Biogenic amines and pheromones as feed attractants for the freshwater prawn *Macrobrachium rosenbergii*

R. MENDOZA[1], J. MONTEMAYOR & J. VERDE *Universidad Autonoma de Nuevo León, Facultad de Ciencias Biológicas, Aptdo. F-56, San Nicolás de Los Garza, 66450 Nuevo León, México*

Abstract

The use of attractants in formulated feeds has become paramount to economic success owing to the need to optimize feed conversion rates by maximizing consumption and reducing feed waste so as to lower production costs. Taking into account the importance of attractants, a series of experiments designed to evaluate the potential of natural molecules was carried out with the freshwater prawn, *Macrobrachium rosenbergii* (De Man, 1879). Two biogenic amines (putrescine and cadaverine) and two sexual pheromones (crab urine and freshwater prawn green gland extracts) were compared with reference products proven to be major attractants, such as squid extracts and a commercial product. These were incorporated in a basal diet designed to be non-attractive. Results were obtained by three different approaches. First, a laboratory bioassay was conducted to evaluate the time that lapsed as an animal presented the different alimentary stages (perception, orientation, movement, arrival and ingestion). A second approach was developed in a commercial farm, to test the performance of the attractants in the presence of other stimuli and in conditions of water movement that may cause rapid dilution. The test consisted of placing a quantity of feed on a tray, which was submerged in a cage (1 m^3) in which 10 animals (five males and five females) of 20 g mean weight were placed. The tray was lifted at different times (10, 20, 40 and 80 min) and the number of pellets left was counted. Three replicates were performed for each treatment. A third approach consisted of incorporating an antibody in the feed. Following a methodology similar to the above-mentioned, hepatopancreas and mouth parts of the prawns were collected at different times. Later, immunodiffusion tests were executed to assess the actual ingestion of the feed. The results obtained from the different approaches indicated that cadaverine included at 0.2% was the best attractant. On the other hand, the crab urine and freshwater prawn green gland extracts exhibited good results only with males, so their utilization could be recommended for monosexual cultures.

KEY WORDS: attractants, biogenic amines, Crustacea, feed, *Macrobrachium*, pheromones

Received 28 May 1996, accepted 23 October 1996

[1]*Correspondence and present adress: Dr Roberto Mendoza, Universidad Autonoma de Nuevo León, Facultad de Ciencias Biológicas, Aptdo. F-56 Cd. Universitaria, San Nicolás de los Garza, 66450 Nuevo León, México* (e-mail: RMENDOZA@CCR.DSI.UANL.MX)

Introduction

The needs to assure that all the food offered to the animals in a commercial operation is consumed and to reduce formulated food waste have been considered as some of the most relevant aspects to lower production costs on aquatic farms. Considering the importance of chemical stimuli in the development of the crustacean life cycle, it is logical to assume that by adding attractants to the food, the animals would find potential food in a shorter period of time, increasing the likelihood of ingestion. Additionally, this measure would enable the incorporation of protein from vegetable sources that, like soy bean, when included in high percentages in the formulation, lead to reduced levels of food intake (Lee & Meyers 1996a,b). Moreover, because of faster ingestion (Mendoza 1993), this approach would imply the reduction in the diet of binders, which are expensive, non-nutritious and often contribute to poor overall digestibility.

To study these aspects, readily available chemicals and extracts of natural tissues such as biogenic amines and pheromones were assayed. The fact that several secondary metabolites produced during *post mortem* changes (adenosine monophosphate, AMP; inosine monophosphate, IMP; hypoxanthine, Hx; ammonia; trimethylamine, TMA; lactic acid) have been proven to be effective attractants (Costa-Pierce & Laws 1985; Carr & Derby 1986; Harada 1986; Harpaz *et al*. 1987) has led us to evaluate biogenic amines, considering the hypothesis that freshwater prawn, *Macrobrachium rosenbergii* (De Man, 1879), like many crustaceans, are scavengers and often eat decayed

material. Two amines that came to our attention are putrescine and cadaverine, due to the attraction efficacy of their precursory amino acids (McLeese 1970; Hindley 1975; Derby & Harpaz 1988).

In the case of pheromones, we considered that if they were powerful enough to attract other individuals during mating (Bauchau 1986), they could act as attractants. Thus urine of blue crab, *Callinectes sapidus* (Rathbun, 1896), and green gland extracts of the freshwater prawn *M. rosenbergii* were tested as a raw material source of pheromones.

