Chemoattraction and the Development of an Artificial Bait for the Western Rock Lobster (*Panulirus cygnus*)

Associate Professor Emilio Ghisalberti

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2000/255 Chemoattraction and the development of an artificial bait for the western rock lobster (*Panulirus cygnus*)

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OBJECTIVES:

1. To contribute to the development of an efficient and cost-effective artificial bait for western rock lobsters within the context of an understanding of chemical communication within the species and the response of lobsters to external chemical stimuli.
2. To undertake and collate the results of searches of the recent scientific and patent literature in relation to the development of artificial bait for the western rock lobster.
3. To isolate and identify attractant compounds present in normal commercial rock lobsters bait.
4. To formulate and conduct trials to test the efficacy of identified attractants for western rock lobsters.

NON TECHNICAL SUMMARY:

OUTCOMES ACHIEVED TO DATE

A bioassay to measure the effect of different compounds on the western rock lobster has been developed. Using this, it has been possible to compare the relative attractant ability of some commercial baits. Abalone was the most effective bait. Chemical analysis has allowed the identification of some of the compounds contributing to the attractant activity of abalone. Of these, pyruvic acid, proline, taurine betaine and ATP appear to be the most active. Preliminary tests on mixtures containing some of these compounds revealed one that stimulated locomotion of the lobster as rapidly as abalone, but less efficiently. Overall, the results indicate that the development of effective artificial baits can now be monitored systematically.

The western rock lobster fishery is the largest single-species fishery in Australia and is worth approximately A$ 370 million/annum. The catch has varied between 8,000 and 14,500 tonnes.

Lobsters and crabs have been caught with baited pots for centuries. Traditionally, for the western rock lobster the bait has consisted of waste fish or parts of fish, such as herring, salmon head, and the discarded parts of animal carcasses, e.g. cattle hide and hocks. From both an economic and an ecological perspective, the development of suitable artificial baits is desirable. Throughout the years, many attempts have been made to produce an effective artificial bait for

(chemical signals) to identify and orient towards potential prey, but the nature of these compounds has not been determined.

In the present study, attempts have been made to identify the number and type of such compounds. A successful outcome would identify a compound, or mixture of compounds, that could be incorporated into a slow release matrix to provide an efficient artificial bait. Other desirable characteristics include low cost of preparation, long shelf-life and specificity of action.

The approach taken in this study was to identify which of the recognised baits showed the most potent activity in attracting lobsters. To determine this, a reliable assay to measure the relative attractant activity of different baits was developed, and significant information on the physiological and behavioural aspects of lobster detection of food was obtained.

From the experiments undertaken, it became clear that abalone was the most effective bait for lobsters. A detailed analysis of a leachate from abalone muscle showed that it also had chemoattractant activity and that it contained a mixture of amino acids and other small molecular weight compounds, most of which are common in tissues of aquatic animals. Of these, pyruvic acid, proline and taurine betaine hydrochloride were found to be the most active and were considered to be good candidates for inclusion into a mixture that might be the prototype of an artificial bait.

A number of mixtures containing different combinations of the compounds with chemoattractant activity were also tested. None of the synthetic mixtures tested showed the level of attractant ability of either abalone or the leachate from it. It is interesting to note that a mixture containing glycine betaine, lactic acid, phenylalanine, taurine and tryptophan induced locomotion more quickly than the abalone leachate, but with a lower percentage response.

The inability to mimic the chemoattractant ability of abalone muscle to lobsters is not unexpected given the limited number of tests conducted. There are a number of possible explanations to rationalise this. The simplest is that the selection of compounds chosen is incomplete and/or does not contain the appropriate relative ratio of compounds. In the present work, attempts have also been made to determine the effect of different oils on the chemoattractant activity of artificial mixtures. While there were some indications of a positive effect, these were not consistent enough to warrant further studies. On the other hand, the non-eugenol portion of clove oil is worthwhile investigating as the basis for an attractant.

All the objectives have been addressed and have been achieved to a greater or lesser extent. The major factors limiting the number of positive outcomes were the labour-intensive nature of the bioassay, the number of animals required to obtain reasonable statistics, and the chemical complexity of the leachates from commercial baits.

In conclusion, it appears that the development of an efficient bait for the rock lobster requires more information on the sensitivity and specificity of the appropriate chemoreceptors

Background:

Current status of commercial bait for lobster fishery

The western rock lobster fishery is the largest single-species fishery in Australia and is worth an ~ A$ 370 million/annum. The catch has varied between 8,000 and 14,500 tonnes per year. The 1999/2000 season produced a record catch of 14,500 tonnes. This fishery operates between Shark Bay and Cape Leeuwin spreading up to 60 km off the coast. The western rock lobster fishery is based upon lobsters of both sexes entering baited traps (pots). Traditionally, the bait has consisted of waste fish or parts of fish, such as herring, salmon head, and the discarded parts of animal carcasses, e.g. cattle hide and hocks. A total ban on the use of hide bait in both recreational and commercial rock lobster fisheries came into force in the 2001/2002 season. Since the supply of local fish used by the industry has decreased, the western rock lobster fishery is heavily dependent on imported fish baits which comprise 65 to 70% of bait used and are mainly from New Zealand, the Netherlands and Scotland. The total quantity of bait used in the commercial fishery is estimated to be about 15,000 tonnes at a cost of A$ 20 million for the 2001/2002 season. Although the risk of introducing an exotic disease from the importation of frozen fish used for bait in Western Australia is virtually non-existent (Jones and Gibson, 1997), one cannot discount the possibility of a disease risk associated with the importation of frozen fish baits. The reliance upon imported fish may be problematical for the rock lobster industry if a change in Australian quarantine policy were to restrict supply. In addition, significant quantities of recreational fin fish species are used as bait in the rock lobster fishery, leading to environmental concern with the high exploitation rate on these stocks.

Requirement for effective bait in lobster fishery

Although lobsters and crabs have been caught with baited pots for centuries, progress on the chemical basis of food detection and the development of reliable baits has been slow. Use of artificial bait for lobster pots is an attractive alternative for lobster fishermen because of the high cost and limited shelf life of fresh natural bait. Throughout the years, many attempts have been made to produce an effective artificial bait for the western rock lobster (Appendix 2). Generally speaking, these attempts have been unsuccessful. There is, however, a need to develop a cost-effective and safe alternative bait to the fish/animal material. Such bait should have a long storage life, be pathogen-free and water stable. Ideally, it should remain effective in the water for the whole period between pot-pulls (1-2 days or more) and be selective in attracting lobsters. In spite of the complexity of the marine environment, lobsters and other aquatic animals can recognise certain chemicals that guide them towards potential prey. A search for the components of an effective artificial bait should start with an animal food preferred by (attractant to) the lobster.

all baits is related to the rate of diffusion of attractant chemicals from the insoluble materials into the surrounding environment. The most important factors influencing the effectiveness of the artificial baits are the choice of matrix and the rate of diffusion of the mixture into the environment. A slow and continuous diffusion rate, mimicking the breakdown of fresh tissue, is optimal (Lee and Meyers, 1997). Daniel and Bayer (1987) have tested the leaching rate of different bait types including fresh, frozen and salted herring over 45 h. They found different leaching rate among the baits for the first 12 h, but after this all types of baits had similar low leaching rates. The efficacy of a bait also diminishes as tissues decompose and bacterial degradation may give rise to products which have lower or no attractant activity (Trott, 1999; McLeese,1973; 1974).

Research on the chemoattractants of crustaceans

Despite of the chemical complexity of aquatic environments, decapod crustaceans, such as lobsters, crayfishes and crabs, utilise water-borne chemical signals to detect potential prey, to escape predators and to locate mates (Ryan, 1966; Rittschof, 1992). In recent years, much has been learnt on how crustaceans locate sources of odour and on the role of multiple sensory appendages (Grasso and Basil, 2002). Considerable information is now available on the many types of biological responses to chemical stimuli (Zimmer-Faust and Butman, 2000) that are postulated to play a role in mediating the various stages from initial detection or orientation to sustained feeding. The exterior surface of crustaceans is covered by a diversity of sensory hairs with chemosensory and/or mechanosensory properties. Chemoreceptor cells are mainly located on the flagella of the antennules, the dactyls of the pereiopods and the mandibles. Although there is disagreement on the distribution of these chemoreceptors, it seems clear that there are at least two types. The first are highly specific distance chemoreceptors approximating a sense of smell, and a second are contact chemoreceptors that control food handling and ingestion (taste).

One of the behaviours activated in lobsters by water-borne chemical stimuli is ‘antennule flicking’. This is a stereotypical activity that involves movement of antennules in a rapid downward (and then upward) movement and is followed by wiping the antennules through the maxillipeds, the feeding appendages located near the mouth (Lee and Meyers, 1997). During flicking, the boundary layer surrounding the antennule narrows, shortening the distance between the sensor and the ambient fluid (Grasso and Basil, 2002). It is interesting to note that a much more compound specific behaviour has been observed as a response to glutamate (Barbato and Daniel, 1997). Lobsters with two antennules also can determine the direction of the smell by comparing the difference in concentrations between the two antennules. Their food-finding ability includes detection, identification and discrimination of natural food odours above natural background levels, and finally locomotion toward the food sources (Zhou and Rebach, 1999).

Chemoreception is an important component of search behaviour and food detection is

chemoattraction or feeding stimulation of lobsters including the genus of spiny lobsters *Panulirus* (Lee and Meyers, 1997; Coman *et al.*, 1996). Most studies have concentrated on amino acids, such as glutamine, glycine, alanine, arginine, the sulphonic analogue of glycine, taurine, glycine betaine, and even ATP as chemoattractants (Table 1). Chemoreception is a concentration-oriented behaviour, and some chemicals can act as attractants at low concentrations, but as repellents at high concentrations. Synergistic interactions of chemoattractants have also been observed for crustaceans (Shelton and Mackie, 1971; Mackie, 1973; Coman *et al.*, 1996).

It is important to note that, in addition to chemical attractants, lobsters are also attracted by conspecific odours (Nevitt *et al*., 1995; Nevitt *et al*., 2000). These serve not only as general aggregation cues, but may also communicate chemical information between individuals.

Table 1. Baits and chemicals known for their chemoattraction to spiny lobsters.

Compound or bait	Stimulus	Species	Reference
Abalone	Attractant	*P. interruptus*	Zimmer-Faust and Case, 1982, 1983
Crab, mullet, oyster and shrimp	Incitant	*P. argus*	Daniel and Derby, 1988 Fine-Levy and Derby, 1991
Glycine	Attractant	*P. interruptus*	Zimmer-Faust, 1991
Taurine	Incitant	*P. argus*	Olson *et al.*, 1992, 1995
AMP	Incitant	*P. argus*	Olson *et al.*, 1992, 1995
ATP	Attractant	*P. interruptus*	Zimmer-Faust, 1993
Ammonia	Repellent	*P. argus*	Zimmer-Faust, 1987

Biology and research on the Western Rock lobster (Panulirus cygnus)

P. cygnus, one of eight species of rock lobsters found off the Western Australia coast, is endemic to the south-west coast of Western Australia, occurring along the continental shelf from North West Cape to Cape Leeuwin (George *et al.*, 1979). These lobsters detect foods by the many different types of sensory hairs (setae) that project through the exoskeleton on the antennae, antennules, mouthparts and last segments (dactyls) of the walking legs. The antennules, each with hundreds of thousands of receptor cells, are thought to be responsible for reception at low concentration and so would be useful for sensing food at a distance. The receptors of the legs and mouthparts are responsive only to higher concentrations and respond when closer to the food source, determining the exact nature and quality of the food encountered and selecting precisely which food items to eat.

In their diet and foraging activity, they are ‘opportunistic’, searching for a wide variety of small live prey and scavenging dead remains when opportunities arise (Joll and Phillips, 1984).

declines during the night and increases just before dawn. There is minimal activity immediately after dawn, and little during the day (Jernakoff, 1987). The first and second legs are used to search and probe the top centimetre of the sediment of the seagrass beds or the reef surface where an abundance of small animals live. Slow-moving or sedentary bottom-dwelling invertebrates such as worms, small molluscs and crustaceans are selected. The strong crushing jaws of the mandibles are capable of gnawing into the hard shells of most molluscs and worm tubes.

