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Marine Biology / Oceanography Joint
Hons.

**OXB 1007 Marine Biology Practical
Skills.**

Observing the use of chemoreceptors in
Panaeus monodon (tiger prawns).

Introduction

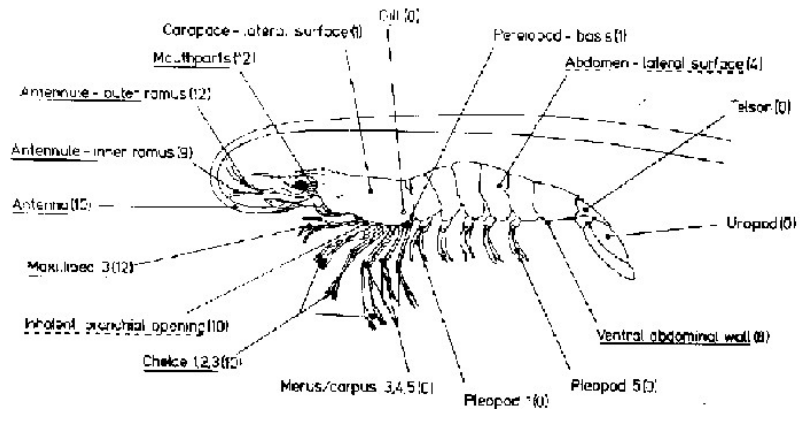
Penaeus monodon (*P.monodon*) is in the Crustacea phylum and is a decapod. In the animal kingdom in general chemical stimuli is important to the detection, location and recognition of prey and predators. Taste and smell are very important. Animal sensitivity to chemical stimuli is controlled by peripheral and central neural mechanisms (Zimmer-Faust, 1995), stimuli are detected by chemoreceptor cells, which translate into electricity flowing along neurones.

Studies by Bateson, 1889; Hazlett, 1968, 1971; Symonds, 1964 have all shown that chemosensory stimulus is probably much more important than visual stimulus in provoking a feeding response. Chemoreceptor cells can tune into and respond to an array of structurally different substances belonging to different classes of molecules. This fine-tuning allows the animal to distinguish the scent of prey from the interference of other chemical noise in the sea environment and follow changing fluctuations of concentration of a particular chemical to its source. Groups of cells that work together to fine tune the distinguishing form a receptor. Receptors p1-like and p2-like in particular respond to Adenosine mono phosphate and Adenosine triphosphate. (Carr *et al* 1987). McLeese (1970) showed that a wide range of amino acids some with concentrations as low as 0.7ppm provoked a feeding response in some crustaceans. (Hindley, 1975) It has also been reported that Glutamergic and Taurinergetic chemoreceptors are present in some marine decapods.

In the genus *Penaeus* of which *Penaeus monodon* is a member it is known that the antennular filaments, pereopod dactyls and the mouthpart appendages are the prime chemoreception sites.

The antennules act as distant chemoreceptors and the dactyls and the mouthpart are high and moderate level receptors (Carr *et al* 1984b)

Figure 1. Chemosensory organs of genus *Penaeus*. Words underlined are responsive loci, underlined in dotted line slightly responsive loci with non-responsive parts remaining.



P.monodon' diet consists mainly of

small crustaceans and molluscs with some annelids and algae. It is thought that *P.monodon* is a predator rather than a scavenger (Motoh, 1984). Lesley (MSc Thesis, University of Wales, Bangor) and Duffin (Honours project, School of Ocean Sciences, menai bridge) showed that when starved *P.monodon* were presented with extracts of *Nereis virens* it provoked a strong feeding response in comparison to other natural extracts that form *P.monodon*' diet such as mussel or squid flesh.

Black Tiger Prawn (*P.monodon*) is also known as Leader prawn or Asian Tiger shrimp. It is the most commonly farmed prawn species in Australia and Southeast Asia. *P.monodon* have been intensively cultured for more than 15 years in Thailand. Success in culture depends on good management, nutrition and environmental awareness. Much research has been done on the development of diets for penaeids. The increase in world markets for prawns and shrimp still depend a lot on diets of live foods, which are difficult to maintain (Kurmaly, Jones, Yule and East 1988). Shrimp feed quality has been developed to the optimal nutritional level with long water stability. During shrimp feeding approximately 10% of the diet will be lost to the

water and becomes nutrients for bacteria and micro-algae. There has been a lot of research and study concerning chemo-attractants present in animal flesh and manufactured diets.

