Proc. Indian Acad. Sci., Vol. 87B (Animal Sciences-2), No. 6, June 1978, pp. 145–149,

Effect of the epidermal secretions of Hemichordate, *Ptychodera flava* on growth of *Amphora coffeaeformis* and *Cyclotella meneghiniana* (Diatoms)

JAYAPAUL AZARIAH, V N R RAO* and V T SRIDHARAN*
Department of Zoology, University of Madras, Madras 600 005
*Centre of Advanced Study in Botany, University of Madras, Madras 600 005

MS received 13 August 1977; revised 8 February 1978

Abstract. The effect of epidermal secretions of *Ptychodera flava* collected during non-breeding and breeding seasons on growth of two diatoms *Amphora coffeaeformis* and *Cyclotella meneghiniana* is studied. Differential effect of the secretions on growth rate of the diatoms indicates differences in their potency. Low concentrations tend to stimulate growth. The ecological significance of the secretions has been briefly commented upon.

Keywords. *Ptychodera flava;* epidermal secretions; *Amphora coffeaeformis; Cyclotella meneghiniana.*

1. Introduction

The epidermis of *Ptychodera flava* is studded with three types of glandular cells, namely reticular gland cells, goblet gland cells and granular gland cells (Bullock 1945; Rao 1951; Hyman 1959). Although the exact nature of secretions is not precisely understood it is known that mucus from these glands is helpful in feeding and burrow formation. Studies of Azariah *et al* (1975) indicated the possible occurrence of a ' conditioning factor ' which helps to normalize the metabolic rate in the organism. Recent studies of Azariah and Ahamed (unpublished) revealed the presence of a third type of secretion which interferes with the metabolism of its own members and thus appears to be a toxic irritant. As a result, not more than one specimen of *P. flava* is found per burrow in the natural habitat.

A peculiarity of the habitat of *P. flava* at the Galexea lagoon of Krusadi Island is the limited occurrence of fauna and flora when compared to other areas in the same region. It is possible that metabolites of *P. flava* may in some way control growth and distribution of other plants and animals of the habitat. It was thought worthwhile to study the effect of epidermal secretions of *P. flava* on growth of two diatoms that are components of first trophic level of the ecosystem.

2. Materials and methods

2.1. *The epidermal secretion*

Full advantage was taken of the behaviour of *P. flava* when collecting epidermal

secretions. It is known that when specimens of *P. flava* are kept together, away from the natural habitat, they intertwine with each other and this process triggers epidermal secretions (Azariah and Annamalai, unpublished). In less than 30 minutes the secretion may colour sea water golden brown with a characteristic 'iodoform' odour.

Animals with approximately the same size and weight were selected and kept in a plastic bucket containing filtered sea water. The number of specimens per container was adjusted to give a constant ratio of one animal per 100 ml of sea water. As mentioned above, within an hour there was copious secretions which dissolved into the surrounding sea water and this secretion was used in culture experiments. To study seasonal variations, if any, in its potency, the secretion was obtained from specimens collected during breeding and non-breeding seasons.

2.2. *Test diatoms*

Amphora coffeaeformis and *Cyclotella meneghiniana* were used as test organisms. Both diatoms have previously been shown to have the same growth intensity in a wide range of salinities (Desikachary and Rao 1972). Any osmotic change in the medium, caused by addition of different amounts of epidermal secretions will, therefore, not affect their growth rate. Furthermore, they suspend uniformly in the medium without clumping or sticking to sides of the glass tube. This minimizes self-shading of the light received and is a great advantage during cell counting.

The diatoms were maintained in modified Guillard's f/2 sea water medium (McLachlan 1973; see Guillard and Ryther 1962). The epidermal secretion was filter-sterilized by filtering through a millipore filter of 0·45 μm pore size using a millipore syringe adapter filtration unit. Graded concentrations were then added aseptically to autoclaved Guillard's medium to give a concentration range of 0·05, 0·1, 0·5 and 1·0 ml of secretion per 10 ml of medium. Preliminary studies showed that the effect of secretion was not so pronounced when it was obtained from specimens collected during breeding season. Therefore, secretion of such specimens was added in concentrations ranging from 0·05, 0·1, 0·5, 1·0, 2·0 and 5·0 ml per 10 ml of medium. Unamended Guillard's medium served as control. Proper care was taken in these trials to see that the final salt composition of medium was unaltered.

The inocula for these experiments were from a 7-day old exponentially growing culture. The experimental tubes were incubated under continuous light of 1500 lux and a temperature of 26°C$\pm$1°C. The experiments were run for 14 days. Pigments were cold extracted in 90% acetone on alternate days and the relative optical density was read in a Nephelometer against an acetone blank. Haemocytometer cell counts were also taken on these days. Cell counts were counter checked with the corresponding optical density measurements. Cell counts and the O.D. obtained were the average of three replicates.