Little information is available on the chemoattraction of western rock lobster. However, a previous study by Dr. Kagi, Curtin University (unpublished data) presented some preliminary information on the response of lobsters to small molecular weight acids and amino acids. Other FRDC projects (such as 96/337, 99/372) have also carried out trial-and-error based tests with cheap proteins containing fish and animal offal. One of these studies (FRDC 99/372; Caputi *et al.*, 1999) indicated that the catch rates for undersize, legal size and combined rock lobsters, for all of the artificial baits alone, were significantly lower (26%) than the catch rates for the two control baits of fish alone and the fish plus cowhide bait. This strongly suggests that the attractant bait was the fish, not the artificial baits. Hence the artificial baits do not appear suitable as stand alone baits without further development.

Aim

This project proposed further study for the development of artificial bait through a study of the fundamental chemistry and biochemistry of bait attractiveness in rock lobsters. This included identification of the attractive and stimulatory components of food.

Need:

The need for artificial baits for the western rock lobsters industry is increasing. Western rock lobsters fisheries consumed 15,000 tonnes of frozen imported baits worth almost A$ 20 million in the 2000/2001 season. This constitutes about 70% of the rock lobster bait market and, thus, the industry is very vulnerable to quarantine policy changes which might restrict bait supply from overseas. Unlike frozen bait that requires high maintenance, artificial bait with long shelf life would contribute considerable benefits to the operation of the lobster industry. Furthermore, a total ban on the use of hide bait in both recreational and commercial rock lobster fisheries has placed more pressure on the development of an artificial bait. It is now clear that a range of compounds act as feeding attractant to marine crustaceans and it is likely that this applies to the western rock lobsters. Identification of these substances could lead to the application of this information in lobster fisheries across Australia and in other countries. In addition, a cost effective

Objectives:

- To contribute to the development of an efficient and cost-effective artificial bait for western rock lobsters within the context of an understanding of chemical communication within the species and the response of lobsters to external chemical stimuli.
- To undertake and collate the results of searches of the recent scientific and patent literature in relation to the development of artificial bait for the western rock lobster.
- To isolate and identify attractant compounds present in normal commercial rock lobsters bait.
- To formulate and conduct trials to test the efficacy of identified attractants for western rock lobsters.

General Methods

All the experiments were carried out in the aquarium facilities at the Western Australia Marine Research laboratory in North Beach, WA. The basic methods used for the experiments in relation to aquarium set-up and animals used are described below.

1. Experimental aquaria

To assess the chemoactivities of test samples, two systems were assembled: a single animal assay system and an animal group assay system.

The aquarium for the single animal assay system was a modification of that used by Mackie and Shelton (1972). The test apparatus consisted of a series of 7 troughs (Fig. 1B), each 115 cm long, fabricated from plastic pipe, 25 cm diameter and with wall thickness of 0.7 mm (Fig. 1A) filled with filtered seawater (*ca*. 40 L) with a constant water flow (*ca*. 800 ml/min). A section 110 cm long and 18 cm wide was cut from the top of the pipe, leaving a section 7 cm long at the outlet end to serve as a shelter for the lobster. The troughs were enclosed with black plastic film to block outside light. Light was provided by 40W red bulbs in the middle of the troughs situated 50 cm above the water level. Plastic mesh was glued on the bottom of the trough to provide a rough surface for lobsters to grip or walk. One lobster was placed in each aquarium to acclimatise for 24 hours prior to carrying out the experiment.

The aquarium for the group animals assay system was already available at the Marine Research laboratories (Fig. 2). The test apparatus was divided into two sections. Each section, 100 cm long and 25 cm wide, was filled with filtered seawater (*ca.* 120 L) with constant water flow (*ca.* 1000 ml/min).

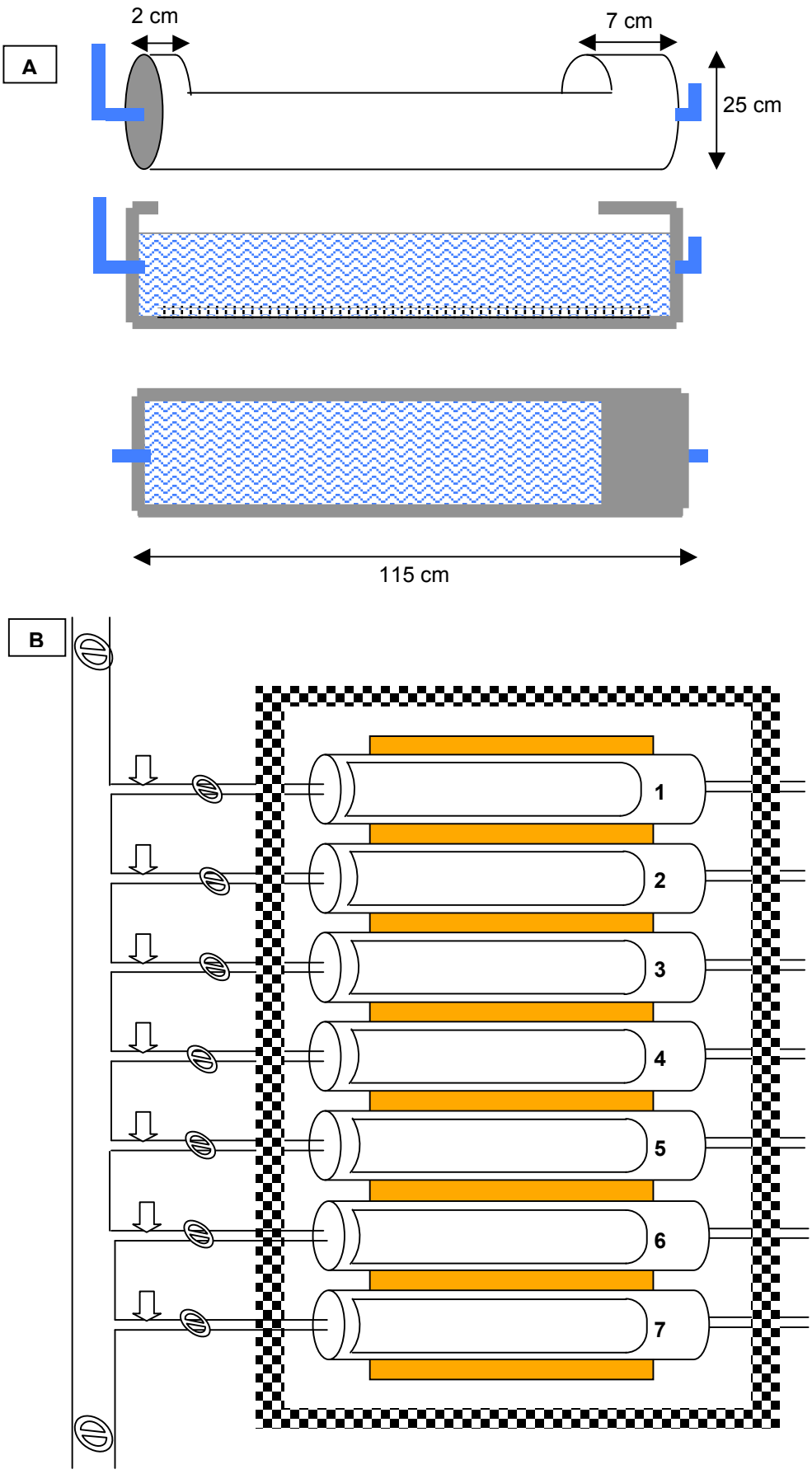


Fig. 1. Diagram of the aquaria used for the single animal assay system.

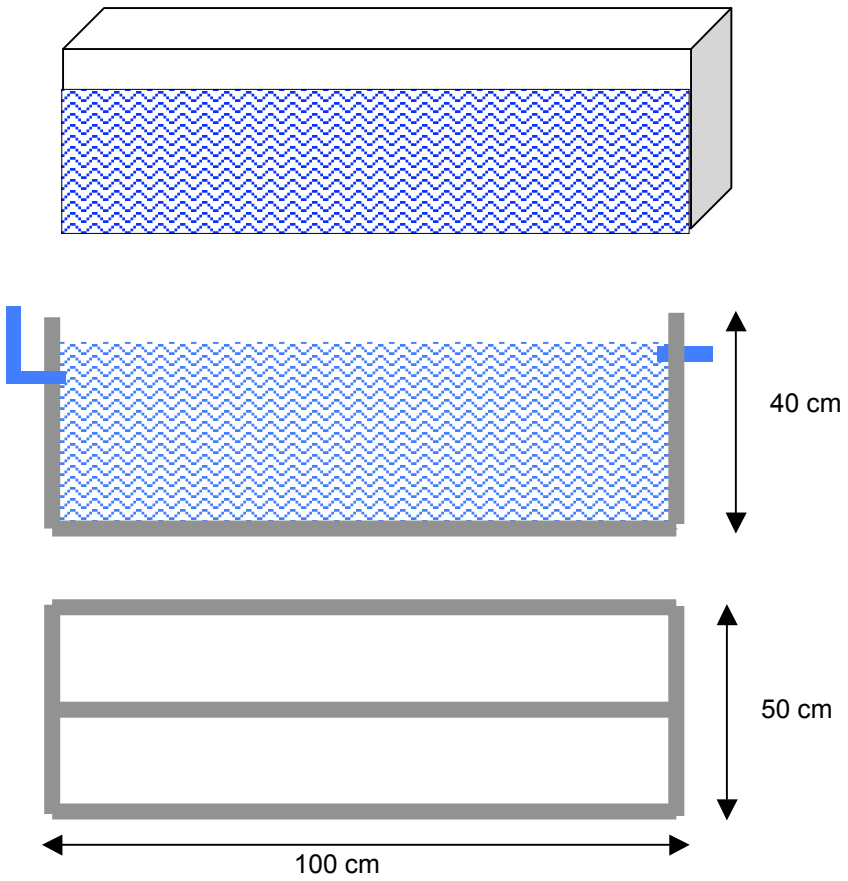


Fig. 2. Diagram of the aquarium used for the group animals assay system.

2. Western rock lobster, Panulirus cygnus

Lobsters caught locally were tagged with individual numbers after measuring their size, sex and any damage occurred on their body (examples of tagging is shown in Table 2) and were maintained in the holding tank for two weeks prior to each experiment. The animals were all below legal size; carapace length ranging between 71 mm and 75 mm. Lobsters were held in the seawater aquarium with constant flow and ambient temperature and fed on mussel every second day. All lobsters tested were held without food for 3-5 days prior to each experiment. To minimise learned behaviour effects, the same lobsters were used for three experiments not involving the same test sample or compound. Their response towards baits was dependent on the stage of their moulting cycles. To overcome this problem, 'chemically active' lobsters were selected following an initial test run.

Table 2. Example of logbook entry recording information about lobsters used.

Tag No.	Size (C L. mm)	Sex (M/F)	Damage (old)#		Damage (new) #		Note*
			antenna	leg	antenna	leg	
Y001	71.0	F	0	2	0	0	

3. Presentation of test samples to lobsters

Laboratory studies on chemoattraction have involved the use of a number of tanks or chambers, including static tanks, straight raceways with no choice options, Y-mazes or divided tanks with two choice options and circular chambers with multiple choice options. Static aquarium tanks have been used for some descriptive and quantitative behavioural studies (Lee and Meyers, 1997). Generally, the tanks are small (<20 L) and hold only one animal. For reasons presented below, we were forced to use the multiple animal static aquarium.

Solid test samples were presented in a mesh tea ball. Leachates, single compounds and artificial mixtures (solutions in seawater) were added through a burette or a syringe at the corner of the aquarium directly opposite the corner in which the animals were resting. Simple diffusion was allowed to carry the sample to the test animals. This eliminated rheotropism, the orientation of the animals towards a current and movement into it. However, the threshold for locomotion in response to a chemoattractant is several orders of magnitude greater than that of detection (Lee and Meyers, 1997). While this results in a less sensitive assay, it provides a system that allows detection and locomotion to be clearly distinguished.