Burke *et al* (1997) proposed that for a diet to be effective it must in addition to providing the animal with the required proteins and nutrients the diet must also be attractive to the organism as food, maintain stability and have appropriate buoyancy.

With optimal feed management, shrimp and other organisms will live well in an equilibrium environment. Over-feeding leads to nutrient load and eutrophication, with severe consequences for the water, which may become difficult to manage and may necessitate the early harvesting of shrimp. Successful commercial rearing of prawns in the future will depend upon the efficient and economic use of suitable diets. See studies on energy and growth by Kurmaly *et al* (1988) and Zimmer-Faust (1987)

In a standard reference diet developed for Crustaceans by Castell (1989) a crabmeat protein concentrate was used as a main ingredient because this was known to contain a chemo-attractant. Diets, which included chopped fresh frozen green-lipped mussel flesh and squid mantle have also been used. One of the sample diets used in studies for *P.monodon* larvae by Kurmaly *et al* (1988) even included Cod Liver Oil.

The aim of the experiment was to observe *Penaeus monodon* when the organism was placed within a Y-Maze and to monitor its behaviour (whether it goes to the left or to the right) under a series of different conditions; placing different food sources in the arms of the maze. The experiment involved a control to establish the bias of the Y-Maze and then was followed by four separate experiments investigating the responses of the prawn to the different stimuli. The hypothesis (the null hypothesis) is that *P.monodon*' behaviour will not be changed or affected by the introduction of potential food sources into the Y-maze. We are using a Y-

maze to test this. The alternative hypothesis i.e. when conditions are changed (potential food sources are placed in Y-maze) the probability of the *P.monodon* going in a certain direction is not the same as calculated for the unbiased maze, the control test (approx 0.5).

P.monodon will be offered a variety of stimuli including *N.virens* extract and fresh *N.virens* flesh. The *N.virens* extract is called NCA and was developed by Genesis for use in the farming industry to be included in pellet feed which is freeze dried + solvent extract of whole *N.virens* (not including the body wall). Studies on this food indicate that *P.monodon* prefers wild *N.virens* to farmed *N.virens*. These studies also showed that chemo-attractant properties were also significantly reduced when the worm was processed possibly indicating that the attractant is a water soluble substance present in the body fluids of the polychaete lost in the extraction and freeze drying process. (Mascarenhas, RAP 1990).

Compounds in the chemo-attractants are believed to be primarily amino acids, though quaternary ammonium bases, nucleotides and nucleosides as well as organic acids are also thought to illicit a response (Zimmer-Faust, R 2004)

Method

A Y-Maze was constructed made of clear Plexiglas. The Y-maze measures 60cm in length along the straight 'run', and then each arm measures 50cm each in length. The prawn is given room to move sideways, giving the maze the width of 25cm. The flow speed through the maze is set at 1cm/s using a small flow device (regular aquarium pump). The flow is towards the straight run of the Y-maze down the two arms. After each separate experiment we ensure that the Y-Maze is thoroughly cleaned as chemoattractant from the previous experiment may linger in the Y-maze. A control experiment is set up using a sample size of 51 *P.monodon*.

One organism at a time is placed in the end of the Y-maze and observed to see which direction the prawn will choose to go when it reaches the junction of the Y-maze. Does it go down the left arm or down the right arm of the maze? If it does not move or go in either direction after 20 minutes the organism is discarded and the experiment is continued with a different *P.monodon*.

A further 4 experiments are set up. Experiment 1: place 100g of large pieces of chopped *N.virens* in the left arm of the maze and then place 100g of *Fucus serratus* into the right arm of the maze. For this experiment a sample of 20 *P.monodon* are used. Record how many go to the left (1) and how many go to the right (0).

Experiment 2: place 100g of large pieces of chopped *N.virens* in the left arm of the maze and leave the right arm empty. Use a sample of 20 *P.monodon*. Record how many go to the left (1) and how many go to the right (0)

Experiment 3: place 100g of *Fucus serratus* into the right arm of the maze and leave the left arm empty. Use a sample of 20 *P.monodon*. Record how many go to the left (1) and how many go to the right (0).

