions were all greater than for the prestimulus period (Dunn's Method, Control 1 vs. Prestimulus, Q=2.672, P<0.05; Fish vs. Prestimulus, Q=3.186, P<0.05; Control 2 vs. Prestimulus, Q=3.154, P<0.05), but were not significantly different from each other.

There was no significant difference detected in the number of antennule movements performed by Non-Ablates following the presentation of the various stimuli (Page Test, z_L=386, k=4, N=15, P>0.05).

Individual antennular movements performed following the ejection of fish filtrate were next examined and compared (Fig. 2.11). There was no significant overall difference.

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Fig. 2.11. Comparison of Twitches performed by treatment groups. Bars represent median values; measures of variance represent 25% and 75% quantiles. Outer and Total Ablates were excluded, as they lack the only antennular rami capable of Twitch movements.
in the number of Twitches made by the Cross Ablates, Inner Ablates and Non-Ablates (Kruskal-Wallis, H=0.126, df=2, P=0.939). Both the Outer Ablate and Total Ablate groups were excluded from analysis, since they lack the only rami capable of Twitch movements. Surprisingly, the Cross Ablates performed equivalent numbers of Twitches to those of both the Inner and Non-Ablate groups, despite missing one outer ramus. Corrections for the absence of an outer ramus have been made in a previous study (Dunham et al., 1997), but were not made here. Inner and Non-Ablate groups also did not differ from each other (Dunn’s Tests used in all comparisons).

Small Amplitude Flicks (SAF) were performed by all crayfish (Fig. 2.12). Since SAF and the larger amplitude movements involve movement of the entire antennule, the Outer Ablate group was included in analyses. Total Ablates could not be analysed. There were no significant differences found between groups, in either the overall performance of SAF (Kruskal-Wallis, H=1.843, df=3, P=0.606), or in pairwise comparisons (Dunn’s Test, P>0.05 for all comparisons).

Large Amplitude Flicks (LAF) were also performed by all groups following the ejection of fish filtrate (Fig. 2.13), with a significant overall difference detected (Kruskal-Wallis, H=19.602, df=3, P≤0.001). Outer Ablates performed significantly more LAF Inner Ablates (Dunn’s Test, Q=4.056, P<0.05), and Non-Ablates (Dunn’s Test, Q=3.562, P<0.05), but not Cross Ablates. Cross, Inner and Non-Ablate groups did not differ from each other (Dunn’s Test, P>0.05 for all comparisons).
Fig. 2.12. Comparison of Small Amplitude Flicks performed by treatment groups. Bars represent median values; measures of variance represent 25% and 75% quantiles.
Fig. 2.13. Comparison of Large Amplitude Flicks performed by treatment groups. Bars represent median values; measures of variance represent 25% and 75% quantiles.
Chapter 2: Responses to a Food Stimulus

Cross Ablate  Inner Ablate  Non-Ablate  Outer Ablate

Median Number of LAFs

0  1  2  3  4  5  6
A significant difference was also observed in the number of Large Amplitude Depression (LAD) movements performed (Fig 2.14, Kruskal-Wallis, $H=11.508$, df=3, $P=0.009$). Although it appears that the Outer Ablates performed many more LADs than the other groups, they were only significantly greater than the Inner and Non-Ablate groups (Dunn's Test, $Q=2.906$, $P<0.05$ for both), and not significantly different from the Cross Ablates (Dunn's Test, $Q=2.314$, $P>0.05$). Cross, Inner and Non-Ablate groups did not differ from each other (Dunn's Test, $P>0.05$ for all comparisons).

**Experiment B**

There was an overall significant difference in the ability of the different treatment groups to locate the food source ($\chi^2=18.653$, df=4, $P<0.001$; Roscoe & Byars (1971) discuss the use of the chi-square statistic with small sample sizes). The Inner Ablates, Cross Ablates and Non-Ablate groups were almost identical in the number of crayfish which were able to successfully locate the source of the food odour, while only 7 of the 15 animals lacking outer rami were able to perform the same action. Only a third of the 15 crayfish with all rami ablated were able to find the food source (Table 2.2; Table 2.3 lists the results of pairwise comparisons).
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Fig. 2.14. Comparison of Large Amplitude Depressions performed by treatment groups. Bars represent median values; measures of variance represent 25% and 75% quantiles.
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There was an overall significant difference among groups in the distance from the source at which substrate searching first occurred (Kruskal-Wallis, $H=38.96$, $df=4$, $P<0.001$). Pairwise comparisons showed that the Total Ablates started substrate searching at a closer distance to the food source than Cross Ablates (Dunn’s Test, $Q=29.5$, $P<0.05$), Inner Ablates (Dunn’s Test, $Q=43.85$, $P<0.05$), Non-Ablates (Dunn’s Test, $Q=33.4$, $P<0.05$) and Outer Ablates (Dunn’s Test, $Q=30.6$, $P<0.05$). Inner Ablates were observed to start substrate searching behaviour at a greater distance than Cross Ablates (Dunn’s Test, $Q=14.4$, $P<0.05$), Non-Ablates (Dunn’s Test, $Q=10.45$, $P<0.05$) and Outer Ablates (Dunn’s Test, $Q=13.25$, $P<0.05$). Table 2.3 lists the median distances for each group.

Moore et al. (1991b) report that chemoreceptors located on the walking legs of the lobster *H. americanus* play an increasingly important role in responses to chemical odours at distances less than 22 cm from the source. Twelve of the 15 Total Ablates tested in this experiment detected or first found the food at a median distance of 10.22 cm, well within this proximity. These results, from both the present study, and the one by Moore et al. (1991), suggest that crayfish which lack rami are not able to successfully perform long-distance chemoreception. As a result, the Total Ablate group was excluded from further analyses. One individual from the Inner Ablate group was also excluded, for the same reason.

The searching behaviour of the four remaining groups was next examined in greater detail. Data were square root transformed in most cases using a procedure found in...
Table 2.1. Number of crayfish which located the food source

<table>
<thead>
<tr>
<th></th>
<th>Cross Ablate</th>
<th>Inner Ablate</th>
<th>Non-Ablates</th>
<th>Outer Ablates</th>
<th>Total Ablates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Found Source</td>
<td>13</td>
<td>13</td>
<td>12</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Did Not Find</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 2.2. Fisher Exact Tests comparing success in locating food source

<table>
<thead>
<tr>
<th>Comparison</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross Ablate vs. Inner Ablate</td>
<td>1.000</td>
</tr>
<tr>
<td>Cross Ablate vs. Non-Ablate</td>
<td>0.598</td>
</tr>
<tr>
<td>Cross Ablate vs. Outer Ablate</td>
<td>0.014</td>
</tr>
<tr>
<td>Cross Ablate vs. Total Ablate</td>
<td>0.002</td>
</tr>
<tr>
<td>Inner Ablate vs. Non-Ablate</td>
<td>1.000</td>
</tr>
<tr>
<td>Inner Ablate vs. Outer Ablate</td>
<td>0.028</td>
</tr>
<tr>
<td>Inner Ablate vs. Total Ablate</td>
<td>0.004</td>
</tr>
<tr>
<td>Non-Ablate vs. Outer Ablate</td>
<td>0.075</td>
</tr>
<tr>
<td>Non-Ablate vs. Total Ablate</td>
<td>0.025</td>
</tr>
<tr>
<td>Outer Ablate vs. Total Ablate</td>
<td>0.487</td>
</tr>
</tbody>
</table>
Table 2.3. Median distances at which substrate searching first began.

<table>
<thead>
<tr>
<th></th>
<th>Median (cm)</th>
<th>25th percentile</th>
<th>75th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross Ablate</td>
<td>58.80</td>
<td>54.28</td>
<td>69.10</td>
</tr>
<tr>
<td>Inner Ablate</td>
<td>70.71</td>
<td>61.87</td>
<td>79.08</td>
</tr>
<tr>
<td>Non-Ablate</td>
<td>63.53</td>
<td>54.07</td>
<td>72.88</td>
</tr>
<tr>
<td>Outer Ablate</td>
<td>60.53</td>
<td>53.49</td>
<td>67.46</td>
</tr>
<tr>
<td>Total Ablate</td>
<td>10.22</td>
<td>6.51</td>
<td>13.77</td>
</tr>
</tbody>
</table>
Chapter 2: Responses to a Food Stimulus

SigmaStat (1995). No significant difference was found among the four remaining groups (Non-Ablate, Cross, Inner, and Outer Ablates) in the latency between the exit from the mesh container and the first performance of substrate digging (Fig. 2.15; ANOVA, df=3, F=1.711, P=0.177). No significant differences were found in pairwise comparisons (Tukey’s Test, P>0.05 in all comparisons).

Similarly, no overall significant difference was found in the distance from the source at which substrate digging was first performed (ANOVA, df=3, F=2.149, P=0.106). No significant differences were detected in pairwise comparisons (Tukey’s Test, P>0.05 for all comparisons).

