Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 91, Number 4, July 1982, pp. 367-373.

Sex pheromone in a stomatopod crustacean *Squilla holoschista*

M DEECARAMAN* and T SUBRAMONIAM**
* Department of Zoology, Sri Theagaraya College, Madras 600 021, India
** Department of Zoology, University of Madras, Madras 600 005, India

MS received 25 January 1982 ; revised 29 May 1982

Abstract. The stomatopods are well-known for their aggressive and agonistic encounters. The males are normally aggressive ; the females too in the non-reproductive condition show such a behaviour with males. In *S. holoschista* mating is frequent as well as repetitive. The present paper explains whether there is any involvement of sex pheromone. The sex pheromones are considered to be present in ovaries, cement glands as well as oviducal extractions. These substances were tested for their pheromonal activity. The results indicate that there may not be such attraction as evidenced by the lack of mating gestures from the isolated males in the presence of these substances. It is therefore suggested that the mating in the stomatopod, *S. holoschista* is indiscriminate. The physiological effect of such a repeated and indiscriminate mating on the female is discussed.

Keywords. Pheromone ; natural sex attractants ; Squilla ; premating gestures.

1. Introduction

The accumulation of evidences drawn from insects led to the introduction of pheromone concept (Karlson and Lüscher 1959). Recently sex attractants in the form of pheromones have been found to exist in several crustaceans (Dahl 1975). However due to lack of proper controlling methods, the mere existence of pheromones in Crustacea is questioned (Dunham 1978).

In decapod crustaceans the behavioural movements may be due to chemical or visual stimuli (Salmon 1965, 1971 ; McLeese 1971 ; Ryan 1966 ; Teytaud 1971). Initial behavioural contact between male and female is followed by mutual exchanges of communicating signals. This helps in transmitting information of species, identification of sex and reproductive drive from one animal to another (Hazlett 1975). Alteration in the agonistic behaviour has been shown to result in the pair of reproductive male and female (Hazlett and Winn 1962 ; Nolan and Salmon 1970). The act of copulation in marine Crustacea varies from one species to another. In general, mating occurs in the freshly moult condition. But there are exceptions to this rule (Hartnoll 1969). In lobsters and anomuran species mating occurs normally between a fresh moult female and an intermoult male (Berry 1970 ; Hazlett 1970, 1972). In some hermit crabs, Hazlett (1972) observed frequent mating and copulation was prolonged in hard-shelled crabs. Dingle and Caldwell (1972) have observed in a stomatopod, *Gonodactylus breedini* that mating is not preceded by moulting.

In spite of the elaborate mating processes reported in some decapod crustaceans, not much is known on the pheromonal attraction among the males. Kittredge *et al* (1971) have pointed out that "the closest parallel to insect pheromone communication observed in marine organisms are the sex pheromones of marine Crustacea." The available information on crustacean sex pheromone indicates that their behavioural assays accepted as admissible evidence for sex pheromone are as follows : (i) chemokinetic reactions, (ii) chemotaxic reactions, (iii) releaser reactions (Dunham 1978). The presence of non-diffusible stimulating substances have been found by Carlisle (1959) and Forster (1951) in *Pandalus borealis* and in *Leander serratus* respectively. They have found that the stimulant is not restricted to any particular part of the body ; instead even antennal contact seems to be sufficient for exciting the male. Ryan (1966) reported on the water soluble sex pheromone released through the urine of *Portunus sanguinolentus* during premoult stage. Atema and Engstrom (1971) and McLeese (1971) have also demonstrated the existence of water-soluble sex pheromone released by moulted mature female lobsters. Kamaiguchi (1972) has shown in *Palemon paucidens* that such sex attractant is released from the sternal glands during prepaturial moult. Sex pheromonal activity of the moulting hormone (crustecdysone) itself has been indicated by Kittredge *et al* (1971). However Atema and Gagosian (1973) found no evidence for the pheromonal activity for ecdysone or its analogue in the mature males. Perhaps the occurrence of sex pheromone in more crustacean species should be demonstrated in order to draw conclusions on its physiological specificity on the males. It is of interest to note in this connection that in a male crab *Emerita asiatica* mating occurs indiscriminately without the involvement of any sex pheromone (Subramoniam 1979). The aim of the present paper is to find out whether the extracts of various female reproductive organs as well as the "female water" possess pheromonal activity on the mature males kept in isolation in the laboratory.