Experiment 4: place a 100g block of wood in the right arm of the maze (this is a visual stimulus that has no smell), and place 100ml of *N.virens* body fluid into the right arm of the maze (a non visual but chemoattractant stimulus). Use a sample of 20 *P.monodon*. Record how many go to the right (0) and how many go to the left (1).

Results

Results were first collated from our control test to determine whether there was any bias to the y-maze. The results show a probability of 0.481 of choosing right over left as a direction for the prawn to take in the y-maze. This is approximately half of the

Title of Experiment	N	Min	SEMin	SD
Control Experiment	51	0.481	0.007	0.05
Experiment 1	20	0.750	0.093	0.413
Experiment 2	20	0.850	0.089	0.063
Experiment 3	20	0.400	0.112	0.633
Experiment 4	20	0.850	0.089	0.063

Figure 2. Summary of control experiment showing p-value of 0.005 and mean of 0.481 (probability)

P.monodon choosing left and half choosing right. Achieving a probability average of almost 50% (0.5) gives us a nearly unbiased maze. This is important for the remainder of our experiments. With an average probability of 0.5 the results for our control form a fairly normal distribution, it is binomial data so there are only two possible outcomes but the chances of getting one or the other are almost even. (Figure 2 for statistics)

Title of Experiment	N	Probability	Mean	Variance	ST Dev
Control Experiment	51	0.481	9.62	0.519	2.23
Experiment 1	20	0.750	15.00	0.250	1.93
Experiment 2	20	0.850	17.00	0.150	1.60
Experiment 3	20	0.400	8.00	0.600	2.19
Experiment 4	20	0.850	17.00	0.150	1.60

Table 1 – Statistical Data for various experiments including the control test. Mean = $P \times n$. Variance = $1 - p$. St Dev = $\sqrt{m \times v}$.

Using the probability distribution outcomes from the control this data can be used to calculate probabilities for other numbers of trials. If 25 *P.monodon* were released into an

Table 2 – Numbers of *P.monodon* that chose either left or right in the y-maze.

Title of Experiment	Left	Right
Control Experiment	25	26
Experiment 1	15	5
Experiment 2	17	3
Experiment 3	8	12
Experiment 4	17	3

empty y-maze as set up in our methods using the control results the mean number of individual *P.monodon* likely to go to the right would be 12.975. The standard deviation for this is approximately 2.50.

In the results for experiment 1 15 *P.monodon* chose the left arm of the y-maze. According to the control results the probability of 15 *P.monodon* choosing the left arm of the y-maze is 0.018 the probability of getting 15 *P.monodon* in our experiment using the mean and probability results from the experiment is 0.177. Probability of 15 or more *P.monodon* turning left to successfully locate food in experiment 1 is 0.741 this is significantly greater than 0.5. The probability of 15 *P.monodon* or more choosing the left path in our control is 0.027 this is a difference of 0.713. Table 2 shows the numbers of *P.monodon* that chose left or right in the specified

experiment. Table 3 indicates clearly the probabilities of getting the results we obtained according to our control and according to the particular experiment. The overall probabilities for *P.monodon*

choosing to go left are: experiment 1 – 85%, experiment 2 – 75%, experiment 3- 40%, and experiment 4 – 85%.

The difference between the actual probability result of the resulting number of *P.monodon* choosing the left arm of the Y-maze and the probability of that number choosing left in the control in each case is significantly greater than 0.05. This also applies to the same number or more of the animals making those choices.

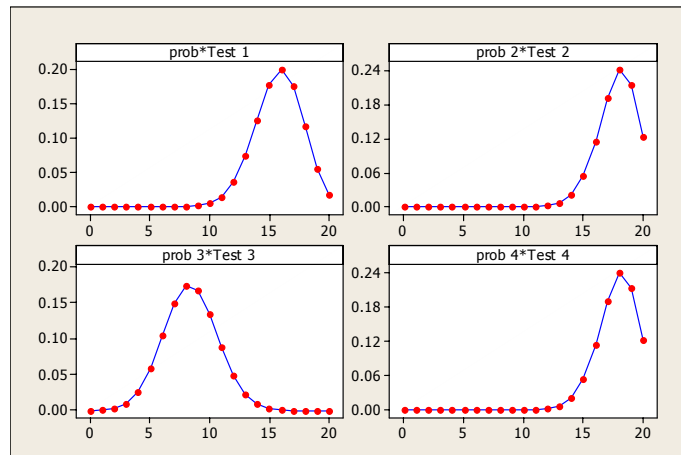


Figure 3. Probability Density curves for our four experiments. Test 1 – Test 4 equivalent to Experiment 1 – Experiment 4.