A meander ratio was calculated for those animals which were successful in locating the food source, by dividing the actual distance travelled by the shortest distance between the food source and the point at which searching began (Fig. 2.17). No significant difference was found, either overall (ANOVA, df=3, F=1.142, P=0.344), or in pairwise comparisons (Tukey’s Test, P>0.05 for all comparisons).

The search durations of successful crayfish (Fig. 2.18) was significantly different across groups (ANOVA, df=3, F=3.517, P=0.03). Successful Outer Ablates required a longer period of time to locate the food source than Non-Ablate groups (Tukey’s Test, p=4, q=4.202, P<0.05). Comparisons between other groups were all non-significant (Tukey’s Test, P>0.05 for all comparisons).

Data on walking speed was transformed using a log10 procedure, as the square root transformations used in other analyses did not yield normal distributions when
Fig. 2.15. Latency to perform substrate digging. Bars represent median values; measures of variance represent 25% and 75% quantiles.
Fig. 2.16. Distance from the source at which substrate digging was first performed. Bars represent median values; measures of variance represent 25% and 75% quantiles.
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[Diagram showing median distance to source (cm) for Cross Ablate, Inner Ablate, Non-Ablate, and Outer Ablate conditions.]
applied here. A significant difference was found in the average walking speed used by the various groups when locating the food source (Fig. 2.19; ANOVA, df=3, F=3.803, P=0.017). Outer Ablates were significantly slower than the Cross Ablates (Tukey's Test, p=0.30, q=3.788, P<0.05), Inner Ablates (Tukey's Test, p=0.30, q=3.822, P<0.05), and Non-Ablate groups (Tukey's Test, p=0.368, q=4.581, P<0.05). Cross, Inner and Non-Ablate groups did not differ significantly from each other.

**DISCUSSION**

The antennules of decapod crustaceans are used in long-distance chemoreception, and the movement of these organs facilitates the detection and identification of olfactory cues following a change in the surrounding chemical environment (Gleeson et al., 1993a; Moore et al., 1991b; Schmitt & Ache, 1979). Pearson & Olla (1977) report that antennular movement will increase following the introduction of a stimulus.

Dunham et al. (1997) report that, in *C. bartonii*, a specific pattern of antennule movements occurred following the presentation of particular stimuli. All ablate groups in that study were observed to increase the total number of antennule movements following the presentation of a sucrose solution, and then decrease again after the second control solution. A consistent pattern was not found in the present study, although the movements performed by the Cross and Outer Ablates were similar to those in Dunham et al. (1997). Differences between the two studies could be a result of species and sex
Fig. 2.17. Comparison of meander ratios (calculated by diving the distance travelled by the shortest distance). Bars represent median values; measures of variance represent 25% and 75% quantiles.
Fig. 2.18. Search durations. Bars represent median values; measures of variance represent 25% and 75% quantiles.
Fig. 2.19. Average walking speed used when locating food source. Bars represent median values: measures of variance represent 25% and 75% quantiles.
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![Median Walking Speed](image)

- Cross Ablate
- Inner Ablate
- Non-Ablate
- Outer Ablate

Median Walking Speed (cm/s)
Chapter 2: Responses to a Food Stimulus

differences, as Dunham et al. (1997) used Form I male *C. bartonii* in their experiment, and female *P. clarkii* were tested here. The use of different test stimuli in the two studies may have also played a role, as a sucrose solution was used by Dunham et al. (1997), and fish filtrate used here.

However, differences in the methodology used in the two studies may also explain the contradictory results. Dunham et al. (1997) presented crayfish with both control and sucrose solutions only after the test crayfish was interacting with another crayfish held within a water-tight container. Social interactions were used to standardize motivation across the different treatment groups, and thus reduce the amount of variance found in the response. This standardisation procedure was not used in the present experiment, and it is possible that differences in motivation could have increased the variance found in the level of response.

The frequency of certain antennule movements following the presentation of a fish solution did not appear to be affected by ablation. Neither the Cross nor Inner Ablate groups differed significantly from the Non-Ablates in the number of Twitches or SAFs performed, a result that is consistent with observations made by Dunham et al. (1997). Interestingly, despite missing one outer ramus, the Cross Ablates performed the same number of Twitches as both the Inner and Non-Ablate groups. This suggests that the Cross Ablates compensated for the absence of an outer ramus by doubling the activity of the remaining one. It is unclear if this action is species-typical, as no increase was noted in *Cambarus bartonii*, following exposure to a sucrose solution (Dunham et al., 1997), or if this difference can be attributed to methodological differences between the two studies.
Chapter 2: Responses to a Food Stimulus

Outer Ablates differed from the other groups in the number of large antennule movements that were performed, as they performed more LAFs and LADs than the Non-Ablates. This result was also observed by Dunham et al., (1997). In that study, however, the two movements were not kept separate, and it was thus not possible to determine if the Outer Ablates used one specific movement more than the other.

Antennule movements, such as Twitches, have been characterized as equivalent to “sniffing” in vertebrates (Schmitt & Ache, 1979). This movement allows water to be exchanged over various receptors on the antennule, such as the aesthetasc hairs found on the outer rami (Tierney et al., 1984), facilitating the access to, and removal of, odour stimuli between sensilla (Gleeson et al., 1993b). Large antennule movements, such as LAFs, sweep through the greatest volume of water. The increased number suggests these animals may have been trying to compensate for the lack of sensory input from the missing outer ramus.

Crayfish in all treatment groups performed substrate searching behaviours, indicating that antennules were not required for the detection of the fish solution. Chemosensitive sensilla located elsewhere on the body, such as on the maxillipeds (Corotto et al., 1992; Derby & Atema, 1982a) or walking legs (Borroni et al., 1986; Derby & Atema, 1982a; Hazlett, 1971; Hodgson, 1958), may have been used in place of antennular input and mediated the initiation of substrate searching behaviour.

Results from Experiment B, however, support the hypothesis that the antennules may not be needed for short-distance chemoreception, but are required for chemoreception over long distances. All groups, with the exception of the Total Ablates,
began substrate digging at a median distance of approximately 60 cm from the food source. The fact that the Total Ablates only began digging within a median distance of 10 cm indicates that the walking legs, despite being chemosensitive (Derby & Atema, 1982a; Derby & Atema, 1982c; Johnson et al., 1984), play a more “local” role. A similar result has been found in *H. americanus*, where the walking legs play a subordinate role in search path control (Devine & Atema, 1982) but become increasingly more involved the closer the animal is to the source of the stimulus (Moore et al., 1991b).

Although there is evidence that crustacean antennae are similar to the antennules in receptor cell composition and can thus be considered a major chemoreceptor organ (Voigt & Atema, 1992), they do not appear to have played a major role in either the detection or localisation of the food source. If these organs were important in long-distance chemoreception, the Total Ablate group should not have differed so dramatically from the other treatment groups. It isn’t clear, however, whether the antennae may have played a role in mediating searching behaviour at close distances.

Other results obtained in the present study both support and contradict the findings of previous research. As in Moore (1991b), no typical search pattern was used by any of the treatment groups. The Cross Ablates were not significantly different from Non-Ablates in meander ratio, search duration, or average walking speed, and had only a marginally shorter latency to the performance of substrate digging behaviours. Unfortunately, most studies examining the role of specific rami in orientation have not used a cross ablation technique. Devine & Atema (1982) created a different class of Cross Ablates by examining the responses of *H. americanus* following the ablation of one outer
or inner ramus on one side, and then again after the contralateral ramus was removed.

Comparing the mean (± s.e.) of their Cross Ablates (labelled either “L + M” or “M + L”) and Non-Ablates shows no change in the latency to alert responses. Differences were found in search path length, but without a measure of the most direct distance between start and end points, this cannot be directly compared with the meander ratio calculated in the present study.

Outer Ablates differed the most from the other ablation groups tested. Although they were all able to detect the piece of fish from a distance, and at a latency similar to that of the other treatment groups, only half of the animals tested were able to successfully locate the food source. These successful animals, however, took a longer period of time to find the food source than Non-Ablates, and also had a slower average walking speed than the other treatment groups. Interestingly, the meander ratio calculated for this group was not significantly different than that for the other ablation treatments. Similar results were obtained by Reeder & Ache (1980) in experiments using the spiny lobster *Panulirus argus*. They found that animals which had the outer rami ablated had longer search times, but not a longer latency to search. Devine & Atema (1982) observed that *H. americanus* with either the aesthetasc hairs shaved off, or with an outer ramus ablated, had a latency no different than that of control animals. Walking speed was not reported in that study.

The number of Inner Ablates which successfully located the food source (13 out of 15) was almost identical to the number of Cross Ablates (13 of 14) and Non-Ablates
(12 of 15) which were able to do the same. Inner Ablates were also similar to these two groups in the latency to substrate digging, meander ratio, search duration and average walking speed. Devine & Atema (1982), using H. americanus, and Reeder & Ache (1980), using P. argus, also found that ablation of the inner rami had no effect on the animals' ability to reach a stimulus source, the latency to the performance of searching behaviours, search duration, or any other reported behaviour.