4. Assessment of lobster responses

The response of the chemoattraction of lobsters was measured using various assessments: (1) detection, (2) orientation, (3) locomotion and (4) continuation. The detailed behaviour responses are listed below (Table 3). The activities of baits were determined to be attractant or repellent if lobsters moved towards or away from the test chemical or food sources, respectively. For samples that were attractant, the time taken for the lobster to reach the point at which the sample was introduced was recorded. If the lobsters did not display detection behaviour within a certain time limit (usually 10 min), the sample was recorded as inactive.

Table 3. Phases and behaviour observed

Phase	**Behavioural response**
(1) Detection	Recognition of a chemical signal by the chemoreceptors on the antennules. Antennule flick or wipe (1 flick > 1 sec)
(2) Orientation	Change in position; turning either toward or away form the test sample
(3) Locomotion	Decisive movement either toward or away from the test sample
(4) Continuation	Ingestion of food in the case of baits

tank.

5. Effects of water temperature

In the various tests conducted with lobsters, it was noticed that the response of the animals was dependent on the temperature of the seawater. At temperatures <20^{o} C, the lobsters were sluggish and relatively unresponsive, whereas between 22-25 ^{o} C they were more alert. The impact oftemperature on catchability has also been observed in the field (Morgan, 1974). This is an important consideration when comparing response time for the same test sample in different experiments. As a comparison, the mean sea water temperature at Shark Bay varies between 21.8 ^{o} C (November) and 24.3 ^{o} C (April) (Pearce *et al*.,1999), whereas at Cape Leeuwin mean temperatures of 19.2 ^{o} C (November) and 21.3 ^{o} C (April) were observed (Pearce and Pattiaratchi, 1999).

Experiment 1.

Response of western rock lobsters to various commercial baits

Introduction:

In the initial phase, the task was to develop a reliable bioassay to allow the classes of chemical stimuli to be related to the lobster's behavioural responses. It has been pointed out that of the many studies on the influence of chemical stimuli on behaviour, only a few have demonstrated chemoattraction over a distance. This is because, frequently, no clear distinction was made between chemoattraction and feeding stimulation. For the present work, the behavioural model outlined by Lee and Meyers (1997) was adopted. In this model, five phases are recognised for the animal's responses to chemical stimuli: (1) detection, when the lobster becomes aware of the chemical stimuli (excitant), (2) orientation, when the animal prepares for movement (attractant, repellent, or arrestant), (3) locomotion (attractant or repellent), (4) initiation of feeding (incitant or suppressant), (5) continuation of feeding (stimulant or deterrent). Thus, a chemoattractant is a chemical that causes an animal to orient and move towards the source. An incitant is one that triggers feeding and a stimulant causes the animal to continue feeding. Specifically, our interest focused on the first three behaviours to identify and classify different baits and other test samples.

Since no such assay had been developed before for the rock lobster, a considerable amount of time was spent observing lobster behaviour and qualitatively determining the effect of outside stimuli, e.g. light, noise, disturbance of the water surface, on the test animals that may elicit false positive or false negative responses. Factors such as period of starvation and the effect of moulting on the feeding behaviour of lobsters were also examined. Frequent replacement of animals was required to minimise the risk of learned behaviour.

Although western rock lobsters have been described as opportunistic or scavenging feeders, they show some preferences for food sources (George *et al.*, 1997). They feed on the flora and fauna (molluscs, worms, small crustacea, coralline algae, seagrass) associated with seagrass beds and reef flats.

A point of interest was to establish if different types of foods could be classified (attractant, repellent and incitant) according to the feeding behaviours elicited in lobsters and quantified under controlled conditions. To this end, a number of commercial baits were selected to test for their "attractant" effect. Other test samples (e.g. lobster blood and octopus) known to repel or not to be attractant to lobsters were also tested. The responses of the lobsters were measured by (a) increased movement of the antennules, followed by (b) movement of the lobster towards the

The aims of this experiment were (1) to examine the response of the rock lobster toward different baits and (2) to rank the effectiveness of baits tested by measuring the time of the initial response and frequency of the behaviour.

Methods:

Baits

Commercial baits and other foods were tested in this experiment. These included north-sea herring, salmon head, mackerel, mussel, and abalone. In addition, lobster blood and octopus were also used to assess their chemoactivity to lobsters. All the samples were frozen until required and thawed completely prior to use for each experiment.

Each bait (*ca*. 3 g) in a mesh tea ball (75 mm) was introduced at the corner of the aquarium opposite that where lobsters were resting. The mesh ball was used to prevent the lobsters from consuming the bait provided which might interfere with the next set of experiments.

Experimental design

Five replicates were conducted for each treatment using 3 animals in each selected at random from the holding tank. All lobsters were held without food for 3-5 days prior to experiments. To minimise learned behaviour effects, the same lobsters were used for 3 experiments, not involving the same test sample. Differences between means were examined with unpaired Student's t test.

Results and Discussion:

Surprisingly, lobsters did not respond to commercial baits and other foods sources in the single animal assay. Although these assays were carried out in the dark and with minimum disturbance, the lobsters did not respond to any bait provided when the observer was present. Various alterations were made including use of a video camera and mirrors. Videotaping was not viable as the red light provided above the aquarium was inadequate. Individual mirrors were located in front of the trough in a way that an observer was able to see a lobster's response in the mirror standing at the back of trough. This method also failed and no response from the lobsters was observed. Although other workers have carried out the single animal assay, we were unable to elicit any response under the conditions described.

In contrast, varying degrees of lobster activity were observed in the animal group assays. Of the baits and other foods selected, lobsters showed consistent preference towards abalone, even when this was presented in very small amounts (0.1 g/ 120 L). The average time taken from presentation of the abalone test sample to the animals reaching the food (point source) was 240±225 sec, with a 94% response. Mussel, herring, salmon head and mackerel, in that order,

Although octopus in nature is a predator of lobsters, dead octopus proved to have strong attractant activity to lobsters. It is well known that lobsters are repelled by dead remains of animals of the same species (Hancock, 1974). In keeping with this, lobsters were strongly repelled by lobster blood. A vigorous detection response was accompanied by the animal moving away from the test sample. On the basis of these experiment, a more detailed study of an abalone seawater leachate was undertaken as described in Experiment 2 and 3.

It is interesting to note that, regardless of the quality of different foods, the lobsters showed detection responses by flicking the antennules soon after the food source was introduced into the aquarium. On the other hand, in the case of abalone, the time interval between detection and locomotion was minimal. It follows that measuring detection response (antennule flicking or wiping) is not an appropriate method to screen for chemoattraction of lobsters since they showed detection activity even toward the repellent food sources and demonstrated indistinctive detection behaviours within a very short time frame.

An interesting observation was made in the group assays experiments. It was noted that one particular lobster in the group consistently showed dominant activity and was the first to move toward the food source. On removal of the dominant lobster, another lobster replaced the previous one after some delay. The dominant lobsters were not always the larger in size among the animals in a group. This observation stands in contrast to the statements that “aquatic invertebrates are less likely to move towards the source of a feeding attractant if a conspecific is upstream” (Pratt, 1974) and that “the presence of a feeding competitor may suppress the effect of an attractant” (Lee and Meyers, 1997).

Although no statistical data is available to support the following observation, female lobsters seemed to eat more food (mussel) than males when they were kept in a holding tank separately. Little information is available for the sexual or size differences (different developing stages) in relation to feeding behaviours and food preference. However, all the animals used in this project were just below legal size so that differences compared to legal size animals would be minimal.

Experiment 2.

Study of chemoattractant effects of different extracts of abalone

Introduction:

The detection and identification of chemoattractants or feeding stimulants of lobsters, including the genera of spiny lobsters *Panulirus*, has been the subject of considerable research (Lee and Meyers, 1997). However, as mentioned previously, many of these are marred by a loose interpretation of the behavioural response regarded as locomotion towards the food source. As a consequence, some contradictions in research findings have been reported. For example, Lee and Meyers (1997) reported that low molecular weight compounds (i.e. amino acids, nucleotides and organic acids < 1000 MW) caused the highest behavioural responses among Crustacea. In contrast, Zimmer-Faust *et al.* (1984) concluded from a chemical analysis of the seawater extract of abalone muscle that the molecular weight fraction > 1000 and < 10,000 (i.e. peptides and polypeptides) contained the principal stimulants to the spiny lobster *Panulirus interruptus*. However, in this work, locomotion was defined as "a laterally or anteriorly directed movement of the body to a distance >1/2 carapace length". On the other hand, there seems to be little doubt that the high molecular weight fraction contained compounds with incitant and feeding stimulatory activity.

Given the results obtained in Experiment 1, abalone was selected for further investigation to determine the nature of the chemoattractants released from the fresh tissue. A significant locomotive response followed the presentation of the abalone food source at a point furthest from the animal under static conditions. This was indicative of rapid diffusion of attractants from the food source. It is well known that a slow but continuous diffusion rate of chemoattractants accompanies the breakdown of fresh tissue baits. It is surprising, therefore, to find that studies aimed at the identification of chemoattractants from animal tissues most frequently utilise the total extract from the food source. We have adopted the approach of leaching the chemoattractants by simply stirring the source tissue in seawater. The aqueous solution generated, after filtration, is referred to as the extract and should contain the chemoattractants, *inter alia*.

The aim of this experiment was (1) to prepare different extracts from abalone muscle and fractionate the extract, (2) to screen the chemoactivities of these extracts towards the lobsters and (3) to examine the chemical nature of individual fractions, especially those that caused strong attraction.

Methods:

in a 1 L beaker containing 500 ml of seawater and stirred for 1 h at room temperature prior to placing in a cold room (4 °C) overnight. The aqueous portion was filtered through a filter paper (Waterman No. 1) to remove particulate material. The filtrate was frozen in small lots (50 ml) for later use. In separate experiments, it was observed that the thawed AMSE did not loose its chemoattractant activity to lobsters even after 7 days.

Fractionation of seawater extract of abalone muscle (AMSE-AE) using amberlite

Fresh AMSE (100ml) was eluted through amberlite (XAD-4: 20-60 mesh, 80 g) using a gradient from water to methanol (Fig. 3). Six fractions were eluted from the column. Each fraction was concentrated using a rotary evaporator and brought to dryness using nitrogen. For the bioassay, dry samples were dissolved in 1 % methanol in seawater. In addition, a sample containing equivalent amounts of each of the six fractions was prepared for bioassay. AMSE was used as a control.

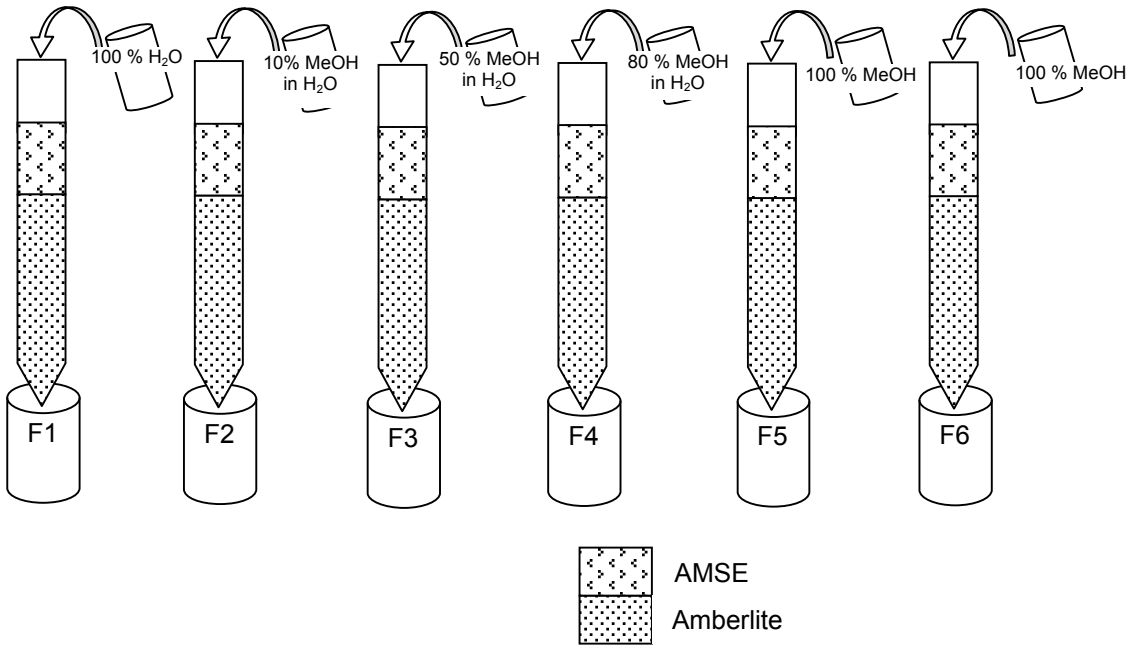


Fig. 3. Details of amberlite fractionation of AMSE.

