A PRELIMINARY STUDY OF THE EFFECTS OF GONYAULAX TAMARENSIS ON FEEDING IN BIVALVE MOLLUSCS

TERRY L. CUCCI*, SANDRA E. SHUMWAY*'**, RICHARD C. NEWELL*** AND CLARICE M. YENTSCH*

*Bigelow Laboratory for Ocean Sciences, W. Boothbay Harbor, Maine 04575,
**Department of Marine Resources, W. Boothbay Harbor, Maine 04575,
***Institute for Marine Environmental Research, Plymouth, England

ABSTRACT

Filter feeding bivalve molluscs accumulate poisons from toxic dinoflagellates such as Gonyaulax tamarensis during feeding. Previous investigators have reported reduced feeding rates in the presence of shellfish toxin and interspecific differences in accumulation levels. Since selective feeding is well documented for several species of bivalves, a series of experiments were carried out using flow cytometry to determine what effects G. tamarensis has on the feeding regimes of several species of molluscs. The results indicate that the effects are species-specific and include 1) a reduction in feeding rate in the presence of G. tamarensis for Mya arenaria and 2) preferential ingestion, digestion and egestion of various phytoplankton species.

INTRODUCTION

Several authors have indicated that various dinoflagellate species have an adverse effect or are actively selected for/against by filter-feeding molluscs. Mytilus californianus has been shown to selectively ingest non-toxic dinoflagellates in natural seawater [1,2], although these cells may pass through the gut unchanged and in viable conditions within the fecal material [2]. Mussels exposed to toxic dinoflagellates usually show a reduction in clearance rates associated with direct toxic effects from the dinoflagellates and not by the toxins released into the water [3]. It has been reported, however, that M. californianus shows a vigorous uptake of toxins when exposed to Gonyaulax catenella [4].

Unlike mussels, other bivalve species have exhibited more negative reactions to the presence of toxic dinoflagellates. Closing of the shell valves [4-8], reduced pumping/filtering rates [4,9,8], and selecting against these toxic dinoflagellates by forming pseudofaeces [4,5] seem to be the major responses of bivalve molluscs when exposed to toxic dinoflagellates.

Further, toxicity levels are known to vary between species at a given locality, e.g. Mytilus edulis were found to be more toxic than Mya arenaria [9]. It has also been observed that it takes approximately 10 days longer for Mya to become toxic than for M. edulis from the same location [10], probably due to the retraction of the siphons demonstrated by Mya [11].

The scope of this paper and our ongoing research is to compare the effects of Gonyaulax tamarensis on feeding in commercially important species of bivalve molluscs. With the addition of G. tamarensis to the food source, our objectives are to determine a) pre-ingestive vs. post-ingestive selection, b) clearance rates, c) preferential selection for or against G. tamarensis and d) the possible role of G. tamarensis as a food organism for those species which actively select for dinoflagellates.

TOXIC DINOFLAGELLATES, Anderson, White, and Baden, Editors

MATERIALS AND METHODS

Ostrea edulis, Placopecten magellanicus, Mya arenaria and Mytilus edulis were collected locally. In addition, a group of M. edulis were collected from the Sakonnet River, Tiverton, R.I. These animals were assumed to have no prior exposure to Gonyaulax tamarensis. Experimental methods are as described in Cucci et al. [12] and Shumway et al. [13] with the additional comment that the same individuals for each species were used in experiments with and without G. tamarensis (clone GT429) added to the food source.

Algal cultures consisted of Thalassiosira pseudonana (clone 3H), Chroomonas salina (clone 3C), Prorocentrum sp. (clone Exuv), and Gonyaulax tamarensis (GT429). Cultures were grown in f/2 media at 15°C with a 14:10 photoperiod. All three clones (3H, 3C, Exuv) were mixed just prior to the experiment to obtain equal cell densities with a final cell concentration of 10^4 cells·ml^{-1} in each of the test jars. GT429, when used, was added to the mixed culture at a cell density of 5×10^5 cells·l^{-1}, simulating bloom conditions.

Cells were analyzed by differences in their fluorescing intensities from the photosynthesizing pigments of chlorophyll (3H, 3C, Exuv, GT429) and phycoerythrin (3C). To analyze the samples, a Coulter EPICS V flow cytometer/sorter was used. Descriptions of analysis methods [12,13] and the general use of flow cytometers [14] are reported elsewhere.