To establish a clear response of the prawns under study to a chemical stimulus originating from the feed, a series of five critical behavioural response descriptors were used; these allowed us to recognize different sequential phases (Costero & Meyers 1993; Lee & Meyers 1996b).

Materials and methods

A series of experiments was performed to elucidate the attraction of different molecules that were compared with reference products proven to be major attractants, such as squid extracts and a commercial attractant. Three different approaches were carried out.

1 A laboratory bioassay in which different attractants were added to a basal diet, the response to which was evaluated by means of a series of behavioural descriptors.

2 A field bioassay where the consumption of different pelleted diets (treatments) proffered to the animals was evaluated at different times.

3 A field bioassay consisting of tracing reference molecules (antibodies) that were included in the pelleted diet so as to assess the actual ingestion of the food.

Laboratory bioassay

Animals and experimental system: Seventy adult (20 ± 3 g mean weight) freshwater prawns (35 males, 35 females) were chosen from local aquaculture farms and stocked during 4 days in round tanks (40 cm H × 200 cm D). A natural light cycle was maintained and the water was replaced at a rate of 50% day^{-1}. Animals were carefully conditioned to the basal diet and comparative trials were run with prawns from which feed had been withdrawn for 24 h prior to each chemical trial. The order of diet presentation was established using a random number table.

The methodology adopted for the chemoattraction bioassay was similar to that described by Costero & Meyers (1993), which entails the use of tanks of 120 × 30 × 40 cm (L × W × H) without water flow. These tanks have a movable division on one end to hold the animals while the food was placed at the other end of the tank. Prior to the testing sessions, the aeration tubes were removed from the aquaria. The water temperature was maintained constant at 26 ± 1°C by means of automatic aquarium heaters and the pH during the experimental period was 7.5–8. After each test, the tanks were emptied, washed thoroughly and refilled with fresh water with the same pH and temperature, in order to exclude any possible interference of previously tested attractants dissolved in the water that could affect the response of the animals in a subsequent test. Single animals were used in each trial to avoid possible group responses and were not reused after testing to avoid any preference for a particular attractant. All tested animals were at intermoult phase of the moulting cycle because it has been suggested that the level of responsiveness varies from stage to stage of the cycle (Harpaz *et al*. 1987). Designation of the different stages was according to Peebles (1977).

Descriptors: Five critical behavioural response descriptors (perception, orientation, displacement, arrival, ingestion) as described by Costero & Meyers (1993) were considered.

Categorization of the test: The test was considered negative if there was no response after 500 s. This period was established in a preliminary bioassay to maximize differences in responses considering the five different phases of behaviour observed.

Treatments: Seven treatments were compared: (1) a hydro-alcohol soluble extract of squid (positive control); (2) a commercial attractant, *Langobuds*, manufactured by Quali Tech[1] (Quali Tech Inc. Chaska, MN, USA) (positive control); (3) blue crab urine; (4) freshwater prawn green gland extracts; (5) cadaverine; (6) putrescine; (7) basal diet (negative control).

Blue crab, *Callinectes sapidus* (Rathbun, 1896), urine samples with 'pheromone-like' activity were kindly supplied by Dr Richard Gleeson, from the Withney Laboratory of Florida State University. Freshwater prawn urine gland extracts were obtained by homogenizing a pool of green glands of females in a precopulatory stage, characterized by the development of the ovaries, which can be seen by external examination. To assess the correct extraction of the material with pheromonal activity, a series of preliminary bioassays were carried out, where the extracts (300 mL) were placed in contact with receptive males. These are the dominant males in the population and are characterized by brilliant blue claws. To confirm that the response was exclusively caused by the presence of pheromones, other kinds of

1. Use of trade or manufacturers' names does not imply endorsement. *Langobuds* contains: shrimp extracts, butylated hydroxytoluene and ethoxyquin (preservatives), diacetyl and other cetonic compounds, isobutyric acid and other organic acids, ethyl lactate and other esthers, betaine, L-proline, L-glutamic acid, disodium guanilate and disodium inosianate, fish meal, calcium silicate and different by-products.

stimulii were tried such as water from aquaria where no animals had been kept, small pieces of flesh from prawns and crabs, and fresh and marine water. The material was selected only after a positive excitatory response was observed (pre-copulatory behaviour). The biogenic amines (putrescine [L(+) Ornithine hydrochloride ($C_5H_{12}N_2O_2$ – HCL)] and cadaverine [1, 4 diamino-butane 99% ($C_4H_{12}N_{12}$)]) were acquired commercially from Janssen Chimica, Belgium.