The results of the present experiment and previous studies demonstrate the importance of the outer rami in the detection and localisation of food odours over long distances by P. clarkii and other decapod crustaceans. The outer rami are likely the primary olfactory organ by which detection and localisation occur when the animal is some distance from the odour source, with input from the walking legs (and possibly the antennae) being of greater importance at closer distances. It appears that the inner rami can also be used in distance chemoreception and localisation, although the large number of crayfish which possessed only these rami and did not successfully locate the stimulus source suggests that this is not the primary organ used in this process.

Rutherford et al. (1996) report that LAD behaviour occurs most often during social encounters, and that this movement would place an antennule in a position ideal for encountering an opponent's maxilliped currents and possible chemical information that they contain (e.g. Cowan, 1991; Karavanich & Atema, 1991). The inner rami, which are also moved during large antennule movements, may be used specifically to detect and identify the source of chemical stimuli released during social interactions. An investigation of this possibility is reported in Chapter 3.
CHAPTER 3

Use of the inner rami in the localisation of social odours by female red swamp crayfish, *Procambarus clarkii*
Antennules are used by decapod crustaceans in long-distance chemoreception. The ablation of specific rami was used to determine if the inner (medial) rami are used by female red swamp crayfish, *Procambarus clarkii*, to locate social stimuli. Antennule rami were not required for the initiation of searching over short distances, but crayfish with all rami ablated were unable to locate a social stimulus over long distances. No significant difference was detected among or between crayfish with at least two rami in the latency to search, search duration, meander ratio, or average walking speed. Crayfish possessing only inner rami were as successful as those with only outer rami. These results indicate that the inner rami are used to locate the source of social stimuli, and confirm that there is redundancy in the organs used for this purpose.
Chemical cues are used by a large number of organisms, both terrestrial and aquatic, when searching for resources (Atema, 1988; Bell & Carde, 1984; Rittschof, 1990; Svensson, 1996). The ability to detect chemical stimuli is therefore quite common, and organs specialized for this function are found in many animal groups (Price & Ache, 1977; Schmitt & Ache, 1979). Decapod crustaceans rely heavily on chemical information, and are able to detect practically every class of biological molecule (Rittschof, 1992). Mellon et al. (1992) report that 30 to 40% of crayfish brain volume is devoted to the processing of olfactory input.

Chemical cues are important in the social behaviour of many decapod crustaceans. Previous studies have shown that chemical stimuli are used by a variety of crustaceans in species recognition (Rose, 1986; Tierney & Dunham, 1982), as well as sex recognition (Dunham & Oh, 1996; Hazlett, 1985; McLeese, 1970; Snyder et al., 1993). Little (1975) demonstrated that larval crayfish use chemical cues to distinguish brooding females from non-brooding ones. Chemical cues are also known to be used by the lobster Homarus americanus in courtship behaviours (Atema, 1986; Atema & Cowan, 1986; Atema & Engstrom, 1971; Cowan, 1991; McLeese et al., 1977), and in addition, evidence has been found suggesting that chemical stimuli may play an important role in the aggressive interactions of crustacean species (Breithaupt & Atema, 1993; Kaplan et al., 1993; Karavanich & Atema, 1991; Rutherford et al., 1996; Thorp & Ammerman, 1978). These results have not been without controversy. Itagaki & Thorpe (1981) reported no
transmission of sexual or species identity, nor of agonistic state, in tests using the red swamp crayfish, *Procambarus clarkii*. The methodology of that particular study has been called into question, however, and may explain the results obtained (see Hazlett (1984); Rose (1984); and response by Thorpe (1984)).

The flexible antennules of crayfish and other decapod crustaceans are considered the most important organs used in distant chemical communication (Ache, 1975; Devine & Atema, 1982; Hamner & Hamner, 1977; Hazlett, 1971; McLeese, 1973a; McLeese, 1973b; Moore et al., 1991b; Pearson & Olla, 1977). Each antennule is bifurcated, with one outer (lateral) and one inner (medial) ramus. The two rami differ morphologically, with aesthetasc hairs being found on the outer ramus but not on the more slender inner ramus (Tierney, 1985; Tierney & Dunham, 1982). Other sensilla found on both rami are believed to serve both a mechanosensory and chemosensory function (Derby, 1989). The chemosensory function of the aesthetasc hairs is enhanced through the flicking of the antennules, which increases their exposure to the surrounding chemical environment (Schmitt & Ache, 1979).

The functional role played by the two types of rami is controversial, because conflicting results were obtained in different studies. In experiments on the reproductive behaviour of the blue crab *Callinectes sapidus*, Gleeson (1980) found that crabs with all four rami ablated did not perform courtship behaviours. Ablation of specific flagella demonstrated that it was the outer rami which were used in the detection of possible pheromones, as well as for the initiation of courtship behaviour. Ablation of the inner rami did not change the performance of these behaviours.
A similar result was found by Tierney et al. (Tierney et al., 1984), using male crayfish *Orconectes propinquus* and female conditioned water. A significant reduction in response was observed following the ablation of the outer rami; these animals spent very little time within 3 cm of the chemical source. Crayfish with the inner rami ablated, however, did not appear to suffer any disadvantage. It is unclear, however, if the ablation of the outer rami affected the ability of the animals to either detect, or localise the source of the stimulus, or if both abilities were affected.

Ameyaw-Akumfi & Hazlett (1975) performed ablation experiments on *P. clarkii* to determine the site of chemoreception. Animals with only the inner rami ablated, or with all rami removed, did not respond to test solutions, suggesting this ramus is the site of reception. However, results for animals missing only outer rami were not reported.

Oh & Dunham (1991) found that one ramus of each type was required for *P. clarkii* to distinguish self-conditioned water from stranger-conditioned water. Although locomotion was one of the behaviours examined, analysis of this measure was not reported. It is therefore unclear if the ability of the crayfish to locate the stimulus source was inhibited.

Evidence that the inner rami may be used in localisation can be found in Dunham & Oh (1992). Non-ablated and cross-ablated *P. clarkii* were able to discriminate between male and female conspecifics, and were also able to localise the source of the stimuli. Outer ablates were not able to localise the source of stimulation, but were able to discriminate between the two sexes. Crayfish with the inner rami ablated, however, were able to discriminate, but could not locate the stimulus source.
Cross-species differences may account for the different results obtained in the various studies, in some way as yet not understood. Flaws in experimental design, however, may have also masked the role of the inner rami, leading to the suggestion that they are not used in distant chemoreception, despite being very chemosensitive (Tierney et al., 1988). Studies have not always separated detection from localisation (e.g. Dunham & Oh, 1992; Tierney et al., 1984). In addition, Moore (1991b) reports that receptors found on the walking legs may initiate search behaviours at distances less than 22 cm from the source. Small testing tanks can therefore influence the results obtained, as they may not have been large enough to properly test the abilities of Crustacea to locate the source of an odour over greater distances (e.g. Dunham & Oh, 1992; Oh & Dunham, 1991).

The role played by the inner rami during the localisation of social stimuli is thus still unclear. This study was conducted to determine if the inner rami are used by female red swamp crayfish, *Procambarus clarkii*, to mediate their response to conspecific odours over long distances.

**METHODS**

**Housing**

Adult female *Procambarus clarkii* measuring approximately 5 cm in carapace length were obtained from a wholesaler. Only animals with intact antennules, antennae, and walking legs were used, as these structures are known to have some chemosensory function (Table 1.1). Similarly, any animals which developed eggs or moulted during the
course of the experiment were excluded, since these conditions might affect the responses to be measured.

Crayfish were individually housed in cylindrical 5L glass jars, in 1L of water. Crayfish could see each other through the glass, but were unable to smell or touch each other. Light was provided by fluorescent tubes located above each tank, as well as a 300W incandescent ceiling lamp. Air temperature was maintained at 19°C ± 1°C. A reverse light cycle of 12 hours dark:12 hours light was used, with lights turned on at 2130h.

Animals were fed 5 pellets of Purina Trout Chow every second day, including the day prior to testing. Three-quarters of the water in each jar was siphoned out every second day, and replaced with aged municipal supply water. All crayfish were held under these conditions for three weeks before they were used in any experiments.

Selection and Ablation

Animals were selected at random and placed into sets of 5 animals each. One week prior to their testing, each individual within a set was assigned to one of five treatment groups: (i) a Non-Ablate group, with no antennular rami ablated (N=19), (ii) an Inner Ablate group, with the ablation of both inner rami (N=15), (iii) an Outer Ablate group, with both outer rami ablated (N=15), (iv) A Cross Ablate group, with one inner ramus (either left or right, determined randomly) and one contralateral outer ramus ablated (N=15); or (v) a Total Ablate group, with all four rami ablated (N=15). Figure 2.2 illustrates the different ablate types. All animals were anaesthetised using a 20 minute immersion in ice water, and then had specific rami quickly ablated. Animals which didn’t
have any rami ablated were exposed to the ice water treatment. Following this ablation procedure, crayfish were returned to their individual jars.