2. Material and methods

Squilla holoschista (Woodmason) used in the present study were collected from the Madras coast and maintained in the laboratory in glass aquaria containing sea water. Water was changed and sufficiently aerated every day. The animals were fed regularly with fresh muscles of fish and prawn.

Behavioural sequences were observed in the glass tanks. Before the experiments commenced the matured males and females were fed *ad libitum* and transferred into a tank of dimensions 60 × 25 × 31 cm, with sufficient sea water.

Before the experiment began the males were fully fed. Then the aqueous extracts of ovary (0·5 g of ovary in 1 ml of filtered sea water), cement glands (0·25 g of cement glands in 1 ml of sea water) and the oviduct (from three animals with 0·5 ml of sea water) were tried on the males when it comes to a motionless state. This was repeated many times in order to find out the changes if any in the behavioural patterns towards the premating gestures or agitated or searching beha-

Table 1. Behavioural sequences

Normal behaviour—Male (In isolation within a period of 30 min)	I. Mating—Male and female (Deecaraman and Subramoniam 1981a) (Generally at evening hrs—diffused light)	Aggressive males (Frequently exhibits)
1. Antennule flicking	1. Antennule flicking—male and female	1. Antennule flicking
2. Spreading of the raptorial meri at narrow angle	2. Contact—male initiates	2. Meri spread out widely
3. Telson thrust	3. Male spreads the raptorial meri female remains motionless	3. Strikes the opponents
4. Forward and backward movements	4. Male holds the female by cephalothoracic appendages, grasps and tilts the female	4. Chase
5. Motionless	5. Male erects the intromittent organs and moves towards the female	
6. Cleaning the cephalothorax with telson spines	6. Male exhibits thrusting movements—Female orientates towards the male	Female
7. Coiling by bringing the telson close to the cephalic region	7. Release of male by the female —strikes (indicates to some extent aggressive behaviour)	1. During non-receptive condition
		2. Strikes the male at the end of copulation
	II. Repeated mating	
	1. Both male and female involve	
	2. Some behavioural movements repeated as in column 1.	

vioural patterns as reported by Ryan (1966), Atema and Engstrom (1971) and Kamiguchi (1972).

Similarly the effect of "female water" in changing the behavioural pattern of male was also tested. The female water was obtained by keeping a mature female in a glass tank for six hrs. This female water was tested on males kept in isolation in a glass tank. The behaviour of the males after the addition of female water is compared with the normal mating patterns (Deecaraman and Subramoniam 1981b).

3. Results

In *S. holoschista* normal mating behaviour (Deecaraman and Subramoniam 1981b) could be easily differentiated from the aggressive encounters. The male usually exhibits aggressive behaviour. However, the female also exhibits the same when it is not in the receptive state. This aggressive behaviour by females is also exhibited at the end of copulatory sequences.

The males when introduced into the trough start flicking the antennules in all directions. This movement may last for few sec. Subsequently, the animals spread the raptorial meri on both sides and withdrew them immediately. Then the males move backward using the telson spines and the walking legs. Sometimes, the animals remain motionless up to 10 min, but keep the antennules and the pleopods in motion. Following this the males exhibit forward and backward movements using the thoracic and abdominal appendages, with telson spines planted on the substratum. Frequently the animals clean the maxillipedes with telson spines and also demonstrate "coiling" by bringing the telson and the head close together. These movements may last from a few sec. to some min.

3·1. *Experiments with ovary, oviduct and cement glands extracts*

A mature male was introduced into the tank and its normal behavioural pattern was observed. When the animal comes to a motionless state at one end, the aqueous extract of ovary was introduced at the other end opposite to the animal drop by drop. In the beginning the animal shows a positive response by moving towards the point of application of the ovarian extract ; however, it immediately retreats to its original place without showing any behavioural pattern positive to premating gestures. Repeated application of the ovarian extract failed to show any effect on eliciting the premating gestures. Similarly, the application of oviducal and cement glands extracts did not have any effect on the males (table 2).

3·2. *The female water*

To test the effects of female water on the male behavioural pattern the mature males were introduced in the tank. Even here the males failed to elicit any positive behaviour for attraction.

All these preliminary experiments suggest that there may not be any specific stimulation of the male by the female by way of any pheromonal substances (table 2). It is therefore suggested that mating in the stomatopod under laboratory conditions occurs indiscriminately.

4. Discussion

Many available evidences in invertebrates clearly suggest the involvement of pheromone and one such phenomenon is the settlement of marine larvae of gregarious organisms (Dahl 1975; Crisp 1974). Another phenomenon of sex pheromone is that of "epidemic spawning" (Galtsoff 1938, 1940 ; MacGinite and MacGinite 1949).