Addition of attractants: The attractants were added, as a fine spray, to a basal diet which was designed to be non-attractive. The formulation was characterized by its high content of soy bean (300 g kg^{-1}), known to have an antipalatable effect (Table 1). The commercial attractant was mixed with fish oil and sprayed after the food pellets were manufactured. Other molecules were dissolved in distilled water and sprayed. In the case of the negative control, only distilled water was sprayed. The molecules were incorporated at a dose of 2.0 g kg^{-1} on a volume:volume basis, except for cadaverine which was incorporated on a weight:volume basis.

Recording the test: A remote-controlled video camera was used to avoid influencing the behaviour of the animals. Video-recorded sequences of behaviours of food intake were quantified. A stopwatch was used to record the actual time, simultaneously with picture recording. Time zero was set at the moment that the movable partition of the aquarium was raised. Video recordings were viewed by two independent evaluators.

Experimental design: Five replicates were conducted for each treatment, using five males and five females separately. The animals were chosen at random. The results were evaluated by analysis of variance (ANOVA) to determine differences between treatments. When significant differences were detected, the treatment means were separated by the multiple-comparisons Tukey's test (Steel & Torrie 1980). In the case of the responses between sexes, a Student t-test was used to detect significant differences.

Table 1 Basal diet composition

Ingredient	Quantity (g kg^{-1}, wet basis)
Fish meal	50.0
Soy bean meal	300.0
Shrimp meal	40.0
Wheat meal	551.9
Lecithin	18.3
Fish oil	27.0
Methionine	1.1
Vitamin mix	2.2
Vitamin C	0.3
Sodium monophosphate	9.2

First field bioassay

To confirm the results obtained in the laboratory, an ingestion bioassay under commercial conditions was carried out. This was particularly important to confirm the performance of the attractants when exposed to large volumes of moving water, as in ponds, and the diversity of natural molecules with attractant power present in the benthos and in the water column.

The experiment consisted of placing 10 adult prawns (five males, five females; 20 ± 3 g mean weight) in a 1 m^3 cage (according to the density currently used at the farm). The food (6 g equivalent to 80 pellets of uniform size, 0.4 cm long) was proffered on a tray that was raised at different times (10, 20, 40 and 80 min). At each time, the number of remaining pellets was counted. The trays were designed so as to ensure that the pellets were eaten and not just knocked out of them. Three replicates were performed for each treatment. The treatments compared were the same as those established for the laboratory bioassay, except for the basal diet to which the attractants were added; in this case it was the regular commercial diet used at the farm. This was done to emulate the field conditions. An ANOVA was used to determine the differences between treatments and a Tukey's test was used to separate treatment means.

Second field bioassay

Another experiment was made following the same 'tray-test' protocol as before, but in this case a soy bean basal diet (Table 1) was used. Antibodies generated against proteins of *Artemia salina* were added to the diet to assess whether the animals had indeed ingested the feed. Five animals were killed at each pre-established time (10, 20, 40 and 80 min) and hepatopancreas and mouth parts were analysed by immunodiffusion. Antibodies and not antigens were incorporated in the feed because *Artemia* itself is an attractant. In this case the presence or absence of immunological reaction was considered and a χ^2 test was applied to detect differences among treatments.

Results

Laboratory bioassay

The different behaviours related to feed intake used to quantify chemotaxis were like those identified for other Crustacea such as *Penaeus vannamei* (Boone, 1931) (Costero & Meyers 1993) and *Macrobrachium rosenbergii* (Harpaz *et al*. 1987). Table 2 gives a brief description of these phases.