3. Results

The results on the effect of epidermal secretions of *P. flava* collected during non-breeding and breeding seasons on growth of *Amphora coffeaeformis* and *Cyclotella meneghiniana* are given in tables 1 and 2. It is seen that the effect varied with the two test diatoms. *C. meneghiniana* was less tolerant than *A. coffeaeformis* as

Table 1. Effect of epidermal secretions of *P. flava* collected in the non-breeding season on growth of *Amphora coffeaeformis* and *Cyclotella meneghiniana.*

Treatments		Cell number × 10^4/ml						
		2nd day	4th day	6th day	8th day	10th day	12th day	14th day
		A. coffeaeformis (Inoculum 2·5 × 10^4 cells/ml)						
Control	(T_1)	8·8	17·0	97·4	130·0	178·0	192·0	194·0
Treatments								
0·05 ml	(T_2)	12·6	23·0	131·6	147·0	172·8	145·0	154·0
0·10 ml	(T_3)	7·7	31·0	93·0	106·2	157·4	146·0	116·0
0·50 ml	(T_4)	7·7	19·3	49·0	92·2	125·2	122·0	110·0
1·00 ml	(T_5)	6·3	14·2	54·0	64·2	65·0	110·0	110·0
		C. meneghiniana (Inoculum 2·5 × 10^4 cells/ml)						
Control	(T_1)	6·3	15·4	29·0	39·2	61·6	58·0	79·0
Treatments								
0·05 ml	(T_2)	6·6	13·0	28·0	35·2	48·0	82·0	78·0
0.10 ml	(T_3)	7·7	13·0	31·0	41·6	42·0	46·4	62·0
0·50 ml	(T_4)	5·4	8·0	10·0	9·4	13·0	10·0	24·0
1·00 ml	(T_5)	3·0	3·2	0·4	1·2	1·4	1·4	3·4

Table 2. Effect of epidermal secretions of *P. flava* collected in the breeding season on growth of *Amphora coffeaeformis* and *Cyclotella meneghiniana.*

Treatments		Cell number × 10^4/ml						
		2nd day	4th day	6th day	8th day	10th day	12th day	14th day
		A. coffeaeformis (Inoculum 0·9 × 10^4 cells/ml)						
Control	(T_1)	3·4	14·0	21·7	151·3	178·8	210·4	216·8
Treatments								
0·05 ml	(T_2)	5·0	17·3	108·4	168·8	298·4	228·0	260·8
0·10 ml	(T_3)	6·3	24·8	173·6	219·6	243·0	232·8	282·4
0·50 ml	(T_4)	5·5	17·1	150·0	222·0	236·4	287·6	348·4
1·00 ml	(T_5)	3·0	23·6	125·6	222·0	267·6	302·8	373·0
2·00 ml	(T_6)	4·0	13·9	71·2	174·4	236·0	232·0	418·4
5·00 ml	(T_7)	3·2	5·0	17·5	37·2	96·4	143·0	261·2
		C. meneghiniana (Inoculum 0·5 × 10^4 cells/ml)						
Control	(T_1)	1·6	4·5	13·4	20·0	21·8	33·2	27·0
Treatments								
0·05 ml	(T_2)	1·4	2·7	8·1	20·8	26·4	27·0	26·0
0·10 ml	(T_3)	2·0	3·3	8·5	20·0	23·0	25·2	37·4
0·50 ml	(T_4)	1·6	5·0	15·0	29·0	36·6	31·0	36·0
1·00 ml	(T_5)	1·6	3·3	16·7	25·2	23·0	36·0	29·2
2·00 ml	(T_6)	1·8	4·5	8·3	22·2	30·0	34·0	36·0
5.00 ml	(T_7)	0·7	2·1	2·0	2·5	1·6	6·0	6·4

evidenced by the differential division rate (see table 3). The growth rate of *A. coffeaeformis* was more than that of *C. meneghiniana* in both experimental series.

C. meneghiniana showed no growth in media containing secretions collected during the non-breeding season in concentration of 1·0 ml and only partial growth in 0·5 ml, whereas *A. coffeaeformis* grew in all the treatments though growth was comparatively less in 1·0 ml concentration. Concentrations of 0·05 and 0·1 ml had no inhibitory

Table 3. Division rates per day of *A. coffeaeformis* and *C. meneghiniana* in different concentrations of the epidermal secretions during the two seasons.