Various stomatopod species are known to exhibit agonistic and aggressive behaviour in natural copulation (Dingle and Caldwell 1969, 1976; Caldwell and Dingle 1976). Malacostracans, especially the brachyuran crabs, have been shown to exhibit prolonged premating gestures before the external pairing (Hazelet 1975). Many others have attempted to explain this phenomenon by way of pheromonal

Table 2. Behavioural sequences—Male (within a period of 30 min)

I. Ovary

1. Normal behaviour
2. Motionless
3. Application of ovary extract
4. Antennule flicking
5. Advances towards the point of application
6. Retreats to the normal position immediately
7. No premating gestures or searching behaviour or agitated movements
8. Restore to the normal behaviour

II. Oviduct

1. Normal behaviour
2. Motionless
3. Application of oviduct extract
4. Antennule flicking
5. Avoids the point of application
6. No premating gestures or search behaviour
7. Restore to the normal behaviour

III. Cement glands

1. Normal behaviour
2. Motionless
3. Application of cement glands extract
4. Antennule flicking
5. No premating gestures or searching behaviour or agitated movements
6. Restore normal behaviour

"Female water"

1. Antennule flicking
2. Motionless
3. No premating gestures or searching behaviour or agitated movements
4. Normal behaviour.

attraction (Ryan 1966; McLeese 1971; Kittredge *et al* 1971). Virtually nothing is known about the origin of pheromone in the aquatic invertebrates (Dunham 1978). Recently, Kittredge *et al* 1972 and Kittredge and Takahashi (1972) have reported that the crustecdyzone or the related compound acts as sex pheromone in some decapod crabs, however Atema and Gagosion (1973) have reported negative results to the sex pheromone response to any one of these compounds in *Homarus americanus*. In *Portunus sanguinolentus* Christofferson (1970) has reported that the sex pheromone is of 1000 or less of molecular weight.

A recent study on the mating behaviour of sand crab *E. asiatica* has shown that the tiny males may not be attracted to the female by any sex pheromone as the attachment of the males to the females occur long before the actual deposition of spermatophore and the attachment could also occur at any time of moult cycle (Subramoniam 1977). It was also reported that the mating at least in this crab is indiscriminate and that there may not be any pheromonal attraction involved in it (Subramoniam 1979). It was also suggested that a pheromone may not work in an environment of rapid water movements such as intertidal region inhabited by *E. asiatica*.

The present results have not provided any evidence in support of a sex pheromonal attraction in *S. holoschista*.

Acknowledgement

The authors thank Prof. K Ramalingam, Prof. T K Sudhindran, Prof. S Augustine Chellappa, and Mrs. D Jayalakshmi for provision, facilities and encouragement. One of us (MD) gratefully acknowledge the award of fellowship of U G C.

References

Atema J and Engstrom D G 1971 Sex pheromone in the lobster *Homarus americanus*; *Nature (London)* **232** 261–263

Atema J and Gagosian R B 1973 Behavioural responses of male lobsters to ecdysones; *Mar. Behav. Physiol.* **2** 15–20

Berry P F 1970 Mating behaviour oviposition and fertilisation in the spiny lobster *Panulirus homarus* (Linnaeus); *Invest. Rep. Ocean Res. Inst. S. Afr.* **24** 1–16

Caldwell R L and Dingle H 1976 Variation of agonistic behaviour between populations of the stomatopod *Haptosquilla glyptocercus*; *Evolution* **31** 220–223

Carlisle D B 1959 On the sexual biology of *Pandalus borealis*. The termination of the male phase; *J. Mar. Biol. Ass. U.K.* **38** 381–395

Christofferson J P 1970 *An electrophysiological and chemical investigation of the female sex pheromone of the crab Portunus sanguinolentus (Herbst.)*; Ph.D. Thesis, University of Hawaii, Hawaii

Crisp D J 1974 Studies of barnacle hatching substance; *Comp. Biochem. Physiol.* **30** 1037–1048

Dahl E 1975 Pheromones in aquatic invertebrates, Bio signals Kungl. Fysiografiska Sallaskapet of Lund, Sweden

Deecaraman M and Subramoniam T 1981a Repeated mating and its effect on the female reproductive physiology with special reference to the fate of male accessory sex gland secretion; *Proc. First All India Symp. Inv. Repro. Madras*