The ANOVA revealed the existence of significant differences among the treatments (attractants) during the different phases of the feeding behaviour (F = 10.57; d.f. 10,6; $P < 0.001$; Table 3). When only the males were considered in the analysis, no

Table 2 Sequential phases of the feeding behaviour of *Macrobrachium rosenbergii*

Phase	Behaviour
Perception	Characterized by intermittent flicking movement of the antennae
Orientation	The animal increases the searching activity and orientates itself towards the food source direction
Movement	The animal walks towards the food source, often following a zigzag path, but if the stimulation is powerful it swims
Arrival	The animal arrives at the food source and spends some time exploring it
Ingestion	The animal eats or rejects the food

significant differences were observed during the first phases of the feeding behaviour (perception, orientation and movement) among the biogenic amines, the pheromones, the commercial attractant and the squid extract, the only exception being the basal diet which showed the worst results. In fact, according to the classification of chemical effectors of feeding behaviour proposed by Lindstedt (1971) and Mackie (1982), this diet acted as an arrestant, because animals ceased locomotion when they were near this source; as a repellent, because animals eventually orientated away from this source; and as a suppressant, because the initiation of feeding was inhibited whenever animals touched this diet with their masticatory appendages. The analysis in which only the females were considered gave different results, showing the following categorization: cadaverine, putrescine, commercial attractant and squid extract followed by the pheromones and the basal diet. Generally, cadaverine stands out not only as the best attractant, because it provoked the animals to orientate toward the diets that contained it, but also as the best incitant, stimulating the initiation of feeding; and as the best stimulant by promoting the ingestion and continuation of feeding. However, cadaverine did not differ significantly from putrescine, the commercial attractant and the squid extract, and in the case of males from the pheromones.

Student's *t*-test revealed significant differences between sexes only when the crab urine and the prawn green gland extracts were assayed (Table 4). Indeed, males perceive and ingest the food faster than females. When the total time elapsed from the perception to the ingestion phase was considered (Fig. 1), it could be noted that the progression of these phases was particularly rapid in the case of biogenic amines, the commercial attractant and the squid fraction. In the case of crab urine and prawn green gland extracts, only the males reacted quickly.

Field bioassays

Figure 2 shows the results obtained from this set of bioassays. Significant differences were detected among the treatments ($F = 34.425$; d.f. 6,72; $P < 0.001$) as well as between the different times assayed ($F = 266.839$; d.f. 3,72; $P < 0.001$). No significant differences were observed during the first 10 min, but starting from the 20 min period, differences in the consumption rate could be identified. The greatest differences were obtained with the biogenic amines and the positive controls. However, the pheromones, when added to the diet, did not produce an increased feed intake. These results were confirmed by means of single radial immunodiffusion tests (Table 5) and the χ^2 test revealed the existence of significant differences among treatments ($\chi^2 = 23.57$, 6 d.f., $P < 0.01$).

Table 3 Mean responses ± SD, (time in s) obtained during the different phases of feeding behaviour for each treatment[1]

Treatment	Perception		Orientation		Movement		Arrival		Ingestion	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Basal diet	110.8^{c} ± 15.09	104.4^{c} ± 27.19	231.5^{c} ± 47.06	198.0^{c} ± 32.90	349.0^{c} ± 51.41	268.6^{c} ± 46.67	473.0^{c} ± 37.51	448.4^{c} ± 50.02	496.0^{c} ± 7.15	478.0^{c} ± 37.25
Blue crab urine	96.0^{bc} ± 83.10	25.6^{b} ± 10.43	202.0^{c} ± 33.2	39.0^{b} ± 20.57	257.8^{a} ± 179.8	52.2^{b} ± 13.70	434.0^{bc} ± 88.66	105.2^{ab} ± 16.88	439.4^{c} ± 46.22	112.0^{ab} ± 16.34
Prawn green gland extracts	109.4^{c} ± 12.03	24.6^{b} ± 6.14	127.6^{bc} ± 11.94	34.8^{b} ± 7.91	194.2^{bc} ± 12.23	42.4^{b} ± 8.59	361.8^{b} ± 35.19	95.8^{a} ± 12.04	449.8^{c} ± 21.70	109.8^{ab} ± 17.12
Cadaverine	20.2^{a} ± 4.70	23.6^{b} ± 7.40	28.2^{b} ± 8.16	25.2^{b} ± 7.44	37.8^{a} ± 14.53	38.0^{b} ± 8.30	78.8^{a} ± 24.10	92.0^{c} ± 12.28	100.6^{b} ± 19.20	104.4^{a} ± 9.09
Putrescine	28.2^{ab} ± 4.49	28.4^{b} ± 4.82	42.0^{bc} ± 6.96	43.4^{b} ± 8.96	51.0^{ab} ± 5.87	54.2^{b} ± 8.16	115.8^{a} ± 17.65	118.4^{ab} ± 14.77	126.2^{b} ± 13.62	127.4^{ab} ± 10.76
Commercial attractant	27.8^{ab} ± 11.45	29.2^{b} ± 13.92	46.2^{bc} ± 27.15	45.6^{b} ± 15.63	67.4^{ab} ± 21.91	60.8^{b} ± 4.38	113.4^{a} ± 13.01	102.4^{ab} ± 14.99	130.2^{b} ± 19.30	130.6^{ab} ± 27.89
Squid extract	39.0^{ab} ± 11.37	40.2^{b} ± 12.37	54.0^{bc} ± 21.26	49.2^{b} ± 15.38	65.2^{ab} ± 22.60	68.6^{b} ± 39.19	151.8^{a} ± 39.80	153.2^{b} ± 31.98	162.6^{b} ± 37.77	165.4^{b} ± 40.02