Testing Procedures

Each set of crayfish was tested over two consecutive days. Responses toward different solutions was observed on the first day (Experiment A), and the ability of the animals to localise the source of a chemical stimulus was tested on the second day (Experiment B).

Seven days after ablation treatments, the five crayfish to be tested were placed in individual 10L testing tanks and left for 24 hours. Movement within these tanks was limited to the front third (41 x 9 cm wide) through the use of a clear Plexiglas wall. In addition, a platform angled upwards at 45° from the bottom of the tank to this wall was used to provide a floor upon which the crayfish could move (Fig. 2.3). A substrate was created by attaching a layer of black gravel to the Plexiglas platform with aquarium-grade silicon. The crayfish were more active during the dark period of the light cycle than during the light period, so testing was performed under low light conditions at this time. Since red light did not appear to have a detrimental effect on crayfish activity, low light levels were provided by a 60W red light bulb located above each testing tank.

Experiment A

Experiment A examined the responses of a crayfish following the ejection of chemical solutions (a control solution or one collected from a male P. clarkii) over the
antennular region of the test animal. The responses to a change in the chemical
environment immediately surrounding the test animal were videotaped and later scored.

Preparation of Solutions

Dunham & Oh (1996) observed that female *P. clarkii* are attracted to chemical
stimuli from males, but avoid chemical stimuli from females. To reduce the chance that
the female crayfish tested in this experiment would perform avoidance behaviours, the
social stimulus used was created by collecting water from one of 5 glass jars containing
Form I male *P. clarkii*. Five males were housed individually in glass jars for 48 hours,
under the same conditions as females, before water was gently scooped out using a 500
mL glass jar. Christofferson (1978) found that when restrained, the crab *Portunus
sanguinolentus* inhibited the release of a possible crustacean pheromone for several hours.
Housing the male crayfish for 48 hours should have ensured that the water collected had
detectable levels of any possible pheromone.

A control solution was created using 18 mL of water which was gently scooped
out of the rear portion of the tank used for testing the same crayfish subject. Control
solutions were collected just prior to testing.

Testing

A 60W red lamp positioned directly above the testing tank was turned on 40
minutes prior to testing. The crayfish-being tested was videotaped for an initial 7
minutes. A 14.6 cm disposable Pasteur capillary pipette was then used to eject $2.0 \pm 0.1$
mL of the control solution over the antennules and maxillipeds of the crayfish, at a rate of 
0.5 ± 0.1 mL/s, from approximately 18 cm away (Fig. 2.4). The pipette was inserted 
gently into the water in order to reduce water disturbance. The crayfish’s responses were 
videotaped for the next 7 minutes (see Behaviours Examined below). Immediately 
following this period, a social stimulus was presented, in the same manner as above, and 
behaviours were again videotaped for 7 minutes. The control solution was then presented 
again, and behaviours videotaped for a final 7 minutes. Behaviours were observed to 
return to original levels by the end of each 7-minute observation period. Following 
testing, each crayfish was placed in an individual 10L aquarium (41 (length) x 27 cm 
wide), and remained there until it was used in Experiment B.

Behaviours Examined

Antennular movements are likely used to facilitate the chemosensory function of 
the antennules (Gleeson et al., 1993b; Moore et al., 1991a; Schmitt & Ache, 1979). These 
movements can thus be used as an indication of the animal’s ability to detect a change in 
its surrounding chemical environment (Ciruna et al., 1995). At rest, antennules are held at 
an angle of approximately 45° above the horizontal plane. Since antennules can move 
independently or in tandem (Berg et al., 1992), antennule flicking can involve either or 
both antennules. The following antennule movements (Fig. 2.5) were examined 
(definitions are from Dunham et al. (1997)): 
(i) Small Amplitude Flicks (SAF) are the rapid, fluid and phasic depression and elevation of either or both antennules, and involve both the inner and outer rami. The depression of the antennule does not extend more than 45° below the horizontal.

(ii) Large Amplitude Flicks (LAF) are similar to SAF, but are slower, and extend to a maximum angle of 90° below the horizontal.

(iii) Large Amplitude Depressions (LAD) are depressions of the antennule to a maximum of 90°, as in LAF, but the antennule is held in this position before elevation.

(iv) Antennular Twitches are a very rapid depression and elevation of the outer ramus, to a maximum of 15° below the horizontal. The paired inner ramus is not involved.

Also measured was the time spent with Chelae Raised above the substrate, with claws held either open or closed. Chelipeds were sometimes also pulled backwards during this reaction. Included within this category are behaviours classified in other studies as “meral spread” (Bruski & Dunham, 1987; Dingle, 1969), “raised and extended chelipeds” (Smith & Dunham, 1990), and “claws raised” (Guiasu & Dunham, 1997).

Experiment B

The ability of the different ablate groups to localise the source of a social stimulus was examined in Experiment B.

Testing apparatus

Testing was performed in a large rectangular flow-through Plexiglas tank (100 cm (length) x 60 cm (width) x 30 cm high). Black gravel lined the bottom of the tank, to a
depth of 1 cm, and aged municipal supply water from two head tanks was used to fill the
tank to a depth of 10 cm. A current was created by siphoning aged municipal supply
water from another three head tanks. Water entered the tank via a single entry point at
one end of the tank (the “inflow” end) and was drained through a tube located at the
opposite end (the “outflow” end). Mean (± s.e.) current velocity in the centre of the tank
was 1.7 ± 0.1 cm/s. Plastic grates at both ends of the tank served as collimators, and also
confined the test animal to a smaller area of the tank (80 cm length x 60 cm width); this
area was videotaped using a camera located above the tank (Fig. 2.6).

Two burettes were positioned at the “inflow” end of the tank, with the tip of each
burette directly above a plastic mesh cylinder. Cylinders were each 4 cm in diameter, and
10 cm in height. Cylinders were 8 cm apart, as measured from the centre of each (Fig.
2.7), and were siliconed onto a Plexiglas sheet which was placed 12 cm from the inflow
(Fig. 3.1). One burette contained the stimulus solution; the other contained a control
solution (water collected from the same head tank used to fill the testing tank). The
choice of which burette held the stimulus solution was determined at random, but
balanced over the course of the experiment.

Testing

Crayfish were tested in the same order as in Experiment A. The mesh container
containing the test animal was placed at the “outflow” end of the tank, directly opposite
the current inflow. The water current was then created by siphoning water from a head
tank. Following this, the burettes were opened, and solutions were dripped into the tank at a rate of $2.0 \pm 0.08$ mL/min.

Thirty seconds after the current and solution entry were established, the mesh container was gently tipped over and the crayfish allowed to enter the large testing tank. The entire testing area was then videotaped for the next 20 minutes, or until the crayfish located the social stimulus, whichever occurred first. Once the testing session was terminated, the crayfish was removed, and the burettes closed. The tank was then drained, thoroughly rinsed, and re-filled.

**Analysis**

The specific behaviours used in analyses were observed and recorded directly from video playback.

Successful animals were defined as those crayfish which detected and located the source of the social stimulus. Detection was considered to have occurred if a crayfish paused, increased the angle at which it held its chelae open, and continued walking, but at a slower speed (this decrease in walking speed was not quantified in the present study). An animal was deemed to have successfully located the stimulus if it grabbed the mesh cylinder with the chelipeds, or if it remained motionless next to the cylinder for longer than 20 s. The latency between the point at which a crayfish left the mesh container and the time at which detection and search behaviours were first performed was recorded for each animal. Also recorded for each individual was the location where detection and
Fig. 3.1. Placement of objects in testing tank during Experiment B. Burettes (A) were positioned above cylinders (B) 12 cm from the inflow to the testing tank. The mesh container containing the crayfish being tested was placed at the opposite end of the tank, directly opposite the inflow.
searching behaviours first occurred. The most direct distance between this point and the location of the stimulus source was traced from the video monitor directly onto a transparent plastic sheet. In addition, the pathway used by successful animals was traced from video playback onto the same sheet (Fig. 2.9). Since crayfish can walk sideways and backwards without a change in their overall body orientation, a crayfish’s rostrum was used as a reference point when tracing paths. Using only the rostrum as a reference point allowed the distance travelled, independent of the direction in which a crayfish may have been facing, to be measured. The distances travelled by individual crayfish were measured from the traces using SigmaScan™ (1995) computer software.

A “meander” ratio was created for successful animals by dividing the actual distance travelled by the most direct distance (Zach & Falls, 1976). Also measured was search duration (time elapsed between the moment at which searching behaviours were first performed and the successful localisation of the food source) and the animal’s mean walking speed.