Deecaraman M and Subramoniam T 1981b On the question of sex pheromonal attraction in the crustacean stomatopod *Squilla holoschista*; *Abstr. 10th Ann. Conf. Etho. Soc. Bangalore*

Dingle H and Caldwell R L 1969 The aggressive and territorial behaviour of the mantis shrimp, *Gonodactylus breedini* Manning (Crustacea: Stomatopoda); *Behaviour* **33** 115–136

Dingle H and Caldwell R L 1972 Reproductive and maternal behaviour of the mantis shrimp *Gonodactylus breedini* Manning (Crustacea: Stomatopoda); *Biol. Bull.* **142** 417–426

Dingle H and Caldwell R L 1975 Distribution abundance and intraspecific agonistic behaviour of two mudflat stomatopods; *Oecologia* **20** 167–178

Dunham P J 1978 Sex pheromones in Crustacea; *Biol. Rev.* **53** 555–583

Forster G R 1951 The biology of the common prawn *Leander serratus* Pennat; *J. Mar. Biol. Ass. U.K.* **30** 333–360

Galtsoff P S 1938 Physiology of reproduction of *Ostrea virginica* II. Stimulation of spawning in the female oyster; *Biol. Bull.* **75** 286–307

Galtsoff P S 1940 Physiology of reproduction of *Ostrea virginica*. III. Stimulation of spawning in the male oyster; *Biol. Bull.* **78** 117–135

Hartnoll R G 1969 Mating in Branchyura; *Crustaceana* **16** 161–181

Hazlett B A 1970 Tactile stimuli in the social behaviour of *Pagurus bernhardus* (Decapoda, Paguridae); *Behaviour* **36** 20–48

Hazlett B A 1972 Shell fighting and sexual behaviour in the hermit crab genera *Pagurites* and *Calcinus* with comments on *Pagures*; *Bull. Mar. Sci.* **22** 806–823

Hazlett B A 1975 Ethological analysis of reproductive behaviour in marine Crustacea; *Publ. Staz. Zool. Napoli.* **39** 671–695

Hazlett B A and Winn H E 1962 Sound production and associated behaviour of Bermuda crustaceans. (*Panulirus, Gonodactylus, Alpheus* and *Synalpheus*); *Crustaceana* **4** 25–38

Kamiguchi Y 1972 Mating behaviour in the freshwater prawn, *Palaemon paucidens*. A study of the sex pheromone and its effect on male; *J. Fac. Sci. Hokkaido Univ.* **18** 347–355

Karlson P and Lüscher M 1959 "Pheromones": a new term for a class of biologically active substances; *Nature* (*London*) **183** 55–56

Kittredge J R, Terry M and Takahashi F T 1971 Sex pheromone activity of the moulting hormone, crustecdysone, on male crabs (*Pachytrapsus crassipes, Cancer antennarius, C. anthonyi*); *Fish. Bull.* **69** 337–343

Kittredge J S and Takahashi F T 1972 The evolution of sex pheromone communication in the Arthropoda; *J. Theo. Biol.* **35** 467–471

MacGinitie G E and MacGinitie N 1949 Natural history of marine animals. (New York, London and Toronto : McGraw-Hill Book Co.) 1–473

McLeese D W 1971 Detection of dissolved substances by the American lobster (*Homarus americanus*) and olfactory attraction between lobsters; *J. Fish. Res. Canada* **27** 1372–1378

Nolan B A and Salmon L 1970 The behaviour and ecology of Snapping shrimp (Crustacea : *Alpheus heterochelis* and *Alpheus nomanni*); *Form Funct.* **2** 289–335

Ryan E P 1966 Pheromone: Evidence in a Decapod crustacean; *Science* **151** 340–341

Salmon M 1965 Waving display and sound production in the courtship behaviour of *Uca pugilator* with comparison to *U. minax* and *U. pugnax*; *Zoologica* **50** 123–150

Salmon M 1971 Signal characteristics and acoustic detection by the fiddler crabs, *Uca rapax* and *Uca pugilator*; *Physiol. Zool.* **44** 210–224

Subramoniam T 1977 Some aspects of sexual biology of a crab *Emerita asiatica* Milne Edwards; *Mar. Biol.* **43** 369-378

Subramoniam T 1979 Heterosexual raping in the mole crab *Emerita asiatica*; *Int. J. Invertebr. Reprod.* **1** 197–199

Teytaud A R 1971 The laboratory studies of sex recognition in the blue crab *Callinectus sapidus* Rathbun; *Sea Grant Tech. Bull.* **15** 1–63