Table 3 – Probabilities of the number of *P.monodon* that chose to take the left arm in comparison to the control with difference and the Probabilities of the same number or more making that choice

Title of Experiment	Left	Prob of choosing left	Prob of choosing Left in control	Difference
Experiment 1	15	0.177	0.018	0.159
Experiment 2	17	0.191	0.002	0.190
Experiment 3	8	0.174	0.116	0.059
Experiment 4	17	0.191	0.002	0.190

Title of Experiment	Left	Inv Prob of choosing left	Inv Prob of choosing Left in control	Difference
Experiment 1	15	0.027	0.741	-0.715
Experiment 2	17	0.002	0.770	-0.767
Experiment 3	8	0.872	0.650	0.222
Experiment 4	17	0.002	0.770	-0.767

Discussion

The results of the experiments cause us to reject the null hypothesis and accept the alternative. When conditions are changed in the Y-maze (ie a potential food source is added) the *P.monodon* do not follow the same probability as the unbiased maze of the control experiment. The control experiment showed a 0.481 probability of the *P.monodon* going down the left arm. Our results showed a significant difference (in each case greater than 5%) between what we would expect given our control and the actual results. In the experiments using high strength chemoattractants that the probability of the prawn going down the arm containing the chemoattractant was much more likely. The arms containing *N.virens* had the most effect on the *P.monodon*, as the probability increased of the arm in which they were located.

Our results match other findings. Zimmer R.K (2000) states that sensory perception of chemical signals strongly influences predation, courtship, mating and is used extensively in detection and location of food. Of course it has also been noted that investigators of chemoreceptive behaviour in marine animals should be aware that other environmental

factors such as temperature, light, locomotion etc may all effect an organism behaviour and its chemosensitivity. During the experimental phase every effort is made to try to allow time for organism's to settle before testing and eliminate outside noise or other influences.

The feeding behaviour of the *P.monodon* can also be affected depending on whether the organism is starved or satiated. The optimal foraging theory states that the feeding search behaviour is dependent on the organisms feeding state (Pyke *et al*, 1977). A starved specimen tunes its chemoreceptors to seek attractants of high energy content with nutritional 'payoff'. The prawns in the experiment may have been at different stages of hunger, to reduce this; the prawns could be stored under identical conditions for a week previous to the experiments.

It can not be determined at what distance the *P.monodon* first sensed the chemoattractant as the first part of the maze is straight and it could initially sense the attractant at some point in this area. The experiment deals with individual organisms, which may all show different behaviour from each other.

The result of experiment 4 reinforces that chemical detection by *Penaeus monodon* relies on the chemoreceptor organs and not on the organisms' vision. The Prawn was able to see a 100g wooden block, however it chose to follow the arm, which appeared to contain nothing as a visual stimuli, but did contain an almost clear chemoattractant (body fluid of *N.virens*), 17 out of 20 of the prawns followed the attractant. In most areas where *P.monodon* live unidirectional turbulent flow breaks up the chemical signals, producing odour patches which change size and concentration as they travel through the water; the receptors sense a fluctuating odour signal (Atema & Voigt, 1997).