[1]Means in a row with the same superscripts belong to homogeneous groups (separated by the Multiple-comparisons Tukey's test). Figures with different superscripts in the same row are significantly different from each other.

Table 4 Student's *t*-test values for males and females exposed to the same treatment. Probability values and degree of significance[1] are shown for each feeding behaviour and treatment

Treatment	Perception	Orientation	Movement	Arrival	Ingestion
Basal diet	0.65762 NS	0.63687 NS	0.92542 NS	0.31462 NS	0.30002 NS
Blue crab urine	0.10022 NS	0.11027 NS	0.03421 *	0.00004 **	0.00003 **
Prawn green gland extracts	0.000006 **	0.000005 **	0.000002 **	0.000002 **	0.000007 **
Cadaverine	0.40857 NS	0.58667 NS	0.97934 NS	0.30712 NS	0.69973 NS
Putrescine	0.94760 NS	0.78966 NS	0.49712 NS	0.80699 NS	0.88102 NS
Commercial attractant	0.86653 NS	0.96689 NS	0.52759 NS	0.25045 NS	0.97961 NS
Squid extract	0.87716 NS	0.69586 NS	0.87074 NS	0.95261 NS	0.91224 NS

[1]NS, Non-significant values; *significant values ($P < 0.01$); ** highly significant values ($P < 0.001$).

Discussion and conclusions

In most studies dealing with chemoattraction, no single purified molecule has performed better than artificial or natural mixtures of attractants, as was the case with cadaverine in this study when compared with both positive controls. In particular, the results concerning the attractive power of biogenic amines could be considered as complementary to the theory advanced by Zimmer-Faust (1987), who states that carnivorous crustaceans search for organisms with high energetic levels (in terms of adenylate energy charge) which may assist predators in recognizing their prey. In the present experiments, the biogenic amines have been found to function as potent attractants for *Macrobrachium*, probably because this animal principally scavenges dead animals and detrital materials. Thus the response may be adapted to the dietary mode. Furthermore, biogenic amines have acted not only as chemoattractants (causing the animal to orientate towards the source) but also as feeding incitants (triggering the feeding response) and as feeding stimulants (causing the animal to continue feeding).

Regarding the crab urine and prawn green gland extracts, the attractant molecules attract males of *Macrobrachium*. This finding is controversial because pheromones have often been defined as molecules acting intraspecifically. However, investigations performed in crustaceans (Kittredge *et al*. 1971) and insects (Rojas *et al*. 1990) have shown the possibility of interspecific action. Nevertheless, we do not rule out the possibility that other molecules, such as products of excretion (e.g. urea, tertiary amines, nucleic acids), present in the urine could act as attractants or potentiate the effect of pheromones. A similar phenomenon has been reported in the case of hermit crabs that can locate a new shell from a distance (Rittschof 1990; Kratt & Rittschof 1991). The attraction appears to be due to specific peptides (generated by a degradative process containing a number of neutral residues and a basic residue like arginine or lysine at the carboxy terminus) released from the flesh of dead and dying gastropods that signal the availability of new shells. In the same manner, lobsters are captured more frequently in traps with baits composed from aged abalone, which is more attractive