Statistics were calculated using either the statistical package SigmaStat™ (1995), or through procedures outlined by Siegel & Castellan (1988).
**RESULTS**

**Experiment A**

Raising of the chelae above the gravel substrate was observed in all ablate groups during the two minutes immediately following the ejection of the social stimulus. Overall significant differences were detected within each ablate group in comparisons with the pre-stimulus and each of the two control sessions (Friedman Repeated Measures ANOVA on Ranks, $P<0.02$ in each case). Pairwise comparisons revealed no consistent patterns. There were no significant pairwise differences detected in the Total Ablates (SNK, $P>0.05$ in each case). Inner Ablates were observed to raise their chelae for significantly longer durations following the ejection of the social stimulus than during other observation sessions (SNK, $P<0.05$ for comparisons involving social stimulus, $P>0.05$ in all other cases). Outer Ablates performed significantly greater amounts of Chelae Raising following the ejection of either the control or stimulus solutions than during the pre-stimulus periods (SNK, $P<0.05$ for comparisons involving pre-stimulus; $P>0.05$ in all other cases). All pairwise comparisons, except that between the social and second control sessions, were significant in Non-Ablates. Similarly, all pairwise comparisons were significant in Cross Ablates, except for the comparison between the first and second control sessions.
Figure 3.2 illustrates the median duration of Chelae Raised by each treatment group during the two minutes immediately following the presentation of the social stimulus. Overall, there was a marginally non-significant difference observed among the treatment groups (Kruskal-Wallis, H=9.075, df=4, P=0.059). Total Ablates performed Chelae Raising for shorter durations than Cross, Inner and Non-Ablates (Dunn's Test, P<0.05 in each case), but not Outer Ablates (Dunn's Test, P>0.05). Comparisons made between other pairs yielded no significant differences.

Flicking is believed to enhance the chemosensory function of the antennules (Schmitt & Ache, 1979). In a study examining the integration of antennules in the behaviour patterns used in feeding, Dunham et al. (1997) observed a general pattern in the gross antennule movements performed by Cambarus bartonii. Antennule movements increased from a baseline measure following the presentation of a sucrose solution, and then decreased after the ejection of a control solution. A similar pattern was expected in the present study, with increases in antennule movements occurring after the presentation of the social stimulus. This general pattern was observed for all treatment groups, with the exception of Total Ablates, which could not perform antennule movements.
Fig. 3.2. Duration spent with Chelae Raised during 2 min immediately following the presentation of social stimulus. Bars represent medians; measures of variance represent 25% and 75% quantiles.
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An overall difference was found for the Cross Ablates (Fig. 3.3; Kruskal-Wallis, H=12.247, df=3, P=0.007), with a larger number of movements performed after the ejection of the social stimulus than during the prestimulus period (Dunn’s Method, Q=3.254, P<0.05), or the first control solution (Dunn’s Method, Q=2.696, P<0.05). The number of movements performed following the presentation of the second control solution was not significantly different from responses performed after ejection of the other stimuli (Dunn’s Method, P>0.05 in each case).

Inner Ablates were also significantly different overall (Fig. 3.4; Kruskal-Wallis, H=19.293, df=3, P<0.001). Antennule movements performed following the ejection of the social stimulus were significantly greater than those for the prestimulus (Dunn’s Method, Q=2.821, P<0.05) or Control 1 periods (Dunn’s Method, Q=4.252, P<0.05), but not the Control 2 period (Dunn’s Method, Q=1.743, P>0.05).

There was no overall difference detected in the number of antennule movements performed by Non-Ablates following the presentation of the various stimuli (Fig. 3.5; Kruskal-Wallis, H=6.407, df=3, P=0.093). However, pairwise comparisons indicated that responses to the social stimulus were significantly greater than for the prestimulus and Control 2 (Dunn’s Test, P<0.05), but not the Control 1 sessions (Dunn’s Test, P>0.05).
Fig. 3.3. Total antennule movements performed by Cross Ablates during the 2 min immediately following the presentation of a stimulus. Bars represent medians; measures of variance represent 25% and 75% quantiles.
Fig. 3.4. Total antennule movements performed by Inner Ablates during the 2 min immediately following the presentation of a stimulus. Bars represent medians; measures of variance represent 25% and 75% quantiles.
Fig. 3.5. Total antennule movements performed by Non-Ablates during the 2 min immediately following the presentation of a stimulus. Bars represent medians; measures of variance represent 25% and 75% quantiles.
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The graph illustrates the median number of movements across different conditions:

- Prestimulus
- Control 1
- Social Stimulus
- Control 2

The y-axis represents the median number of movements, ranging from 0 to 18.
The number of antennule movements performed by Outer Ablates to the presentation of the different stimuli were not significantly different overall (Fig. 3.6; Kruskal-Wallis, $H=2.071$, df=3, $P=0.558$), or in pairwise comparisons (Dunn’s Test, $P>0.05$).

Individual antennular movements performed following the ejection of the social stimulus were next examined and compared. There was no significant overall difference in the number of Twitches made by the Cross Ablates, Inner Ablates, and Non-Ablates (Fig. 3.7; Kruskal-Wallis, $H=0.171$, df=2, $P=0.918$). Both the Outer and Total ablates were excluded from analysis, since they lack the only rami capable of Twitch movements. No significant difference were detected in pairwise comparisons (Dunn’s Test, $P>0.05$ in all cases). Cross Ablates performed equivalent numbers of Twitches to those of the Inner and Non-Ablate groups. No correction was made for the absence of an outer ramus in the Cross Ablate group, although this has been done in a previous study (Dunham et al., 1997). A similar response was observed to a food stimulus (Chapter 2).

Small Amplitude Flicks (SAF) were performed by all crayfish (Fig. 3.8). Outer Ablates were included in this and subsequent analyses, since SAF and larger amplitude movements involve movement of the entire antennule. Total Ablates could not be analysed. A significant overall difference was found among the treatment groups (Kruskal-Wallis, $H=19.954$, df=3, $P<0.001$). Outer Ablates performed significantly fewer SAFs than Cross Ablates, (Dunn’s Test, $Q=3.944$, $P<0.05$), Inner Ablates, (Dunn’s Test, $Q=3.302$, $P<0.05$), and Non-Ablates (Dunn’s Test, $Q=3.581$, $P<0.05$).
Fig. 3.6. Total antennule movements performed by Outer Ablates during the 2 min immediately following the presentation of a stimulus. Bars represent medians; measures of variance represent 25% and 75% quantiles.
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Prestimulus  Control 1  Social Stimulus  Control 2

Median Number of Movements

0  0  1  0
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Fig. 3.7. Number of Twitches performed by Cross, Inner and Non-Ablates following the presentation of the social stimulus. Outer and Total Ablates could not be tested, as they lack the only rami capable of Twitch movements. Bars represent medians; measures of variance represent 25% and 75% quantiles.
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The diagram shows the median number of twitches across different conditions:

- Cross Ablate
- Inner Ablate
- Non-Ablate

The values range from 0 to 10 on the y-axis, with Cross Ablate having the highest median number of twitches.
Fig. 3.8. Number of Small Amplitude Flicks performed following the presentation of the social stimulus. Bars represent medians; measures of variance represent 25% and 75% quantiles.
Cross, Inner and Non-Ablate groups did not differ from each other.

Large Amplitude Flicks (LAF) were infrequently performed following the ejection of the social solution (Fig. 3.9). No significant difference was found, either overall (Kruskal-Wallis, $H=0.552$, df=3, $P=0.907$), or in pairwise comparisons (Dunn’s Test, $P>0.05$ for all comparisons).

The performance of Large Amplitude Depression (LAD) movements was even rarer (Fig. 3.10). As was found for LAFs, no significant difference was found in the performance of LADs, either overall (Kruskal-Wallis, $H=0.591$, df=3, $P=0.899$), or in pairwise comparisons (Dunn’s Test, $P>0.05$ for all comparisons).

**Experiment B**

The ability of the crayfish to detect the social stimulus was first examined. There was no overall difference in the number of animals in each treatment group which met the behavioural criteria of pause, movement of the chelipeds, and a decrease in walking speed ($\chi^2=7.398$, df=4, $P=0.116$). All of the Cross Ablates detected the social stimulus.

Twelve of the 13 Inner Ablates, 14 of the 15 Non-Ablates, and 13 of the 15 Outer Ablates also detected the stimulus. Four of the 15 Total Ablates were unable to detect the social stimulus. Pairwise comparisons revealed that Cross Ablates were more likely to detect the social stimulus than were Total Ablates (Fisher Exact, $P=0.035$). No other significant differences were detected (Table 3.1; Table 3.2 lists the results of pairwise
Fig. 3.9. Number of Large Amplitude Flicks performed following the presentation of the social stimulus. Bars represent medians; measures of variance represent 25% and 75% quantiles.
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![Graph showing median number of LAFs across different categories: Cross Ablate, Inner Ablate, Non-Ablate, Outer Ablate. Each category has a median value of 0.]
Fig. 3.10. Number of Large Amplitude Depressions performed following the presentation of the social stimulus. Bars represent medians; measures of variance represent 25% and 75% quantiles.
Table 3.1. Number of crayfish which detected the social stimulus.