Preparation of other extracts of abalone (AFSE and AGSE)

The preparation of abalone muscle freshwater extract (AMFE) and abalone gut seawater extract (AGSE) was as described above for AMSE, except that AMFE was prepared with fresh water and AGSE from abalone gut instead of abalone muscle.

Preparation of lipophilic extract from AMSE (AMSE-EE)

Fresh AMSE (100ml) was extracted with diethyl ether (3 x 150 ml). The organic layer was

Experimental design

All lobsters were held without food for 3-5 days prior to experiments. To minimise learned behaviour effects, the same lobsters were used for 3 experiments, not involving the same test sample. The attractant activity of leachates, AMFE, AGSE and fractions 1-6 was estimated from tests on a group of 3 animals for each sample. The number of animals used to test the other extracts were: AMSE-EE, 18 (14 responding); AMSE, 24 (18); AMSE-AM, 15 (8). Abalone muscle was tested with 14 animals (13). Test samples were injected in a single lot at a point diagonally opposite the resting lobsters in a static aquarium. The time taken for the animal to reach the point source was measured. Differences between means were examined with unpaired Student's t test.

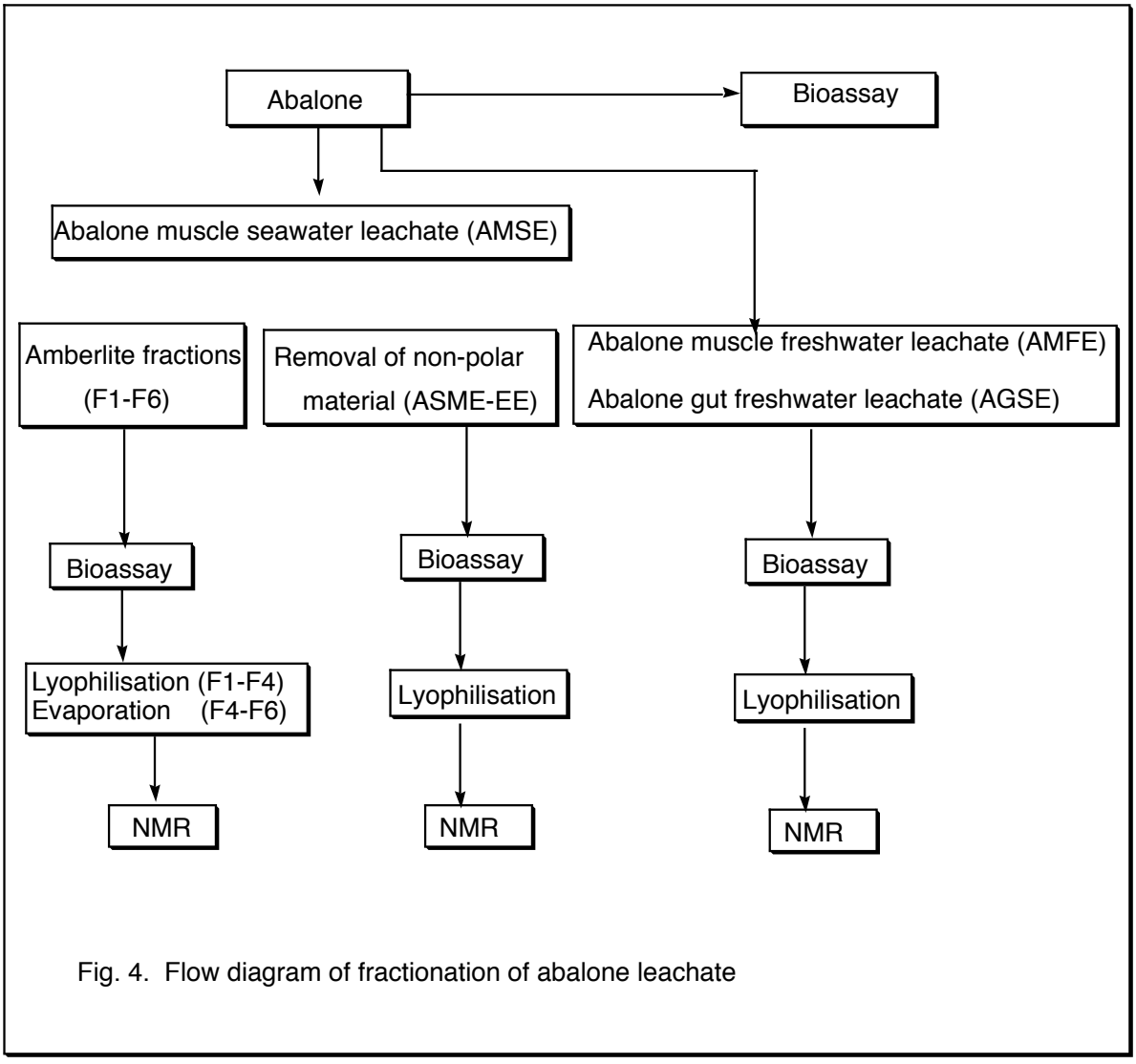


Fig. 4. Flow diagram of fractionation of abalone leachate

Nuclear Magnetic Resonance Spectroscopic Analysis of fractions of abalone leachate

Samples for Nuclear Magnetic Resonance Spectroscopy (NMR) were prepared from each of the fractions by freeze-drying fractions AMSE, AMSE-AE, AMFE, AGSE, AMSE-EE, and fractions 1 to 6 from the amberlite column. ^{1}H-NMR spectra were measured at 500 MHz with a Bruker AM-500 spectrometer, and at 600 MHz with a Bruker AM-600 spectrometer. ^{13}C-NMR

Heteronuclear Multiple Quantum Coherence (HMBC) were carried out with a Bruker AM-600 spectrometer operating at 600 MHz (^{1}H-) and 150 MHz (^{13}C-).

Results and Discussion:

From preliminary tests, leachates AMSE, AMFE and AGSE exhibited strong attractant activity to lobsters. (Table 4). Fraction 1 and 2 obtained by fractionation over amberlite also showed strong attractant activity, whereas fraction 4, 5 and 6 failed to attract lobsters. Fraction 3 caused detection activity in lobsters but failed to cause locomotion. Recombination of the six fraction from the amberlite column gave a sample that still showed attractive activity.

Table 4. Activities of individual and mixed fractions of AMSE to lobsters.

	pH	Salinity	Activity[a]
AMSE	5.3	30.8	+++
F1	8.8	11.6	++
F2	7.3	18.6	++
F3	7.8	6.6	detection
F4	8.1	0.3	inactive
F5	-	-	inactive
F6	-	-	inactive
AMSE-AM[b]	6.2	6.7	+++
AMFE			+++
AGSE			+++

[a]From semi-quantitative tests. [b]Fractions 1-6 recombined

Abalone muscle attracted 94% of the lobsters in an average time of 5 min 48 sec, whereas the other three samples, AMSE, AMSE-AM and AMSE-EE, were significantly less attractive (12-13 min) (Table 5; Fig. 5; Fig. 6). Moreover, the percentage of lobsters responding varied. AMSE-AM attracted 54 % of the lobsters, compared to AMSE and AMSE-EE which elicited 75 % and 78 % response (Fig. 7).

It is clear that none of the extracts show the potency and all inclusive attractant effect of abalone tissue. Somewhat surprisingly, the salt water leachate, after removal of the lipophilic

response, while the other fractions (3-6) failed to attract the lobsters. Fraction 3 caused a detection response in lobsters but failed to elicit locomotion.

Table 5. Responses (time) to abalone muscle and fractions from aqueous leachate.

	Abalone	AMSE-AM	AMSE-EE	AMSE
Average ± sd	5'48" ± 3.45[a]	12'29" ± 2.37[b]	12'30" ± 1.46[b]	13'40" ± 2.49[b]
Min - max	1'45" - 12'51"	8'35" - 15'30"	8'45" - 15'00"	8'08" - 16'45"

Values with different superscripts are significantly different ($P < 0.001$) from each other.

The sample obtained by recombination of fractions 1-6 (AMSE-AM), while still active, attracted fewer animals. This might be the result of a strongly lipophilic compound(s) being retained on the amberlite column.

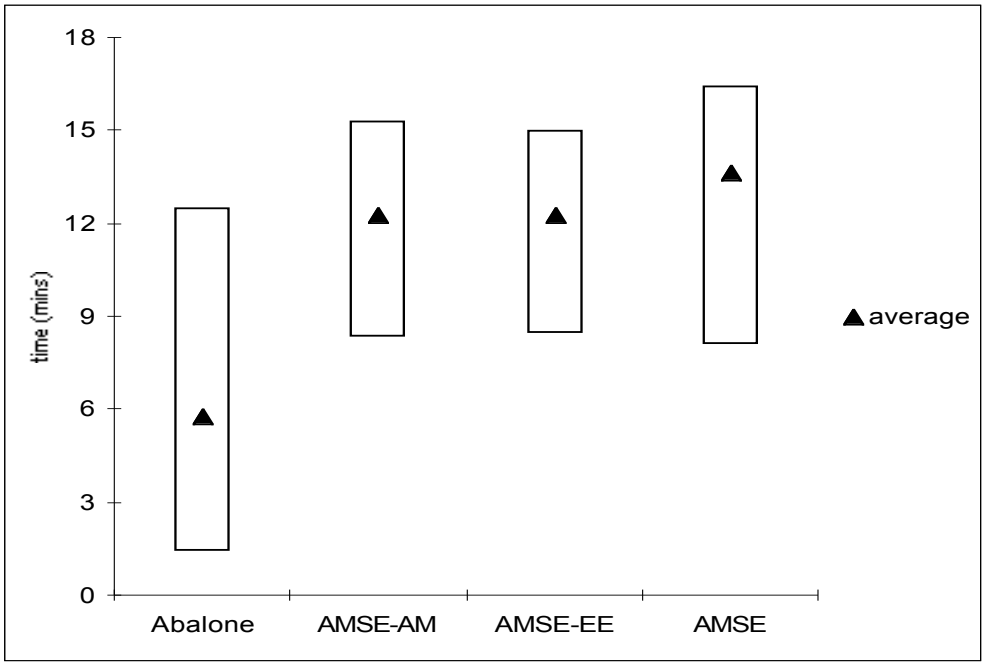
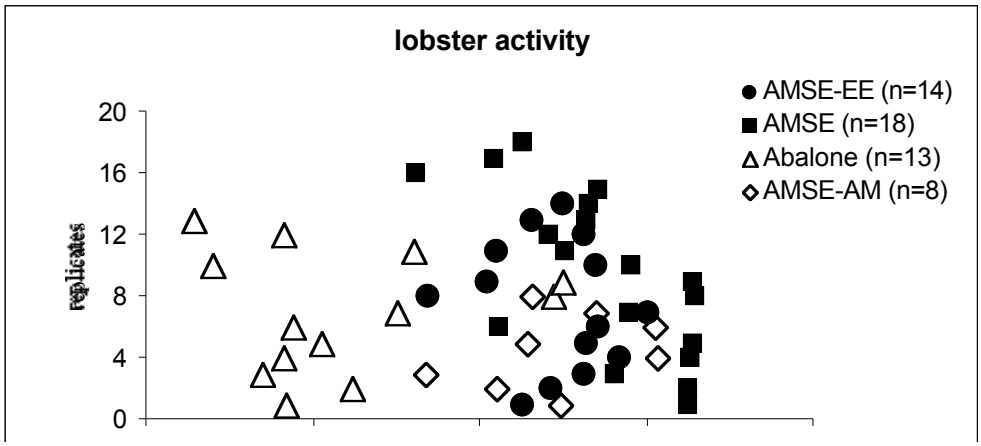


Fig. 5. Response time of lobsters to various extracts



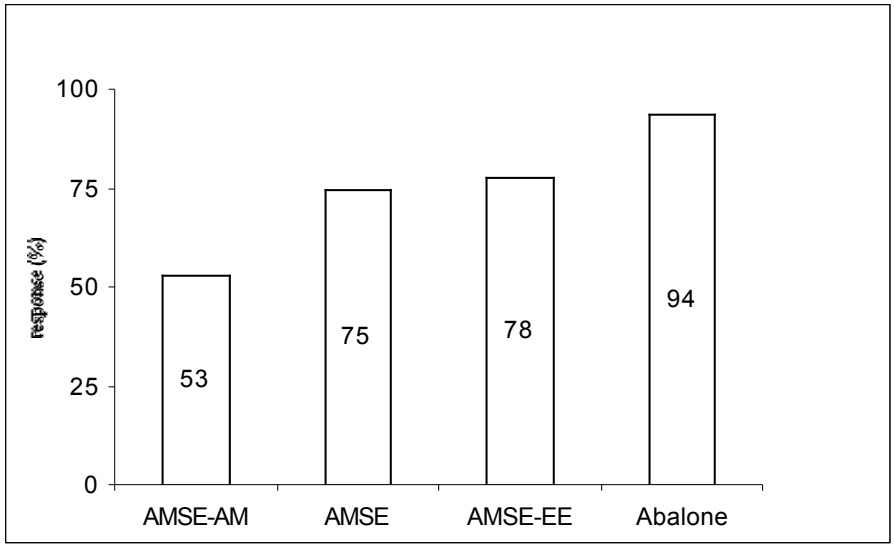


Fig.7. Percentage of lobster response towards various extracts.