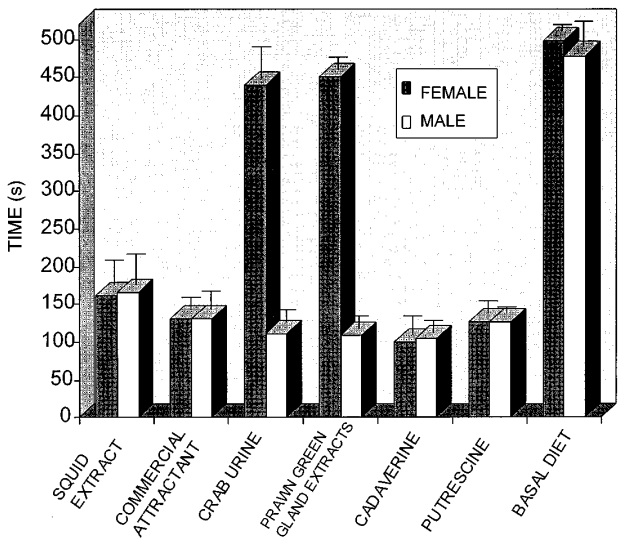

Figure 1 Mean time elapsed (in seconds, + SE) from the perception to the ingestion phase. Standard deviations represented by vertical bars.

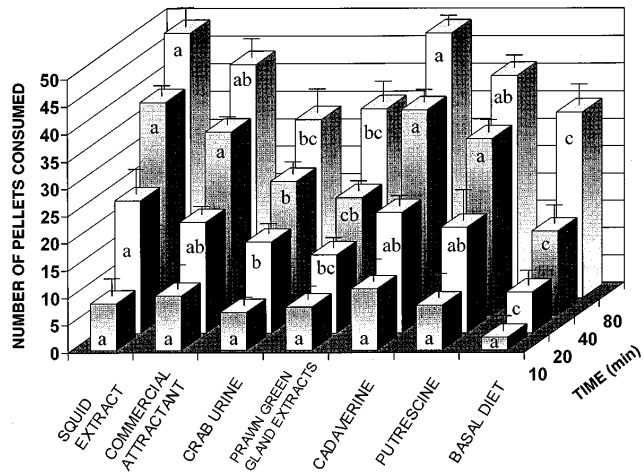

Figure 2 Mean number (+ SE) of pellets consumed per treatment at different sampling times. Standard deviations represented by vertical bars. The means in a row with the same letter belong to homogeneous groups (separated by the Multiple Comparisons Tukey's test).

Table 5 Qualitative responses[1] observed by immunological precipitation in the field experiment

Treatments	Replicates				
	1	2	3	4	5
Basal diet	–	–	–	–	–
Blue crab urine	–	–	+	–	–
Prawn green gland extracts	–	–	–	+	–
Cadaverine	+	+	+	+	+
Putrescine	+	+	+	+	+
Commercial attractant	+	+	+	+	+
Squid extract	+	–	+	–	+

[1]–, No response; +, immunological response.

than fresh abalone (Zimmer-Faust *et al*. 1984; Lee & Meyers 1996b). Other studies have reflected the sensitivity of crustaceans to molecules of these kinds. The ingestion rate of artificial diets by juvenile prawns has been improved with the addition of trimethyl amine hydrochloride (TMAH) to the surface of the pellets (Costa-Pierce & Laws 1985). The TMAH was thought to mimic the odour of rotting fish, thereby inducing a scavanging animal to feed.

From the structural standpoint, it seems logical to assume that the biogenic amines could act positively, because analyses of natural fluids or of extracts of natural materials attractive to crustaceans have generally shown that major stimulants are substances of low molecular weight with properties consistent with the hypothesis that they are amino acids or closely related substances (Heinen 1980). In this regard, it has been reported that the amino group must be unsubstituted and bear a ionic charge so it could react with chemoreceptors, in contrast with the modifications of the carboxyl group including removal of a charge that are tolerated only with some loss of effectiveness (Hatt 1984).

The addition of attractants by spraying seems to be a good method of supplementation, because in this way they are available almost immediately when the food pellets enter in contact with the water, thereby producing a quick perception response.

The results obtained in the field experiment by immunological assessment of ingestion showed that the commercial diet, in spite of containing different sources of natural attractants, such as fish meal and fish oil, was not sufficient to attract the animals and stimulate the feeding process. This aspect was confirmed by the addition of a commercial attractant and the biogenic amines to this diet. The performance of the sources of pheromones in terms of ingestion was rather poor, possibly due to their concentration or to their rapid dissolution. Squid extracts have often been considered as a good source of attractants for crustaceans (Mackie & Shelton 1972; Mackie 1973; Takei 1977), but there is always a risk in incorporating natural extracts, because constancy in the composition of the raw material cannot be predicted and thus some variability should be expected, as was the case in this study.