<table>
<thead>
<tr>
<th>Detected Source</th>
<th>Cross Ablate</th>
<th>Inner Ablate</th>
<th>Non-Ablates</th>
<th>Outer Ablates</th>
<th>Total Ablates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detected</td>
<td>15</td>
<td>12</td>
<td>14</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Did Not Detect</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 3.2. Fisher Exact Tests comparing the detection of the social stimulus.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross Ablate vs. Inner Ablate</td>
<td>=0.464</td>
</tr>
<tr>
<td>Cross Ablate vs. Non-Ablate</td>
<td>=1.000</td>
</tr>
<tr>
<td>Cross Ablate vs. Outer Ablate</td>
<td>=0.483</td>
</tr>
<tr>
<td>Cross Ablate vs. Total Ablate</td>
<td>=0.035</td>
</tr>
<tr>
<td>Inner Ablate vs. Non-Ablate</td>
<td>=1.000</td>
</tr>
<tr>
<td>Inner Ablate vs. Outer Ablate</td>
<td>=1.000</td>
</tr>
<tr>
<td>Inner Ablate vs. Total Ablate</td>
<td>=0.322</td>
</tr>
<tr>
<td>Non-Ablate vs. Outer Ablate</td>
<td>=1.000</td>
</tr>
<tr>
<td>Non-Ablate vs. Total Ablate</td>
<td>=0.153</td>
</tr>
<tr>
<td>Outer Ablate vs. Total Ablate</td>
<td>=0.372</td>
</tr>
</tbody>
</table>
comparisons). Crayfish which did not detect the social stimulus were excluded from further analyses.

Overall, there was a marginal, but non-significant, difference in the ability of the difference treatment groups to locate the source of the social stimulus ($\chi^2 = 9.257$, df=4, $P=0.055$). All of the Inner Ablates, and 14 of the 15 Cross Ablates were able to successfully locate the source of the social stimulus. Nine of the 14 Non-Ablates and 10 of the 13 Outer Ablates were also able to locate the source. Five of the 9 Total Ablates which detected the social stimulus were subsequently able to locate the source (Table 3.3; Table 3.4 lists the results of pairwise comparisons).

There was a significant overall difference in the distance from the food source at which the different treatment groups first began detection behaviours (Kruskal-Wallis, df=4, $H=31.16$, $P<0.01$). Total Ablates detected the social stimulus from a much closer distance than Cross Ablates (Dunn’s Test, $Q=39.73$, $P<0.05$), Inner Ablates (Dunn’s Test, $Q=34.5$, $P<0.05$), Non-Ablates (Dunn’s Test, $Q=28.42$, $P<0.05$) and Outer Ablates (Dunn’s Test, $Q=26.69$, $P<0.05$). In addition, Cross Ablates performed detection behaviours from further away than Non-Ablates (Dunn’s Test, $Q=11.31$, $P<0.05$) and Outer Ablates (Dunn’s Test, $Q=13.04$, $P<0.05$). Table 3.5 lists the median distances at which detection behaviours were first performed.

Moore et al. (1991b) report that chemoreceptors located on the walking legs of the lobster *Homarus americanus* play an increasingly important role in responses to chemical odours at less than 22 cm from the source. The observation that all of the Total Ablates
in the present study detected, or detected and subsequently located, the social stimulus
from a median distance of approximately 14 cm suggests that long-distance
chemoreception requires the presence of antennular rami. As a result, the Total Ablates
were excluded from further analysis.
Table 3.3. Number of crayfish which located the stimulus source.

<table>
<thead>
<tr>
<th></th>
<th>Cross Ablate</th>
<th>Inner Ablate</th>
<th>Non-Ablates</th>
<th>Outer Ablate</th>
<th>Total Ablates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Successful</strong></td>
<td>14</td>
<td>12</td>
<td>9</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td><strong>Did Not Find</strong></td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 3.4. Fisher Exact Tests comparing the number of animals which successfully located the stimulus source.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross Ablate vs. Inner Ablate</td>
<td>=1.000</td>
</tr>
<tr>
<td>Cross Ablate vs. Non-Ablate</td>
<td>=0.153</td>
</tr>
<tr>
<td>Cross Ablate vs. Outer Ablate</td>
<td>=0.311</td>
</tr>
<tr>
<td>Cross Ablate vs. Total Ablate</td>
<td>=0.047</td>
</tr>
<tr>
<td>Inner Ablate vs. Non-Ablate</td>
<td>=0.096</td>
</tr>
<tr>
<td>Inner Ablate vs. Outer Ablate</td>
<td>=0.220</td>
</tr>
<tr>
<td>Inner Ablate vs. Total Ablate</td>
<td>=0.021</td>
</tr>
<tr>
<td>Non-Ablate vs. Outer Ablate</td>
<td>=1.000</td>
</tr>
<tr>
<td>Non-Ablate vs. Total Ablate</td>
<td>=0.662</td>
</tr>
<tr>
<td>Outer Ablate vs. Total Ablate</td>
<td>=0.376</td>
</tr>
</tbody>
</table>
Table 3.5. Median distance at which detection behaviours first occurred.

<table>
<thead>
<tr>
<th></th>
<th>Median (cm)</th>
<th>25th percentile</th>
<th>75th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross Ablate</td>
<td>68.51</td>
<td>62.85</td>
<td>70.79</td>
</tr>
<tr>
<td>Inner Ablate</td>
<td>60.92</td>
<td>53.45</td>
<td>72.93</td>
</tr>
<tr>
<td>Non-Ablate</td>
<td>58.05</td>
<td>45.91</td>
<td>67.10</td>
</tr>
<tr>
<td>Outer Ablate</td>
<td>59.44</td>
<td>45.61</td>
<td>63.38</td>
</tr>
<tr>
<td>Total Ablate</td>
<td>13.63</td>
<td>11.00</td>
<td>18.07</td>
</tr>
</tbody>
</table>
Fig. 3.11. Latency to performance of detection movements. Bars represent medians; measures of variance represent 25% and 75% quantiles.
No significant difference was found among the four remaining groups (Non-
Ablate, Cross, Inner and Outer Ablates) in the latency between the exit from the mesh
container and the first performance of detection movements (Fig 3.11; Kruskal-Wallis,
H=3.311, df=3, P=0.346). No significant differences were revealed in pairwise
comparisons (Dunn’s Test, P>0.05 for all comparisons).

Similarly, no overall difference was found in the distance from the source at which
searching was initiated (Fig. 3.12; Kruskal-Wallis, H=6.704, df=3, P=0.108). Cross
Ablates began searching further from the source than the Non-Ablates (Dunn’s Test,
Q=11.31, P<0.05), or the Outer Ablates (Dunn’s Test, Q=13.04, P<0.05). No other
significant differences were detected in pairwise comparisons (Dunn’s Test, P>0.05 for
all comparisons).

A meander ratio was calculated for those animals which were successful in locating
the food source, by dividing the actual distance travelled by the shortest distance between
the stimulus source and the point at which searching was initiated (Fig. 3.13). No
significant difference was found, either overall (Kruskal-Wallis, H=0.273, df=3, P=0.965),
or in pairwise comparisons (Dunn’s Test, P>0.05 for all comparisons).

The search durations of successful crayfish (Fig. 3.14) was found not to be
significantly different overall (Kruskal-Wallis, H=1.712, df=3, P=0.634). Significant
differences were not detected in any pairwise comparison (Dunn’s Test, P>0.05 for all
comparisons).
Fig. 3.12. Distance to source at which searching behaviours were first initiated. Bars represent medians; measures of variance represent 25% and 75% quantiles.
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The bar chart illustrates the median distance to source (cm) for various conditions: Cross Ablate, Inner Ablate, Non-Ablate, and Outer Ablate.
Fig. 3.13. Comparison of meader ratios (calculated by diving the distance travelled by the shortest distance). Bars represent medians; measures of variance represent 25% and 75% quantiles.
Fig. 3.14. Time spent searching before stimulus was located. Bars represent medians; measures of variance represent 25% and 75% quantiles.
Chapter 3: Responses to a Social Stimulus

![Bar graph showing median duration (s) for Cross Ablate, Inner Ablate, Non-Ablate, and Outer Ablate.]
No significant difference was found in the average walking speed used by the various groups when locating the food source (Fig. 3.15; Kruskal-Wallis, H=2.172, df=3, P=0.538). Pairwise comparisons also revealed no significant differences between treatment groups (Dunn’s Test, P>0.05 for all comparisons).

**DISCUSSION**

Previous studies have demonstrated that the olfactory sense of decapod crustaceans is concentrated in the sensilla of the paired and bifurcated antennules (Fontaine et al., 1982; Ghiradella et al., 1968; Laverack, 1964; Schmitt & Ache, 1979; Tierney & Dunham, 1982; Tierney et al., 1984). An increase in antennule movements indicates an animal has detected a change in the surrounding chemical environment (Pearson & Olla, 1977). Dunham et al. (1997) report a pattern of antennule movements in *Cambarus bartonii* following the ejection of a sucrose stimulus. Antennule movements were observed to increase from a baseline measure following the ejection of a sucrose solution, and then decrease again after the second control solution. It is possible that a similar pattern could occur following the ejection of a social stimulus. The same pattern was, however, not observed in this study, although both the Cross and Inner Ablate groups significantly increased the performance of antennule movements after the presentation of the social stimulus. The maintained high response observed following the second control solution may be explained by the fact that decapod crustaceans are highly
Fig. 3.15. Walking speed used during searching. Bars represent medians; measures of variance represent 25% and 75% quantiles.
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Median Walking Speed (cm/s)

Cross Ablate  Inner Ablate  Non-Ablate  Outer Ablate
aggressive, will readily engage in aggressive interactions (Bovbjerg, 1953; Bovbjerg, 1956), and are also known to be cannibalistic (Hogger, 1988). The reaction of the female following the detection of a Form I male \textit{P. clarkii} may indicate the anticipation of one of two events—a possible mating opportunity, or a potential aggressive interaction. Increased “vigilance” to any change in the surrounding environment would be advantageous under these circumstances.