Identification of components in abalone leachate

- *High Performance Liquid Chromatography*

Attempts were made to develop a method by which the components could be separated as a preliminary step towards analysis by Liquid Chromatography-Mass Spectrometry. Reversed phase HPLC and detection with an Evaporative Light Scattering Detector gave complex unresolved chromatograms. Selective detection by UV Diode array indicated the presence of a few aromatic compounds such as tryptophan, homarine and nucleosides.

(b)^{1}H- and ^{13}C-NMR Spectroscopic Analysis

^{1}H- and ^{13}C-NMR spectroscopy was used to analyse the composition of the various extracts generated. The method of metabolite profiling by one- and two-dimensional NMR analysis of complex mixtures was used. The determination of the compounds present was achieved by reference to the compilations of Tan (1996) and Blunden *et al.* (1986).

The compounds identified in AMSE are listed in Table 6, Fig. 8, together with compounds shown to be present in abalone muscle from the work of others (Konosu and Maeda, 1961; Ha *et al.*, 1982; Watanabe *et al*., 1992a,b; Watanabe *et al*., 1993). Similar analysis of AGSE clearly indicated the presence of glycine (1), arginine (2), alanine (3), taurine (4), taurine betaine (5), glycine betaine (6), lactic acid (7), succinic acid (8) and pyruvic acid (9). The aromatic amino acids, phenylalanine (10), tryptophan (11) and tyrosine (12) were evident in the NMR spectrum of AMSE, but not in that of AGSE.

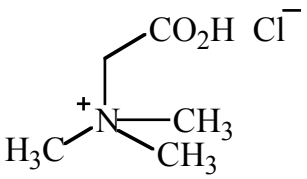
The fractions, F1-F6, derived from amberlite fractionation of AMSE provided confirmation of this. In contrast to the active F1 and F2, F3-F4 showed enrichment of the aromatic amino acids. The non-proteinogenic amino acid homarine (13) was present in AMSE but not in detectable amounts in AGSE. In the amberlite fractionation, homarine was found in fractions F3

Table 6. Compounds from abalone muscle

Amino Acids	**Amount* mg/100g**	**Compounds found in AMSE**
Glycine betaine.HCl (**1**)	975	√
Arginine (**2**)	299	√
Glycine (3)	174	√
Glutamic acid (**4**)	109	
Alanine (**5**)	98	√
Serine (**6**)	95	
Proline (**7**)	88	
Threonine (**8**)	82	
Lysine (**9**)	70	
Tyrosine (**10**)	67	√
Valine (**11**)	37	
Phenylalanine (**12**)	26	√
Leucine (**13**)	24	
Histidine (**14**)	23	
Tryptophan (**15**)	20	√
Isoleucine (**16**)	18	
Methionine (**17**)	13	
Aspartic acid (**18**)	9	
Others		
Homarine (**19**)		√
Lactic acid (**20**)		√
Pyruvic acid (**21**)		√
Taurine (**22**)	946	√
Taurine betaine HCl(**23**)		√
Trimethylamine oxide	3.2	
Trimethylamine	1.1	
AMP	90	√
ADP	12	√
ATP	√	√

*Konosu and Maeda, 1961; Ha *et al*., 1982; Watanabe *et al*., 1992a,b; Watanabe *et al*., 1993

A number of other structurally more complicated compounds have been isolated from abalone species (Hwang *et al*., 1997; Kawabata *et al*., 1978; Kobayashi *et al.*, 1986; Sato *et al.*, 1981), but the presence of these in the leachate investigated was not obvious. However, they might contribute to the chemoattractant activity of abalone to the lobsters.



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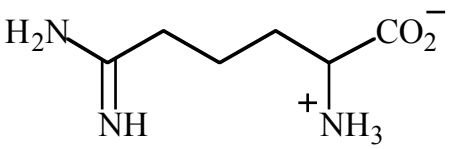

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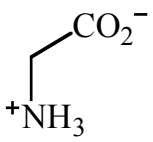

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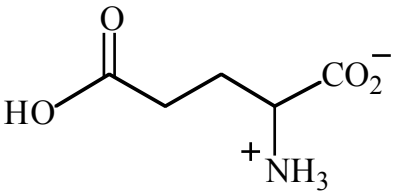

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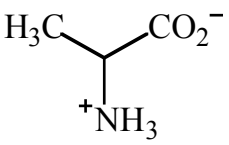

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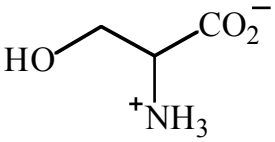

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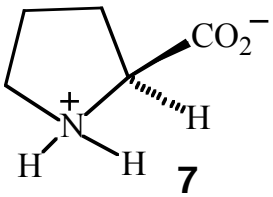

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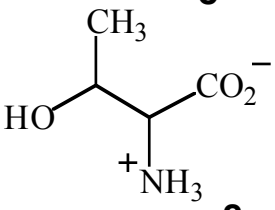

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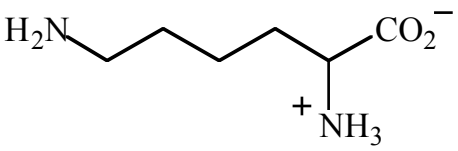

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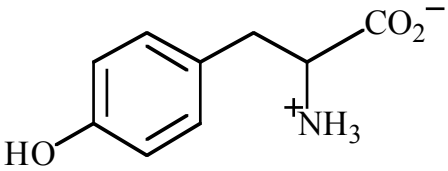

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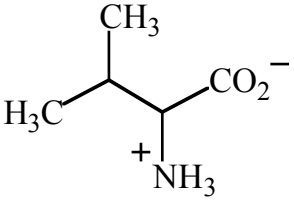

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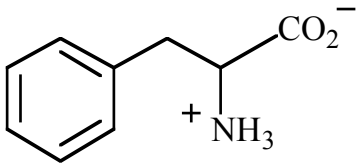

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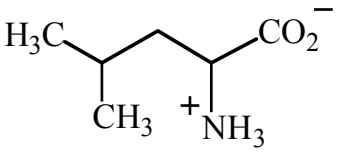

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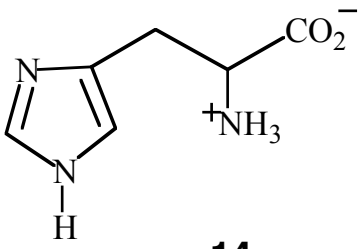

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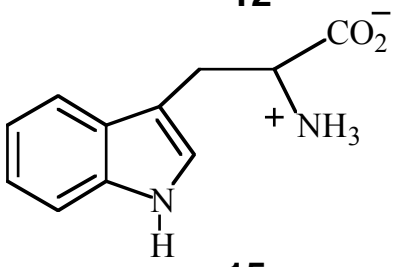

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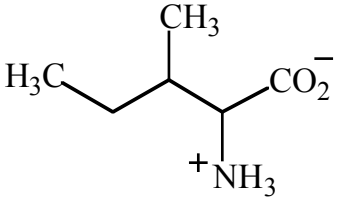

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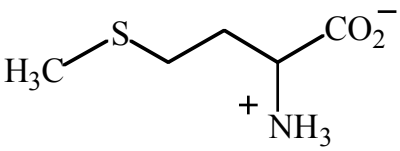

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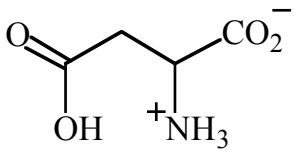

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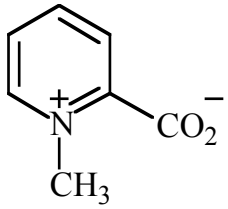

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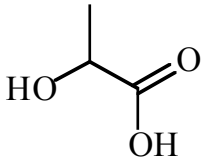

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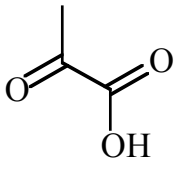

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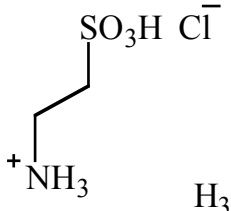

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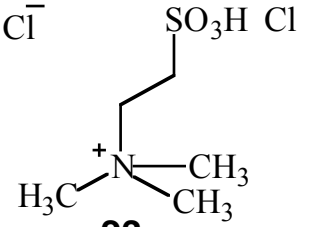

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Experiment 3.

Effect of individual compounds on western rock lobsters

Introduction:

Although abalone muscle was found to be highly attracting to the western rock lobster (Experiment 1), the leachate obtained from it did not appear to contain significant quantities of any unusual metabolite (Experiment 2). Given the scarcity of information on the chemoattractants of the lobster, it seemed important to test a number of compounds, and, in particular, those found in the abalone leachate. It is useful to mention that a previous attempt along the same lines had been made by Chemical Attractants (Australasia). In a research proposal to the Director, WA Marine Research Laboratories, Kagi (1974) disclosed some preliminary results on the effect of seawater solutions of a number of low molecular weight compounds, including many amino acids. However, it is not clear what behavioural effect was being measured. The testing procedure involved delivery of the test solution from a burette aimed "at the region between the sensory antennae and 5-10 cm in font of the feet of the animal. A very positive result was recorded when "pouncing and vigorous feeding movement" occurred, and a positive result if there was "delayed response" and "feeding movements less vigorous". An interpretation is that detection responses and incitant activity were being scored.

The aim of the present experiment was to determine the chemoattractant activity of a number of compounds to the western rock lobster. In particular, the compounds present in the abalone leachate were targeted. Table 7 lists the compounds selected and relevant information on their chemoattractant activity towards spiny lobsters.

Methods:

Experimental design

The 26 compounds tested were selected from the list of compounds identified in abalone leachate and those known to be present from the work of others (Table 6). All were of commercial grade except for taurine betaine HCl which was prepared by the method of Takemoto *et al.* (1964). Pure compounds were dissolved in seawater to obtain a stock solution of 10^{-3} M concentration. All the solutions tested were delivered by injecting 5 ml of solution of the test sample into the aquarium at the corner diagonally opposite that where the lobsters were resting. The lobsters were allowed to settle in the new environment for 1 week, and were held without food for 3-5 days before experiments. Four replicates were conducted for each treatment using groups of 3 animals. Each replicate test was conducted on separate days. The percentage response was calculated from the number of animals reaching the site at which the test sample was injected. The results were

Table 7. Compounds tested and previously reported activity towards crustaceans.