By means of this set of experiments, it was possible to confirm the suspected role of biogenic amines and pheromones in terms of attraction and feeding stimulation. Moreover, the present study confirms that not only antennular flicking but the entire sequence of food searching and grasping behavioural repertoire, as described by Harpaz *et al*. (1987), can be induced by the administration of a single chemostimulant. However, these results can only be considered valid for *M. rosenbergii* adults because patterns of feeding and food selection vary throughout the life cycle of crustaceans. In particular, larval stages have different feeding habits, thus chemoattractants and feeding stimulants to which they respond should change over time (Lee & Meyers 1996a,b). It has been reported that crustacean larval stages rely more on chance encounters to capture food items (Kurmaly *et al*. 1990).

A fact that has to be considered is that the validity of the results is altered by the concentrations used, particularly for the case of biogenic amines. In terms of applicability, the production of amines is simple and inexpensive (Susuki *et al*. 1994) and should be preferred to the aquisition of purified molecules, because their price remains high, even at the tested concentrations (e.g. 1 kg of cadaverine = 203 USD). In the case of pheromones, their utilization implies the need to synthesize the molecules, but they may have certain potential in monosexual cultures or for sex separation. A dose–response experiment should be run, and it is also necessary to find the threshold for the lowest functional concentration and to observe the effect of these molecules when blended with other chemicals that could possibly enhance the response. Additionally, parallel tests of substances prepared from prey or carrion tissues need to be carried out.

Finally, the relevance of this study is reflected in the fact that the addition of the different attractants has provided a significant improvement in the consumption of the commercial diet that already had some source of attractants. This aspect is particularly important because food detection and ingestion ultimately will determine the commercial value of an aquatic feed (Takeda & Takii 1992; Lee & Meyers 1996b).

Acknowledgement

The authors wish to express their gratitude to Dr Richard Gleeson for his kind contribution of the blue-crab urine samples that were used in this study.

References

Bauchau, A. (1986) Sex pheromones in Crustacea. *Adv. Invert. Reprod*., **4,** 337–343.

Carr, W.E.S. & Derby, C. (1986) Behavioral chemoattractants for the prawn, *Palaemonetes pugio*: identification of active components in food extracts and evidence of synergistic mixture interactions. *Chem. Senses*, **11,** 49–64.

Costa-Pierce, B.A. & Laws, E.A. (1985) Chemotactically active feed additive for prawns (*Macrobrachium rosenbergii*). *Progve Fish-Cult*., **47,** 59–61.

Costero, M.C. & Meyers, S.P. (1993) Evaluation of chemoreception by *Penaeus vannamei* under experimental conditions. *Progve Fish-Cult*., **55,** 157–162.

Derby, C.D. & Harpaz, S. (1988) Physiology of chemoreceptor cells in the leg of the freshwater prawn, *Macrobrachium rosenbergii*. *Comp. Biochem. Physiol*. **90A,** 85–91.

Harada, K. (1986) Feeding attraction activities of nucleic acid-related compounds for abalone, oriental weatherfish and yellowtail. *Bull. Jap. Soc. Scient. Fish*., **52,** 1961–1968.

Harpaz, S., Kahan, D., Galun, R. & Moore, I. (1987) Responses of fresh water prawn *Macrobranchium rosenbergii*, to chemical attractants. *J. Chem. Ecol*., **13,** 1957–1965.

Hatt, H. (1984) Structural requirements of amino acid and related compound for stimulation of receptors in crayfish walking leg. *J. Comp. Physiol*., **155A,** 219–231.

Heinen, J.M. (1980) Chemoreception in decapod Crustacea and chemical feeding stimulants as potential feed additives. *Proc. World Maricul. Soc*., **11,** 319–334.

Hindley, J.P.R. (1975) The detection, location and recognition of food by juvenile banana prawns, *Penaeus merguiensis* de Man. *Mar. Behav. Physiol*., **3,** 193–210.