The frequency of certain antennule movements following the presentation of the social stimulus did not appear to have been affected by ablation. Neither the Cross nor Inner Ablates differed from the Non-Ablate group in the number of Twitches performed, a result that is consistent with observations made in experiments using food stimuli by Dunham et al. (1997). Rutherford et al. (1996) appear to have conducted the only study to date which has examined individual antennule movements during social interactions. The number of Twitches were not reported in that study, however, so the results obtained in the present study cannot be compared with their results in detail.

A significant difference was observed in the performance of SAFs, with the Outer Ablates performing fewer of these movements than the other treatment groups. This differs from results obtained by Dunham et al. (1997), although that experiment examined responses to a food stimulus rather than to the social one used here.

Cross Ablates performed an equivalent number of Twitches and SAFs to both the Inner and Non-Ablate groups. This suggests that the Cross Ablates compensated for the absence of an outer ramus, because they doubled the activity of the remaining one. The same response was observed following the ejection of a food solution (Chapter 2). This
compensatory activity may be species-typical, as no increase was observed by Dunham et al. (1997) in their study on antennular response in *C. bartonii* following exposure to a sucrose solution, or it could possibly be the result of methodological differences between the two studies.

The larger antennule movements (LAF and LAD) were seldom performed in the present experiment, the samples being too small to detect possible differences among the groups, or in pairwise comparisons. This result differs from that found by Rutherford et al. (1996), who report that the crayfish *Orconectes rusticus* performed LADs for significantly longer periods of time during social than non-social interactions. This difference may underscore the importance of vision in social interactions. Although vision is not believed to play an important role in aggressive interactions in *H. americanus* (Kaplan et al., 1993), evidence suggests that it is important in enabling the crayfish *O. rusticus* to communicate efficiently during agonistic interactions (Bruski & Dunham, 1987). Bruski & Dunham (1987) observed that visually mediated behaviours were performed less frequently when light levels were reduced. A similar change in behaviour was observed by Smith & Dunham (1990). The use of LADs as a visual display during aggressive interactions, as suggested by Rutherford et al. (1996), may have precluded their use in the present experiment, which was run under low light conditions.

An alternate explanation for the relative absence of larger antennule movements may lie in the concentration of the stimulus used. In a previous study (Chapter 2) which used a methodology almost identical to the one used here, LAFs and LADs were performed following the presentation of a solution created from the homogenising of 20g
of fish. It is conceivable that the “odour concentration” of the fish filtrate was greater than that of the socially relevant chemicals found in the social stimulus used in the present experiment. During social interactions, both animals are in close proximity, with the result that the concentration of urine released during the interaction (Breithaupt & Atema, 1993; Rutherford et al., 1996) is proportionately much greater, relative to the immediate chemical background. LAFs and LADs could therefore be a response to the presence of a high concentration of an odour, regardless of whether that odour is composed of food or social stimuli.

Crayfish in all treatment groups raised their chelae off of the gravel substrate, indicating that antennules were not required for the detection of the social stimulus. Chemosensitive sensilla located elsewhere on the body, such as on the maxillipeds (Corotto et al., 1992), walking legs (Borroni et al., 1986; Derby & Atema, 1982a; Hazlett, 1971; Hodgson, 1958), and antennae (Voigt & Atema, 1992) may have been used in place of antennular input and mediated the observed response. In addition, the Chelae Raising may be a response to the disturbance of the water surface. Experiments using a number of crustacean species have demonstrated that a number of mechanosensory hairs are located on the antennae, allowing these appendages to be used in the detection of water movements (Sandeman, 1989; Tautz et al., 1981; Tazaki, 1977; Wilkens et al., 1996; Zeil et al., 1985). The insertion of pipettes into the tank, through the air-water surface boundary, could have elicited a general “alert” response, involving elevation of the chelipeds, which was enhanced when coupled with the social stimulus.
Although the antennules may not be needed for short-distance chemoreception, the results from Experiment B suggest that they are required for chemoreception over long-distances. Whereas only 1 or 2 of the approximately 15 animals in the rami-possessing groups were unable to detect the social stimulus, almost one-third of the Total Ablates were unable to perform this action. All groups, with the exception of the Total Ablates, initiated searches at a median distance of approximately 60 cm from the source. The fact that all of the Total Ablates used in this experiment began searching at a median distance of 13.63 cm from the source indicates that the walking legs, although chemosensitive (Derby & Atema, 1982a; Derby & Atema, 1982b; Johnson et al., 1984), are only used over shorter distances. A similar result has been found in *H. americanus*, where the walking legs play a subordinate role in search path control (Devine & Atema, 1982), but become increasingly involved the closer the animal is to the source of the stimulus (Moore et al., 1991b).

Other results obtained in this study both support and contradict the findings of previous research. As in Moore et al. (1991b), no typical search pattern was used by any of the treatment groups. In general, no significant differences were observed either among or between treatment groups in the latency to search, the distance from the source at which searching began, meander ratio, or in average walking speed.

The observation that all of the crayfish which possessed rami were able to detect the social stimulus contradicts previous research. Ameyaw-Akumfi & Hazlett (1975) reported that the inner ramus was the site of reception, and that the ablation of this organ precluded responses to social stimuli. Dunham & Oh (1992) observed the opposite
effect, and report that the outer ramus is used for sex discrimination. The results from these two studies, if applied to the present study, indicate that either the Inner or the Outer Ablate group, but not both, should have performed detection behaviours. However, both of the previous experiments examined sex discrimination, a choice which was not available in the present study. The crayfish tested here may have simply detected, and subsequently located, a social stimulus which was different from the chemical background.

Differences can be found in the results obtained in this experiment, and in those which used food, instead of social stimuli. Cross Ablates in this study began searching at a greater distance from the source than Outer or Non-Ablates. Comparisons of other measures, however, show that the Cross Ablates were no different than the Inner, Outer or Non-Ablates in the latency to search, the meander ratio, or average walking speed. The Cross Ablates used in a previous study (Chapter 2) were not significantly different from Non-Ablates in any measure, except in the latency to the performance of substrate searching behaviours. Devine & Atema (1982) found that their class of Cross Ablates (created by examining the responses of *H. americanus* following the ablation of one outer or one inner ramus on one side, and then again after the contralateral ramus was removed) were no different in the latency to alert responses, but had longer search path lengths than controls. This last measure cannot be directly compared to the meander ratios calculated in the present study, as the most direct distance between start and end points was not reported in their study.
In Chapter 2, the Outer Ablates differed the most from other treatment groups. Only half of the animals tested were able to locate the food source, with successful animals requiring a longer period of time, and having a slower walking speed. A similar result was found by Reeder & Ache (1980), who observed that the spiny lobster *Panulirus argus* needed a longer search time to successfully locate a food stimulus. In the present study, Outer Ablates experienced no such disadvantage. Only 3 of the 13 crayfish which detected the social stimulus were unable to locate its source. There was also no difference between Outer Ablates and other treatment groups in the latency to search, meander ratio, or in the average walking speed.

Inner Ablates were also not significantly different from the other treatment groups. A similar result was found in Chapter 2, as well as in studies by Devine & Atema (1982) and Reeder & Ache (1980).

The results of this experiment demonstrate that the inner rami are used by *P. clarkii* in the localisation of social stimuli. It does not appear, however, that this is the only organ by which crayfish localise social stimuli, as animals possessing only outer rami were equally successful. These results may indicate that the organs used to localise the source of social odours are doubly redundant, in that both types of rami can be used to locate social odours, and that crayfish possess two inner and two outer rami. This may be advantageous to crayfish, which can suffer damage to their antennules.
CHAPTER 4

General Discussion
Chemical stimuli are used by a number of animals to identify important objects in the surrounding environment. Decapod crustaceans rely heavily on chemical information, and are able to detect practically every class of biological molecule (Rittschof, 1992). Mellon et al. (1992) have reported that 30 to 40% of crayfish brain volume is devoted to the processing of olfactory input. Studies which have examined crustacean sensory biology have determined that chemical stimuli are used by decapod crustaceans to find food (Weissburg & Zimmer-Faust, 1994), in aggressive interactions (Karavanich & Atema, 1991; Karavanich & Atema, 1993), and in the discrimination between sexes and species (Dunham & Oh, 1992; Dunham & Oh, 1996; Tierney & Dunham, 1982).