Compound	CODE	Animal tested	References
Acetic acid	**ACE**		
L-Alanine	**ALA**	*H. americanus* (+)	Carter and Steele 1982
Adenosine 5'-Monophosphate	**AMP**	*Pa. pugio* (++) *P. interruptus* (-)	Carr and Thompson 1983 Zimmer-Faust 1993
L-Arginine	**ARG**	*H. americanus* (+)	Carter and Steele 1982
Adenosine 5'-Triphosphate	**ATP**	*P. interruptus* (++)	Zimmer-Faust 1993
Glycine betaine HCl	**BET**		
Creatinine	**CRE**		
Fucose	**FUC**		
Glycine	**GLY**	*P. interruptus (+)*	Zimmer-Faust 1991
i-Inositol	**INO**		
Lactic acid	**LAC**		
L-Lysine	**LYS**	*H. americanus*	Carter and Steele 1982
L-Malic acid	**MAL**	*H. americanus* (+)	McLeese 1970; 1973
Phenylalanine	**PHE**		
L-Proline	**PRO**	*H. americanus* (++)	Carter and Steele 1982
Pyruvic acid	**PYU**		
Sarcosine	**SAR**		
Succinic acid	**SUC**	*H. americanus* (+)	McLeese 1970, 1973
Tannic acid	**TAN**	*H. americanus* (-)	Derby *et al*. 1984
Taurine	**TAU**		
Taurine betaine HCl	**T-BET**		
Tetramethylammonium Cl	**TMA**		
Trimethylamine oxide	**TRI**	*Pt. sanguinolentus*	Buch *et al.* 1991
Tryptophan	**TRY**		
Tyrosine	**TYR**		
Urea	**URE**	*Pe. merguiensis* (++)	Hindley 1975
++ Strong activity	*H. = Homarus*	*P. = Panulirus*	*Pe. = Penaeus*
+ Moderate activity	-Repellent	*Pt. = Portunus*	*Pa. = Palaemonetes*

Results and Discussion:

of the compounds.

Table 8. Attractant activity (time in sec) of individual compounds (10^{-3} M) to lobsters

Compound	Bioassay[1] time (sec)[3]	Response[2] (%)	Compound	Bioassay[1] time (sec)	Response[2] (%)
ACE	$332.5^{bc} \pm 79.4$	52	**PRO**	$276.5^{bc} \pm 64.1$	80
AMP	$592.5^{b} \pm 35.6$	50	**PYU**	$327.5^{b} \pm 2.7$	92
ARG	$421.5^{c} \pm 107.9$	75	**SAR**	$528.3^{ac} \pm 5.7$	20
ATP	$567.5^{a} \pm 52.0$	82	**SUC**	$330.0^{bc} \pm 54.8$	46
BET	$441.5^{c} \pm 69.6$	55	**TAU**	$605.0^{a} \pm 87.6$	78
LAC	$381.5^{bc} \pm 1.6$	72	**T-BET**	$455.5^{c} \pm 16.9$	80
MAL	$567.5^{a} \pm 24.6$	55	**TRY**	$670.0^{a} \pm 87.6$	57

[1] Reaction times for animals in groups of 3 (2 replicates).
[2] Percentage of lobster reaching source of compound being tested
[3] Means in the column having the same superscript are not significantly different ($p<0.001$)

Fourteen compounds elicited a positive response from the lobsters (Table 8; Fig. 9). The ANOVA revealed significant differences between the compounds ($F = 27.7$; d.f. 13, 6; $P<0.001$). The variability in the reaction of the lobsters restricted the statistical comparison to two replicates of 3 animals. The times recorded were measured starting from the injection of the sample into the tank until the lobsters had reached the site of injection of the test solution. It is interesting to note that PYU (pyruvic acid) consistently attracted the lobsters, while SAR (sarcosine) showed attracting activity only once in four repeated bioassays. The reason for this inconsistent behaviour is not clear. Since for any one compound replicates were tested on different days, one possibility is that it is due to variations in the water temperature. For each compound, the total number of lobsters reaching the source of the test sample is shown in Table 8, and the ranges in the time taken for this are presented in Fig. 9.

Of some interest is the percentage response for the compounds. Pyruvic acid showed the highest percentage response (92%), followed by ATP (82%), PRO (80%), T-BET (80%) and TAU (78%) (Table 8). The highly attracting nature of pyruvic acid does not appear to have been described before for crustaceans. ATP has been recognised as having potent attracting activity towards crustaceans in general and *Panulirus interruptus* in particular (Zimmer-Faust, 1993). This activity as a chemoattractant (40% attraction at 10^{-6} M) has been rationalised. ATP is

to the unstable ADP which is converted to AMP. This compound has been found to inhibit the chemoattractive effect of ATP and is an indicator of decaying flesh. Spiny lobsters are known to have different types of chemosensory receptors in the olfactory organ specific for ATP, ADP and AMP and it appears the combined inputs of these different receptor cell types govern the behavioural response of the lobsters. It is interesting to note that in the present work, ATP appears to be more attractive than AMP.

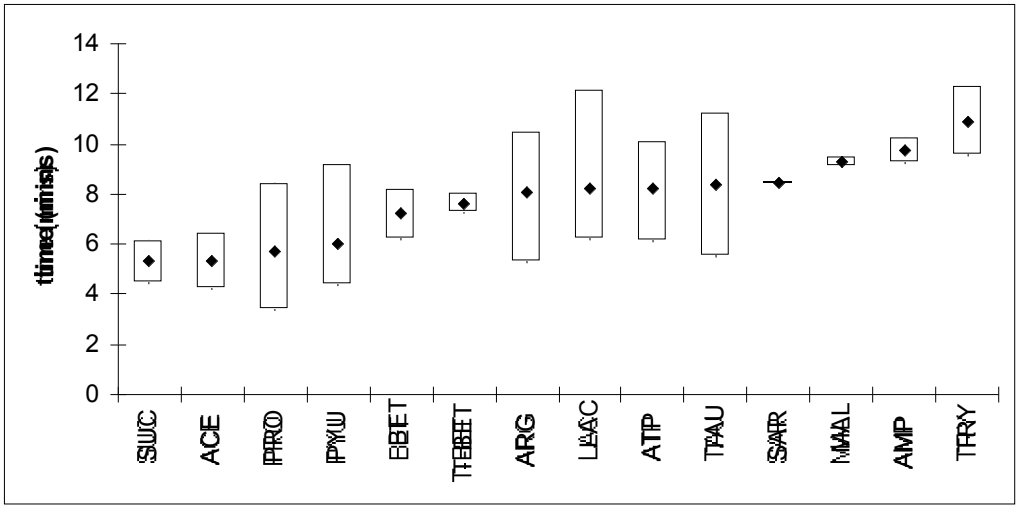


Fig. 9. Chemoattracting activity of 14 compounds

The amino acid proline has been reported as a chemoattractant towards *Homarus americanus* (Carter and Steel, 1982) and *Penaeus merguiensis* (Hindley, 1975) and, as shown here, towards *Panulirus cygnus.* Taurine betaine hydrochloride and taurine also seem to show some promise as chemoattractants. Taurine has previously been found attractive to scavenging and predatory crustaceans (Harpaz *et al*., 1987). Taurine, is the predominant amino acid released from a range of marine invertebrates and is readily leached from damaged tissues. Its detection by lobsters may signal the presence of injured or freshly dead prey.

In crustaceans, specific chemoreceptor neurons have been identified for a number of simple amino acids, including glycine, taurine, hydroxyproline, small peptides, small amines, nucleotides and pyridine (Lee and Meyers, 1997). It is worthwhile noting that complex mixtures of these compounds almost always have a stronger attractive effect than the individual compounds.

Experiment 4.

Combinatorial effect of compounds on western rock lobsters

Introduction:

The synergistic effect of different classes of compounds separated from natural prey extracts on feeding behaviour has been documented by several authors. In all cases, the natural extracts were more effective as feeding stimulants than any of the separated fractions. Fractions containing amino acids were more effective in both natural and synthetic mixtures. Spiny lobsters appear to respond more strongly to mixtures of amino acids (glycine, alanine, serine) and low molecular weight acids (succinic acid, oxalic acids) (Zimmer-Faust et al., 1984). This chemosensitivity in crustaceans probably results from the activation of many receptor sites by different compounds (Coman et al., 1996). On the other hand, the suppressive effects of some compounds has also been described. For example, amino acid receptors are inhibited by ammonium ions. Ammonium and urea have a suppressive effect on glycine and succinate receptors, reducing locomotion and antennule wiping (Zimmer-Faust *et al.*, 1984).

Based on the previous bioassays of individual compounds and the composition of the abalone leachate (AMSE), a suite of chemicals was selected to probe the effect of mixtures of compounds to the lobsters. The aim of this experiment was to test if combinations of compounds with increased chemoattraction to the lobsters could be found.

Methods:

Experimental design

Synthetic mixtures were prepared by combining 1 ml of a 10^{-3} M solution of each compound; BET, LAC, PHE, TAU, TRY, T-BET, BET, PYU, TYR, FUC. Lobsters were maintained in an aquarium for a week and fed on blue mussels. They were held without food for 3 days prior to each bioassay experiment. Lobsters were located in a tank with static seawater at 25 °C and left for at least 24 hours before the experiment. Nineteen animals were used to test each of the artificial mixtures and AMSE. Differences between means were examined with unpaired Student's t test.

Results and Discussion:

The following synthetic mixtures of compounds were tested:

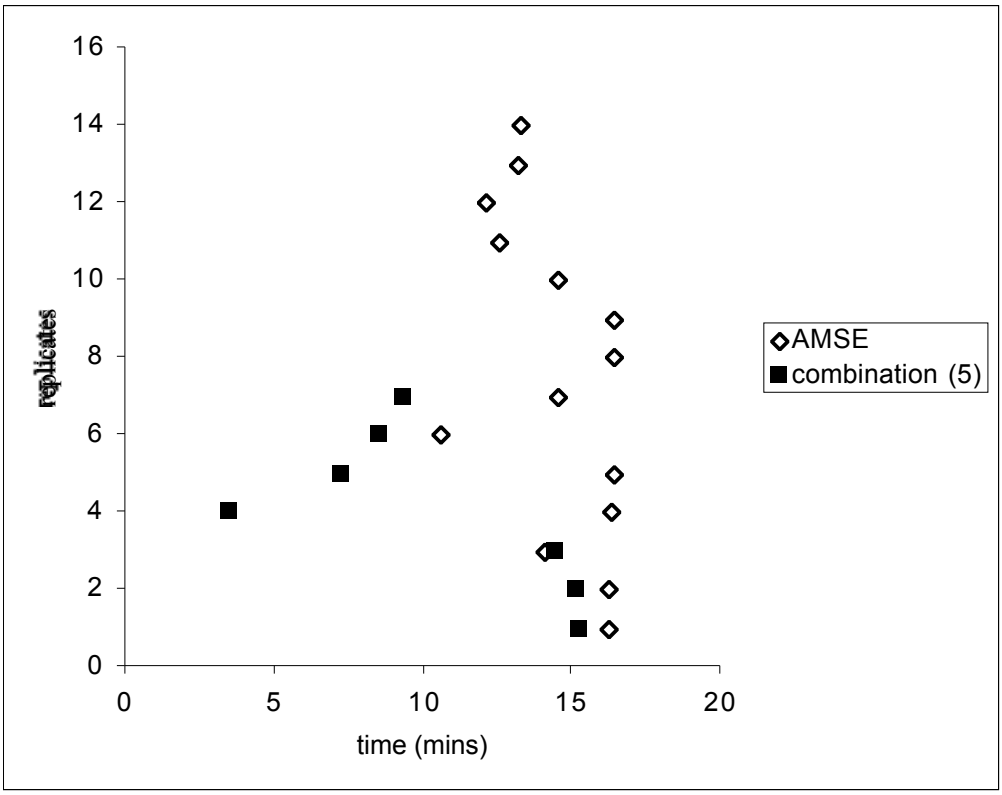
1. TRY:PHE; 2. TAU:T-BET:TRY; 3. LAC.PYU:TRY; 4. LAC:TYR:TRY;