Kittredge, J.S., Terry, M. & Takahashi, F.T. (1971) Sex pheromone activity of the molting hormone, crustecdysone, on male crabs (*Pschygrapsus crassipes*, *Cancer antennarius* and *C. anthonyi*). *U.S. Fish Wildl. Serv. Fish. Bull*., **69,** 337–343.

Kratt, C.M. & Rittschof, D. (1991) Peptide attraction of hermit crabs *Clibanarius vittatus* Bosc: roles of enzymes and substrates. *J. Chem. Ecol.*, **17,** 2347–2365.

Kurmaly, K., Jones, D.A. & Yule, A.B. (1990) Acceptability and digestion of diets fed to larval stages of *Homarus gammarus* and the role of dietary conditioning behaviour. *Mar. Biol*., **106,** 181–190.

Lee, P.G. & Meyers, S. (1996a) Chemoattraction and feeding stimulation in Crustacea. *Aquacult. Nutr*. **2,** 157–164.

Lee, P.G. & Meyers, S. (1996b) Chemoattraction and feeding stimulation. In: *Crustacean Nutrition* (D'Abramo, L., Conklin, D. & Akiyama, D. eds). Advances in World Aquaculture Society Series. Vol. 6. The World Aquaculture Society. Baton Rouge, Louisiana, USA.

Lindstedt, K.J. (1971) Chemical control of feeding behavior. *Comp. Biochem. Physiol*., **39A,** 553–581.

Mackie, A.M. (1973) The chemical basis of food detection in the lobster *Homarus gammarus*. *Mar. Biol*., **21,** 103–108.

Mackie, A.M. (1982) Identification of the gustatory feeding stimulants. In: *Chemoreception in Fishes* (Hara, T.J. ed.), pp. 275–291. Elsevier Scientific Publishing Co., Amsterdam, the Netherlands.

Mackie, A.M. & Shelton, R.G. (1972) A whole animal bioassay for the determination of the food attractants of the lobster *Homarus gammarus*. *Mar. Biol*., **14,** 217–221.

McLeese, D.W. (1970) Orientation of lobsters *Homarus americanus* to odor. *J. Fish. Res. Bd Can.*, **30,** 838–840.

Mendoza, R. (1993) Métodos para evaluar la digestibilidad protéica de los alimentos destinados a los organismos acuáticos. In: *Memorias del Primer Simposium Internacional de Tecnología de Alimentos para Acuacultura*, (Cruz, L., Ricque, D. & Mendoza, R. eds), pp. 155–202. Asociación Americana de Soya. Monterrey, Nuevo León, México.

Peebles, J.B. (1977) A rapid technique for molt staging in live *Macrobrachium rosenbergii*. *Aquaculture*, **12,** 173–180.

Rittschof, D. (1990) Peptide-mediated behaviors in marine organisms. Evidence for a common theme. *J. Chem. Ecol*., **16,** 261–271.

Rojas, J.C., Malo, E.A., Gutierrez, A. & Ondarza, R.N. (1990) Mating behavior of *Triatoma mazzottii* (Hemiptera: Reduvidae) under laboratory conditions. *Ann. Entomol. Soc. Am*., **83,** 598–602.

Steel, R. & Torrie, J. (1980) *Principles and Procedures of Statistics. A Biometrical Approach*. McGraw–Hill Book Co., New York, 633 pp.

Susuki, S., Kobayashi, K. & Takama, K.(1994) Occurrence of biogenic amines at different processing stages of dried herring. *Fish. Sci.*, **60,** 353–354.

Takeda, M. & Takii, K. (1992) Gustation and nutrition in fishes: application to aquaculture. In: *Fish Chemoreception* (Hara, T.J. ed.), pp. 271–285. Chapman & Hall, London, UK.

Takei, M. (1977) Feeding behavior of crabs *Eimacrus isenbekii* and *Neptunus trituberculatus*. *Bull. Tokai Reg. Fish. Res. Lab.*, **89,** 75–82.

Zimmer-Faust, R.K. (1987) Crustacean chemical perception: towards a theory on optimal chemoreception. *Biol. Bull.*, **172,** 10–29.

Zimmer-Faust, R.K., Michel, W.C., Tyre, J.E. & Case, J.F. (1984) Chemical induction of feeding in California spiny lobster, *Panulirus interruptus* (Randall): responses to molecular weight fraction of abalone. *J. Chem. Ecol.*, **10,** 957–971.