The use of the Y-maze controls the flow of the water, reducing the turbulence of the water and should reduce the spread of odour patches. Predatory success is at its peak at a flow

speed of 1cm/s (Zimmer-Faust, 2004), the flow rate in the experiments was set at this speed to maximise the chance of the organism sensing the

attractant. Predatory success is reduced in the absence of flow and in flow speeds of 4cm/s or faster (Zimmer-Faust, 2004). This is partly

because the concentration gradients and dispersal of the odour patches make it harder for the animal to find the source of the chemical signal. Food is unlikely to be detected by sight at a distance of anything more than a few centimetres. Since penaeids diet can also include organic debris a lot of its food will not be visually recognisable. ((Hindley J.P.R, 1975)

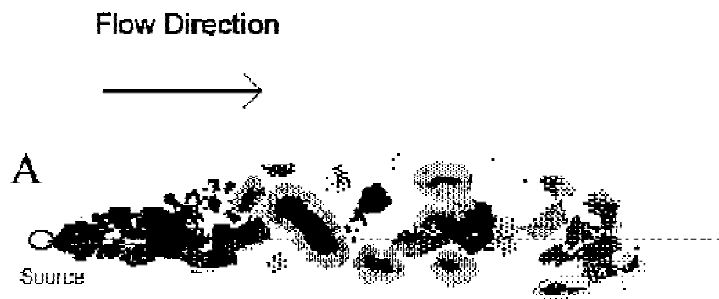


Figure 4 – This shows the chemical plume – signal emanating from its source (e.g. prey) into a unidirectional flow. The flow environment breaks the odor plume into a series of various size patches.

The Y-maze used is ideal as it can effectively regulate the chemical attractants, and control the flow of the water and attractant and artificially straightens the fluid flow, however, it does not allow much free movement of the organism, it is forced to choose only left or right. (Zimmer-Faust R.K, 1996)

The results obtained fitted with research found and showed our null hypothesis to be incorrect. *P.monodon* was influenced in its choice of direction due to stimuli and this stimulus was most likely to be chemical stimuli it detected using its chemosensors. This information and the presence of various chemoattractants in food sources have important impact upon the world farming of animals such as *P.monodon*.

REFERENCES

- Atema, J; Voigt, R.** (1997). Orientation of marine Invertebrates to Odor Sources. Ch12. Boston University Marine Program, Marine Biological Laboratory, Woods Hole.
- Azan, F.; Hodson, R.E** (1977). Dissolved ATP in the sea and its utilization by marine bacteria. *Nature*, Vol 267, 696-697.
- Burke, M.J.; Hansford, S.W.; Marsden, G.Y.; McGuren, J.J** (1997). A moist artificial diet for prawn broodstock; its effect on the variable reproductive performance of wild caught *Penaeus monodon*. *Aquaculture* Vol 149, 145-156.
- Butman, C.A; Zimmer, R.** (2000) Chemical Signaling Processes in the Marine Environment. *Biol Bull* 198, 168-187.
- Carr, W.E.S.; Ache, B.W.; Gleeson, R.A.** (1987). Chemoreceptors of crustaceans: Similarities to receptors for neuroactive substances in internal tissues. *Environmental Health Perspectives*, Vol 17, 31-47.
- Duffin, A.** (1989). The use of polychaete worms as chemoattractants in prawns (*Penaeus monodon*). Joint Honours Project, School of Ocean Sciences, Menai Bridge.
- Hindley J.P.R.** (1975) The Detection, Location and Recognition of Food by Juvenile Banana Prawns, *Penaeus merguensis* de Man. *Mar. Behav. Physiol.* Vol 3, 193-210
- Jones, D.A ; Kurmaly, K; Yule. A.B.** (1998) An Energy Budget for the Larvae of *Penaeus monodon* (Fabricus). *Aquaculture* Vol 81, 13-25.
- Lesley, D.** (1989). Squid inclusion levels in nursery feeds. Msc Thesis, School of Ocean Sciences, Menai Bridge.
- Mascarenhas, R.A.P.** (1990). Chemoattractants and the feeding behaviour of the tiger prawn (*Penaeus monodon*). Thesis. BSc Hons (Wales) 19 Oct 1990.
- O'Neill, P.B; Schar, D.W. ; Zimmer-Faust, R.K.** (1996) The Relationship Between Predator Activity State and Sensitivity to Prey Odor. *Biol Bull* Vol 190, 82-87
- Pyke, G.H.; Chanovel, H.R.** (1977). Optimal foraging, a selective review of theory and tests. *Q.Rev.Biol.* Vol 52, 137-154.
- Zimmer-Faust, R** (2004). Chemical Mediation of Interactions between organisms. *Ocean Ecology: Understanding and Vision of Research*. Natural Science Foundation. Dept of Biology U.S.A.