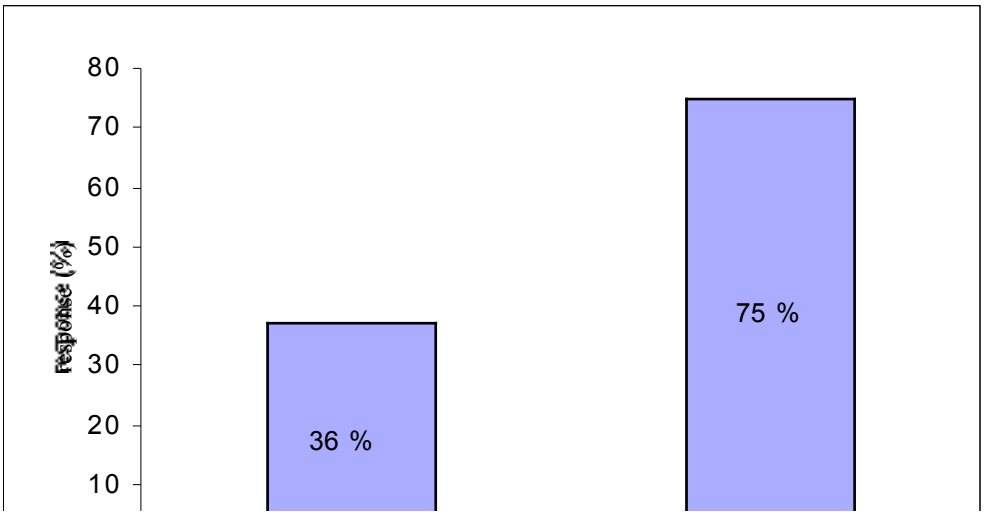
5. BET:LAC:PHE:TAU:TRY; 6. TAU:BET:LAC:PYU

7. BET:FUC:LAC:PHE:TAU:T-BET:TRY:TYR:PYU

quickly (mean 10.50 ± 4.56, n =7) than the standard AMSE (mean 14.48 ± 1.94, n =14; p< 0.01). However, the response rate to the mixture was only 36 %, compared to 75 % with AMSE (Fig. 10).

A problem often faced in determining the chemoattractant activity of pure compounds or synthetic mixtures of compounds is the fact that crustaceans desensitise to chemical stimuli and high concentrations tend to cause desensitisation. Thus, they have been found to detect and respond to low concentrations of chemicals (10^{-9}-10^{-18} M), but higher concentrations are ineffective in eliciting a response (Pittet *et al*., 1996). In an attempt to see if this effect was operating, mixture **7** was tested at concentrations between 10^{-3}-10^{-8} M but no chemoattractant activity was observed.





Experiment 5.

Effects of canola and fish oil on western rock lobsters

Introduction:

The effectiveness of baits is related to the rate of diffusion of chemoattractant from the insoluble materials into the environment. The most important factors influencing the effectiveness of the artificial baits are choice of matrix and rate of diffusion of the mixture into the environment. A slow and continuous diffusion rate, mimicking the breakdown of fresh tissue, is optimal (Lee and Meyers, 1997). Daniel and Bayer (1987) tested the leaching rate of different bait types, including fresh, frozen and salted herring, over 45 h. They found different leaching rate among the baits for the first 12 h. After this, all types of baits had similar low leaching rates and no differences in behavioural responses were noted.

Oily fishes (*e.g.* herring, salmon head, mackerel) have played an important role in the commercial baits for the lobster industry in Western Australia. Oil in the fish baits may reduce the release rates of the attractants from the baits into the environments causing a consistent source of an odour plume that attracts lobsters in the vicinity.

In the generation of extracts from abalone leachate (Experiment 2) no specific assay on the lipophilic portion had been carried out. To test if the addition of a lipophilic component significantly affected the chemoactivities of a number of extracts, canola and fish oil were used as amendments.

Methods:

Preparation of seawater extract of abalone muscle (AMSE)

As previously described for Experiment 2. To determine if volatile compounds were involved, a sample of AMSE was freeze dried (AMSEFD) and its activity was tested for comparison to that of AMSE.

Ether extraction (EE) and AMSE after ether extraction (AMSE-EE)

As previously described for Experiment 2.

Test samples

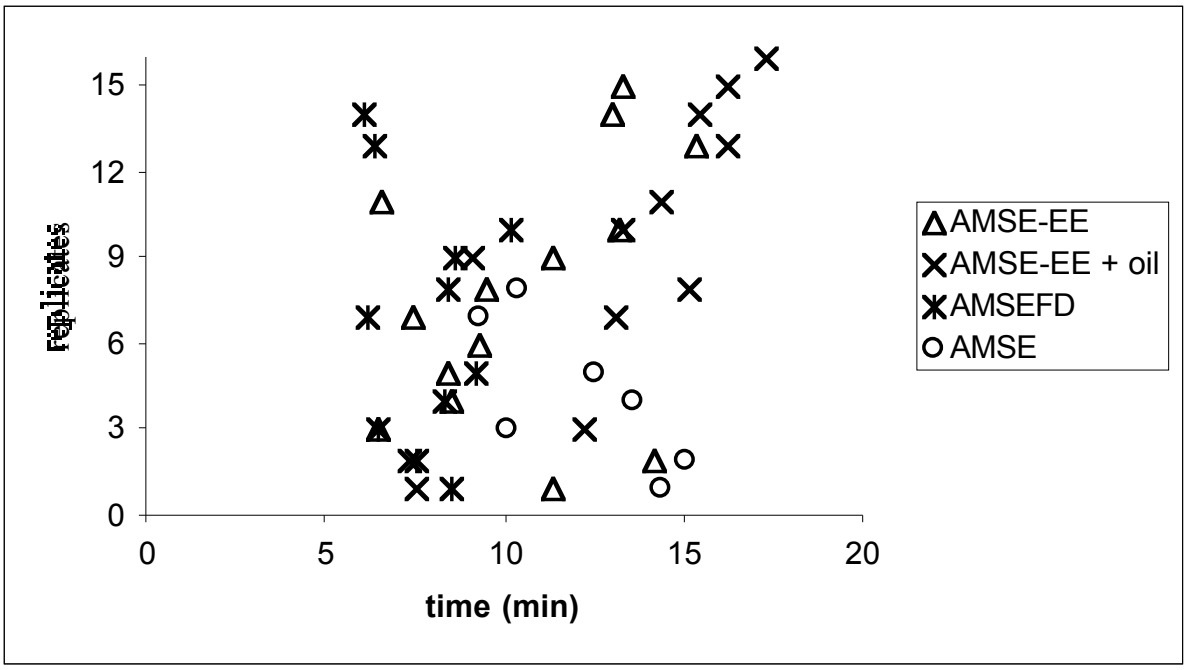
Canola oil and fish oil samples were prepared as emulsions with seawater (1:1 v). The ether extract was mixed with canola oil (1:1 v).

As previously described for Experiment 2. Differences between means were examined with unpaired Student's t test.

Results and discussion

In bioassays involving canola and fish oil, lobsters increased their antennules activities (detection) on the surface of water where oil was flowing due to the lower density. Canola oil didn't induce any other activity in lobsters. Fish oil induced a detection response (antennule flicking) from about 20% of lobsters between 5-10 minutes. The ether extract (EE in 1% methanol/ seawater) caused some degree of agitation and detection activity in the lobsters (22%) which became more general (75%) on addition of fish oil, but no locomotion response was observed. Amendment of AMSE-EE with canola oil showed a slight increase in the percentage of locomotion response (67%) compared to AMSE-EE (58%), but the difference was not statistically significant (Fig. 11). Fish oil also did not affect the activity of the synthetic mixture of 5 compounds (TAU:BET:LAC:PHE:TRY) (Fig. 12) tested in Experiment 4.

Amendment of this mixture with fish oil (10%) resulted in an increased response from 20% to 43% at temperatures (23.4-23.7^{o}C) in which AMSE caused 100% locomotion of the lobsters tested. However the average response time increased from 10'41"±4'30" to 12'51"± 4'25".



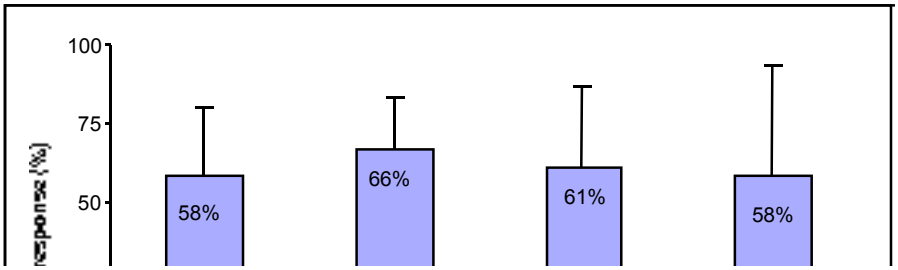
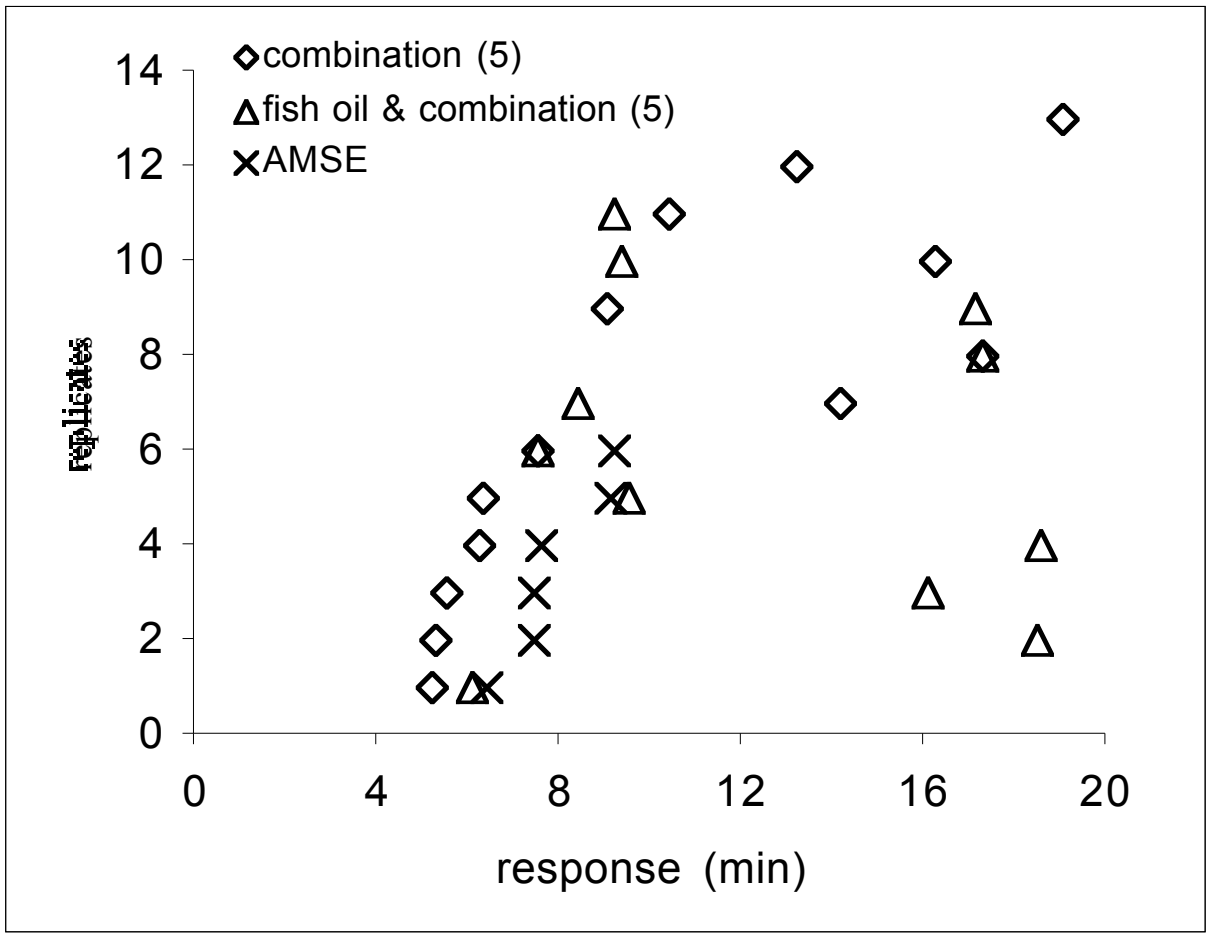
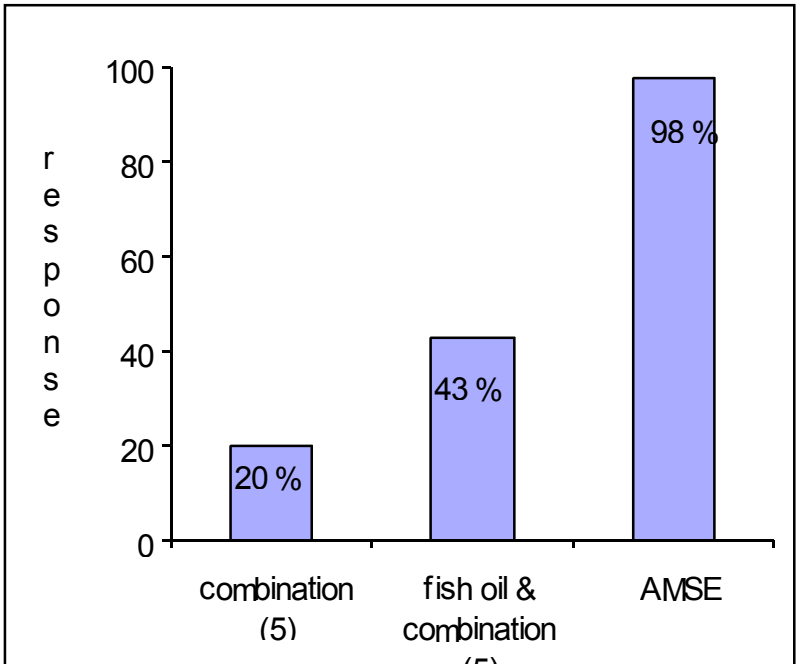