Chemoreceptors are found on every cephalothoracic appendage of a decapod crustacean (Derby, 1982). The walking legs, which are used in a variety of feeding activities, have chemoreceptors housed within thick hairs (Altner et al., 1983; Derby, 1989; Derby & Atema, 1982a; Derby & Atema, 1982b). Antennae possess both mechanoreceptors and chemoreceptors (Altner & Prillinger, 1980; Tautz et al., 1981; Tazaki, 1977; Voigt & Atema, 1992; Wilkens et al., 1996; Zeil et al., 1985). Chemoreceptors found on the maxillipeds appear to determine whether a substance will be ingested (Corotto et al., 1992; Lavalli & Factor, 1995).

The antennules of crustaceans have received the most attention thus far. Known to be used in long-distance chemoreception (Ache, 1975; Devine & Atema, 1982; Moore et al., 1991b), and sensitive to specific chemicals (e.g. Johnson & Atema, 1983; Tierney et al., 1988), each antennule is bifurcated, with one inner (medial) and one outer (lateral) ramus. Tierney et al. (1984; 1986) found that the two types of rami differed...
morphologically, with aesthetasc hairs present on the outer ramus but not on the more slender inner ramus. Flicking of the antennules increases their exposure to the surrounding chemical environment, and is believed to enhance their chemosensory function (Schmitt & Ache, 1979).

The role played by the two types of rami has been controversial, with contradictory results obtained in different studies. Flaws in experimental design, however, may have masked the role played by each ramus in the detection and localisation of chemical stimuli. A summary of these experiments is found in Table 1.2.

One problem with the design of previous experiments has been with the size of the tank used in testing. Moore et al. (1991b) tested the American lobster *Homarus americanus* in a tank measuring 2.5m long, and found that the walking legs became more important in the localisation of food substances when the animal was within 22 cm of the stimulus. In both of the experiments performed in the present study, the vast majority of crayfish lacking any rami (Total Ablates) initiated searches within 22 cm of the stimulus source. These results indicate that tanks with large dimensions are needed if the long-distance chemosensory abilities of crustaceans are to be properly tested. This has not been the case in many of the studies examining this issue.

Previous studies have also not used complete ablation procedures. Ameyaw-Akumfi & Hazlett (1975), for example, ablated the inner rami, but do not report any ablation of the outer rami. Devine & Atema (1982) ablated only the rami from one side, so that test animals still possessed one inner and one outer ramus. Neither Ameyaw-Akumfi (1977) nor Derby & Atema (1982b) investigated the individual types of rami.
separately, but rather ablated or manipulated the entire antennular structure. These incomplete ablations may have prevented the detection of any differential function in the two types of rami.

Detection and localisation have not been separated in other studies (e.g. Oh & Dunham, 1991; Tierney et al., 1984). These two behaviours should be examined separately, as it might not always be clear which ability has been impaired. Animals which do not appear to be able to localise the source of a chemical odour may still be able to detect it. Similarly, the animal’s ability to localise may still be present, but this response has not been “activated”, because the stimulus has not been detected.

The present study examined the role of the inner rami in mediating responses to distant chemical information by *Procambarus clarkii*. To counter the methodological issues of previous studies: (1) a complete series of ablations was performed; (2) detection and localisation were examined separately; and (3) the ability to locate the source of the stimulus was tested in a tank large enough to distinguish between long- and short-distance localisation.

The responses performed by *P. clarkii* following the detection of a chemical stimulus were examined in Chapters 2 and 3 (Experiment A in each chapter). Localisation of the source could not be examined because of the small size of the testing area, and also because the stimulus source, having been ejected from a pipette, was not constantly present. In a study using *Cambarus bartonii*, Dunham et al. (1997) observed a particular pattern of antennule movements following a change in the surrounding chemical environment. Antennule movements were observed to increase from a baseline measure
following the ejection of a sucrose solution, and then decrease after the presentation of a control solution. This pattern was observed for all treatment groups in that study (except for Total Ablates, which could not be tested). No consistent pattern was observed in either of the detection experiments performed in the present study.

Methodological differences between Dunham et al. (1997) and the present experiments may explain the different results obtained. Dunham et al. (1997) used Form I male *C. bartonii*, and standardised motivation by ejecting the sucrose solution over their antennules only after the test animals were interacting with another crayfish. The experiments performed in the present study used females, did not standardise motivation, and used either a social stimulus, or a filtrate collected from a fish filtrate. It is also possible that some of the differences can be attributed to the different species used in both studies. Cross Ablates in the present study appeared to compensate for the absence of one outer ramus by increasing the number of Twitches performed by the remaining one. This response was not observed by Dunham et al. (1997), in *C. bartonii*.

Also of interest is the near absence of the larger antennule movements (LAF and LAD) following the presentation of social stimuli. Both of these movements were performed after the ejection of the fish filtrate. Rutherford et al. (1996) have reported that the crayfish *Orconectes rusticus* performed LADs for significantly longer periods of time during social interactions, in comparison with non-social periods. Bruski & Dunham (1987) observed that visually mediated behaviours are performed less frequently by *O. rusticus* when light levels are reduced. If LADs are a visual display, as suggested by
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Rutherford et al. (1996), then the low light levels used in this experiment may have inhibited their performance.

An alternate explanation for the differences found in large antennule movements may lie with the concentrations of odour used in the two experiments. The fish filtrate used in Chapter 2 may have had a much higher "odour concentration" than the social stimulus, and perhaps elicited a greater response. Further study is needed to determine if LADs and LAFs are performed as visual displays, or if they are also a response to concentration differences of detected chemical stimuli.

The second part of each chapter examined the ability of the different treatment groups to locate the source of a stimulus. The testing tank used was large enough to distinguish between long- and short-distance chemoreception. Total Ablates in both experiments were the most disadvantaged, as those which detected the stimulus began searching behaviours, or searched and subsequently located the stimulus, from distances less than 10.22 cm and 13.63 cm from the food and social stimuli respectively. These results indicate that antennules are required for long-distance chemoreception, but other chemosensitive organs, such as the walking legs, can be used over shorter distances. A similar result was observed in the lobster H. americanus by Moore et al. (1991b).

Observations made in Chapter 2 indicate the importance of the outer rami in locating the source of a food stimulus. Cross, Inner and Non-Ablates were very similar in the number of animals which successfully located the food source, in the latency to the performance of substrate digging, in meander ratio, search duration, and average walking speed. Only 7 of the 15 Outer Ablates were able to find the food source, and these
successful animals took longer, and walked at a slower speed. These results suggest that the outer rami are likely the primary olfactory organ by which *P. clarkii* locates food sources. The inner rami can also be used for this purpose, but the large number of animals which possessed these rami and did not successfully locate the stimulus source suggests they are not the primary organ used for this purpose.

Chapter 3 presents the results of experiments using a social stimulus created by collecting water from a jar holding a Form I male *P. clarkii*. Cross, Inner, Outer and Non-Ablates were similar in the latency to search, the distance from the source at which searching began, meander ratio, and walking speed. These results demonstrate that the inner rami can be used in the localisation of social stimuli. However, it is clear that the localisation of these stimuli is not performed exclusively by the inner rami, as animals with only the outer rami were just as likely to find the stimulus source, and did not differ from the other groups in any of the measured criteria. It is likely that there is redundancy between the inner and outer rami for the detection of social stimuli.

Further study needs to be done on the morphology of the inner rami. Previous studies have focused primarily on the outer rami (Fontaine et al., 1982; Ghiradella et al., 1968; Gleeson, 1982; Gleeson et al., 1996; Grunert & Ache, 1988; Johansson et al., 1996; Spencer, 1986; Tierney et al., 1986), while the inner rami, despite being chemosensitive (Tierney et al., 1988), have been largely ignored. The presence of aesthetasc hairs on the outer rami (Tierney et al., 1986), but not on the inner rami (Oh & Dunham, 1991), may explain the interest given to the one type of ramius over the other. However, the results of the present study, showing that the inner rami are used in the localisation of social
stimuli, indicates that a more detailed examination of the inner ramus should be conducted. In addition to morphological studies, physiological studies should be conducted to determine if chemoreceptor cells on the inner ramus are tuned for chemicals specifically found in social stimuli. Tierney et al. (1988) found the responses of these cells was greatest to hydroxyproline, taurine, and arginine. However, the use of these compounds in possible crustacean pheromones and other social stimuli has yet to be determined.

Previous studies have attempted to examine the functional roles of the antennular rami. Flaws in experimental design, and perhaps interspecific differences, have resulted in contradictory results, with certain abilities demonstrated in some studies but not in others. As a result, there has been no clear understanding of the roles played by the different types of rami. The use of a large testing tank in this study, complete ablation treatments, and the separation of detection from localisation, have helped to clearly demonstrate that the inner ramus can be used for the localisation of social stimuli. Further study needs to be done to determine the morphological and physiological features of the inner rami which make this ability possible.
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IMAGE EVALUATION
TEST TARGET (QA-3)

150mm
6"