Diatoms	Season	Control	Treatments					
			0·05 ml	0·10 ml	0·50 ml	1·00 ml	2·00 ml	5·00 ml
Amphora coffeaeformis	Non-breeding	0·89	1·17	0·87	0·72	0·74	—	—
	Breeding	0·77	1·16	1·27	1·24	1·19	1·25	0·72
Cyclotella meneghiniana	Non-breeding	0·46	0·42	0·41	0·22	0·06	—	—
	Breeding	0·67	0·68	0·67	0·73	0·71	0·69	0·25

effect on both *C. meneghiniana* and *A. coffeaeformis*. In fact, concentration of 0·05 ml had a stimulatory effect. This was reflected in increased growth rate of the diatoms in this concentration (see table 3).

The effect of secretion collected from gravid *P. flava* (breeding season) was at variance with that collected from non-gravid specimens (non-breeding season). The growth rates of both the test diatoms were unaffected in all concentrations up to 1·00 ml. The inhibitory effect was significantly expressed only in concentration of 5·00 ml, which was at the level of 50% concentration in the medium. As in previous experiment, *C. menegniniana* was less tolerant than *A. coffeaeformis*. In this series, concentrations from 0·05 ml to 1·0 ml had a stimulatory effect on growth as compared with the earlier one (non-breeding season) where only 0·05 ml concentrations was stimulatory. This was more significantly expressed in the case of *A. coffeaeformis*.

The results of both series of experiments were subjected to statistical analyses in order to assess their level of significance. Analyses of variance—one way treatment—showed that F was highly significant in both series in the case of *C. meneghiniana*, [($\underline{F}$4, 30)=12·22—non-breeding season: $\underline{F}$(6, 43=53·04—breeding season)], and not significant in *A. Coffeaeformis*. Since F was significant it was possible to grade the treatments using *t* test. In such an analysis it was found to have the following order (T_2 T_1, T_3) (T_4, T_5) during the non-breeding season and (T_4, T_6, T_5, T_1) (T_3, T_2) (T_7) during the breeding season (T_1, stands for control, T_2 to T_7 represent the treatments).

4. Discussion

The effect of epidermal secretions appears to vary with different species of diatoms. *C. meneghiniana* seems to be less tolerant than *A. coffeaeformis*. A survey on the distribution of diatoms in sediments of the habitat of *P. flava* reveals an abundance of *A. coffeaeformis* and a meagre occurrence of *C. meneghiniana*. The secretions from specimens collected from non-breeding and breeding seasons have differential effect on growth of *C. meneghiniana*, which is indicative of the possible differences in its potency between the two seasons and bears a great ecological significance. The growth rate of *C. meneghiniana* was significantly affected during the non-breeding season. However, during the breeding season most concentrations appeared to be stimulatory. The implication of such an effect is two fold. In the breeding season

by being less toxic, the secretion is not likely to be detrimental to the survival of newly settled delicate larvae. By stimulating increased growth of diatom populations, it may, in fact, help sustain them (the larvae).

The present observation indicates that the habitat of *P. flava* is poorly inhabited as evidenced by limited occurrence of animal species. It has been reported that sea birds of the species of *Calidris* prey upon *P. flava* during low tides, resulting in the snapping of the body, which may possibly trigger copious epidermal secretions. It is probable that these secretions are toxic to other animals and therefore, they may not find this habitat suitable for their colonization. Supportive evidence for this proposition was obtained with *Mytilus viridis* (Azariah and Narayanan, in preparation) whose byssus thread formation was affected significantly by the secretions. Later *M. viridis* succumbed to their toxic effect.

Acknowledgement

We thank Prof. A Mahadevan for laboratory facilities.

References

Azariah J, Ismail M M and Najib M A 1975 Investigations on the ecology and respiratory responses of the Hemichordate *ptychodera flava* to tidal cycles and salinity changes; *Biol. Bull.* **149** 455-466

Bullock T H 1945 The anatomical organization of the nervous system of *Enteropneusta; Q. J. Microsc. Sci.* **86** 55-111

Desikachary T V and Rao V N R 1972 Salinity and diatoms; *J. Mar. Biol. Ass. India* **14** 524-538

Guillard R R L and Ryther J H 1962 Studies of marine planktonic diatoms I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran.; *Can. J. Microbiol.* **8** 229-239

Hyman L H 1959 *The Invertebrates;* Vol. **5** (New York: McGraw-Hill) pp. 1-783

McLachlan J 1973 Growth media-Marine. In *Hand book of Phycological methods* ed. J R Stein (Cambridge: University Press) ch. 2, pp. 25-51

Rao K P 1951 The anatomy of *ptychodera flava* and studies on other Enteropneusta of Madras; Ph.D. Thesis. University of Madras pp. 1-150