Fig. 11. Effect of canola oil amendment on the activity of AMSE-EE





Experiment 6.

Chemoattractant effect of clove and other oils

Introduction:

This experiment was initiated by the accidental finding that clove oil seemed to attract lobsters. On the basis of this observation, linseed oil, eugenol, ionone, vetiver and orange oil were also tested their chemoactivities to lobsters.

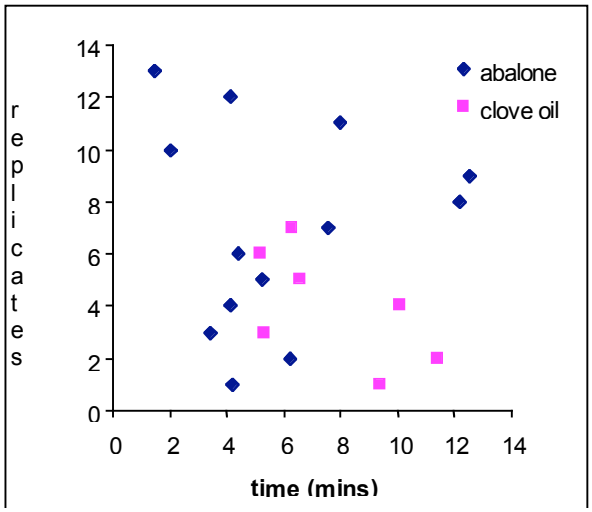
Methods:

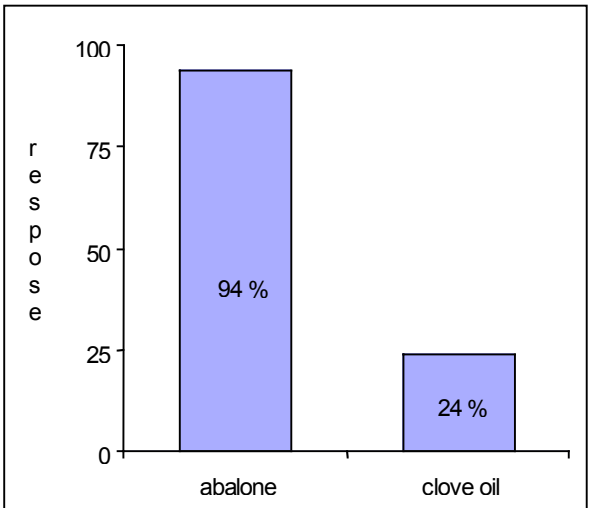
As described previously except that the response to abalone sample was tested with 16 animals (one not responding) and to clove oil with 29 animals (23 not responding).

Results and Discussion:

Of the oils tested, linseed, vetiver and orange oil did not cause any behavioural changes in the lobsters, while the mixture of α- and β-ionones caused some level of agitation of the lobsters (50 % of lobsters were agitated in the time range between 6'30" and 19'30"), but failed to cause locomotion. On the other hand, clove oil proved to have some level of activity.

Although the chemoactivity of clove oil (response time: 7'44" ± 2.30) to the lobsters was similar within the time ranges of abalone (5'40" ± 3.27), the response rate was only 24% which is significantly lower than that of abalone (94%) (Fig. 13). Eugenol, the major component of clove oil (82-87%) failed to elicit any response from the lobsters. It would appear that the sesquiterpene component of clove oil is responsible for the moderate activity exhibited by this oil. It is interesting to note that clove oil has been found to show anaesthetic properties towards fish (Keene *et al.*, 1998; Munday and Wilson, 1997).





Benefits:

The ultimate aim of the project undertaken is the formulation of an artificial bait for the western rock lobster. Although this outcome remains to be achieved, the results arising from this project have some potential benefits, particularly relevant to researchers studying the chemical ecology of lobster behaviour. The development of the lobster bioassay now ensures that a number of test samples can be screened. The identification of some simple compounds, e.g. pyruvic acid, proline, taurine, that show chemoattractant activity provides a starting point for the preparation of active artificial mixtures and/or the investigation of these as useful amendments to some of the more stable commercial baits.

Further Development:

Detection methods

The bioassay developed in this study requires a person with considerable experience in monitoring and recording the activities of lobsters. This is particularly important in the quantification of the effects of attractants on locomotion. The use of some other, more objective, techniques (neurophysiological) (Derby *et al*., 1984; Gomez and Atema, 1996) to measure lobster activity may provide better quantitative data.

Testing of synthetic mixtures of compounds with attractant activity

A more systematic and extensive testing of synthetic mixtures is warranted. Even the limited trials in the present work have disclosed a mixture BET:LAC:PHE:TAU:TRY that is reasonably effective in attracting lobsters. Once the appropriate combination is known, the relative ratios of the compounds could be varied to reflect those in abalone muscle. It is interesting to note that clove oil is detected almost as quickly as abalone tissue, but has a poor percentage response. Modifications, such as reducing or eliminating eugenol and/or amendment with fish oil, could be useful.

Bait delivery system

Different types of delivery methods (sponge, agar) were tested in a preliminary way in the bioassays carried out in this project. The selection of a suitable binding agent will require considerable experimentation.

Field test

The constantly changing environment (water temperature, currents, background levels of

Conclusion:

The results obtained in this project indicate that for the western rock lobster, as for crustaceans in general, chemical stimuli play a major part as inducers of feeding in crustaceans, serving to activate search behaviour and directing patterns of locomotion. In the process, a suitable bioassay was developed that allowed the screening of test solids and solutions of pure compounds and mixtures for their chemoattractant activity. The main attribute of the bioassay is that it clearly measures the effect of these attractants by their induction of locomotion in the animal to the source of the chemoattractant.

Investigations of commercially available baits, mussel, herring, salmon head and mackerel, showed that they all possessed chemoattractant activity, in decreasing order of potency. However, abalone muscle, well-known as a preferred food of rock lobsters, showed the greatest effect as an attractant. Thus, a search for the compound(s) responsible for this activity was initiated. To mimic, as far as possible, the condition in which abalone expressed its activity, a seawater leachate of abalone muscle was tested. This sample retained the attractant activity of the muscle, although the response of the lobsters was somewhat slower. A number of other fractions were generated. Removal of the non-polar components from the leachate did not alter significantly the activity. Fractionation through amberlite gave polar fractions that retained activity, but the non-polar fractions lacked activity. Recombination of the fractions gave a mixture that maintained the activity before fractionation. The overall reduction in activity on manipulation of the abalone tissue could find an explanation if one considers that ATP is the dominant attractant involved. While its concentration in fresh tissues is high, any form of extraction and fractionation would result in the hydrolysis to ADP which is itself unstable and would be converted to AMP.

The next task was to identify the active compound(s) from the leachate. Using HPLC, attempts were made to resolve the constituents of the complex mixture. However, this was not achieved. Modern NMR spectroscopic methods were deployed in the identification procedure, using previous results on the composition of abalone muscle extracts. Fifteen compounds could be identified in the abalone leachate. A total of 24 compounds were selected from the two lists and tested in the chemoattractant activity bioassay. Of these, fourteen showed various degrees of activity, with pyruvic acid, ATP, proline, taurine betaine and taurine emerging as the most active, both in terms of average response time and percentage of response from a population of lobsters. Since it is known that complex mixtures of these compounds almost always have a stronger attractive effect than the individual compounds, the activity of some synthetic mixtures were tested. On the whole, this exercise was not successful, only one combination, BET: LAC:PHE:TAU:TRY, exhibiting an activity approximating that observed for the leachate (AMSE),

Two other experiments were conducted. In one, the effect of adding canola oil or fish oil to the defatted AMSE fraction (AMSE-EE) was investigated. In general, no significant differences were noted. When fish oil (10%) was added to the artificial mixture BET:LAC:PHE:TAU:TRY a greater percentage response (43% compared to 20%) was noted. A serendipitous observation suggested that clove oil had some attractant effect on the lobsters. This oil and some others that were readily available were also bioassayed. Interestingly, clove oil elicited a reasonably fast response and movement, but the lobster participation rate was very low (24%). Interestingly, eugenol, the major component of clove oil, and the compound responsible for the anaesthetic effect of clove oil on fish, was not active. This implicates the sesquiterpene component of clove oil as the chemoattractant.

The present work has contributed to the knowledge of the chemical ecology and chemosensory behaviour of the western rock lobster. The development of a bioassay to determine the relative chemoattractant activity of a wide range of commercial baits, individual compounds, natural and synthetic mixtures of compounds, is an important achievement. The identification of several compounds from abalone with chemoattractant activity has been achieved. In general, it appears that the western rock lobster is very similar to other spiny lobsters in terms of the nature and type of chemical stimuli that induce locomotion towards a potential food source. It is highly probable that these animals have evolved to tune in to a specific combination and ratio of chemicals that signals the presence of food. It is intriguing that synthetic mixtures of chemoattractants, known to be present in a food source, have been found to be inferior to the original bait. It is tempting to suggest that the bait leachate might contain larger molecular weight substances (polysaccharides, peptides) that bind weakly to the more mobile chemoattractant and act in a similar way to the “fixer” elements in perfumes. In this way, small molecules might be maintained in the odour plume

The way is now clear for the assessment of formulations as potential baits for the western rock lobster. However, a number of problems still need to be overcome. The method of delivery of the chemoattractants in the bait to the lobsters is an important consideration. In preliminary attempts, the incorporation of the active components in agar provided a suitable slow-release system. Others have found that chemically impregnated plaster of Paris was superior to agar or gelatin (Mackie *et al.*,1980). The final test of a potential bait is its performance in the field where, under natural conditions, lobsters are in competition with other animals. So, apart from the requirements in a useful bait for stability and effectiveness, there is a need for the bait to be selective for the lobster.

Finally, there is a need for a better understanding of olfactory orientation of the lobsters. The application of chemical visualisation techniques and biomimetic robotics offers some promise in the correlation of stimuli with behaviour and whether differences in plume tracking behaviour

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Appendix 1: Intellectual property

NA

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Appendix 2

Results of searches of recent scientific and patent literature in relation to the development of artificial bait for the western rock lobster (Objective 2)

Remarkably little has been found on this topic, reflecting the lack of systematic investigations and controlled tests. The sequel contains publications directly on the subject, as well as other papers that deal with compounds or preparations that attract related animals.

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