RESULTS AND DISCUSSION

The percent of the total cell count (Fig. 1) for each mollusc species at time zero and subsequent time intervals and concentrations (cells·ml^{-1}) of each of the four algal species were determined. From these data we calculated the reduction in cell concentration·g tissue^{-1}·h^{-1} by each of the bivalve species (Table I). Using the exponential expression from Coughlan [15]:

$$V_w = \frac{M}{n\,t} \log_e \frac{conc_o}{conc_t} \qquad \text{(Equation 1)}$$

where V_w = filtration rate (ml·h^{-1}), M = volume of suspension (ml), n = number of individuals (here n = 1) and $conc_o$ and $conc_t$ = concentration of particles at time zero and time t, respectively, we determined the clearance rates (cells·ℓ$^{-1}$·h^{-1}) and filtration rates (V_w) (Table II) by assuming 100% particle retention efficiency [16].

Ostrea edulis and M. edulis showed relatively little change in clearance rates when exposed to GT429 while increased rates were observed in P. magellanicus. In comparison, M. arenaria was unable to clear cells in the presence of GT429. Filtration rates were slightly higher or remained unchanged for all species except M. arenaria where a 47% reduction occurred.

Figure 2 illustrates a representative sample of bivariate histogram plots of number of cells and log integrated phycoerythrin fluorescence (X) vs. log integrated chlorophyll fluorescence (Y) at time 0 and time 30 min for Ostrea edulis. Also shown are the analyses of the pseudofaeces and faeces resuspended in filtered sea water. There is no preferential clearance of any cell type after 30 min of filtration. Pseudofaeces show all four cell types but a reduction in cell densities of 3C and Exuv. Within the faeces, a predominance of 3H and Exuv, but 3C is absent.

A summary of pre-ingestive and post-ingestive selectivity results are shown in Fig. 1. Selection of food particles has been shown to vary among the four species used in this study [12,13]. With the addition of GT429 to the food source, two changes were observed. First, similar percentages of GT429 to the total cell concentration were observed in the faeces and at

Table I. The decline in cell counts (cells·ml^{-1}) per liter of seawater after 1 h. Values calculated from the difference between cell counts at time 0 and after 60 minutes (calculated regressions lines for cell concentration (Y) versus time in minutes (X) where Y = a + bX) and corrected for volume. Total cell densities at time 0 were 1.05 x 10^3 and 1.0 x 10^3 cells· ml^{-1} for all experiments with and without GT429, respectively.

Species	Dry Tissue (g)	Cell concentrations after 60 min (cells·ml^{-1} x 10^3)				Total cell concentration (cells·ml^{-1} x 10^3)	Reduction in cell conc. per g tissue·h^{-1} (cells·ml x 10^3)
		3H	3C	Exuv	GT429		
Ostrea	0.161 ± 0.035	1.92	2.02	1.84	-	5.78	26.21
Ostrea	0.161 ± 0.035	2.65	0.46	1.03	0.20	4.34	38.26
Placopecten	3.311 ± 0.359	1.68	3.24	2.90	-	7.82	0.66
Placopecten	3.322 ± 0.359	2.32	1.86	0	0	4.18	1.91
Mya	0.694 ± 0.106	2.01	3.05	2.36	-	7.42	3.72
Mya	0.694 ± 0.106	3.02	3.66	3.47	1.05	11.20	0.00
Mytilus (local)	1.461 ± 0.151	*0.68	*0.94	*0.34	-	1.96	11.00
Mytilus (local)	1.461 ± 0.151	*0.30	*0.59	*0.23	*0.06	1.18	12.76
Mytilus (Sakonnet)	4.373 ± 0.735	*1.80	*1.67	*1.58	-	5.27	2.16
Mytilus (Sakonnet)	4.373 ± 0.735	*1.51	*1.80	*1.57	*0.26	5.14	2.44

*cell concentration after 30 min

Table II. The clearance rate and minimum values for V_w and V'_w (filtration rate and weight·specific filtration rates, respectively) estimated assuming 100% retention efficiency of 3H, 3C, Exuv, and GT429. Calculated from Tables I and II, where $V_w = M/n \cdot t \log e (conc_o/conc_t)$ (see Equation 1). Experiments with GT429 introduced into the food source are indicated by (*).

Species	Dry wt (g)	Initial Ration (cells·ml^{-1} x 10^3)	Clearance rate (cells ℓ$^{-1}$·h^{-1} x 10^3)	V_w (ml·h^{-1})	V'_w (ml·h^{-1}·g^{-1})
Ostrea	0.161	1.0	4220	9.17	57.0
Ostrea*	0.161	1.05	6160	11.65	72.4
Placopecten	3.311	1.0	2180	29.40	8.9
Placopecten*	3.311	1.05	5820	35.23	9.7
Mya	0.694	1.0	2580	19.91	28.7
Mya*	0.694	1.05	0	9.31	13.4
Mytilus (local)	1.461	1.0	10000	81.22	55.6
Mytilus (local)*	1.461	1.05	10000	109.29	74.8
Mytilus (Sakonnet)	4.373	1.0	4730	45.68	10.4
Mytilus (Sakonnet)*	4.373	1.05	5360	47.49	10.9

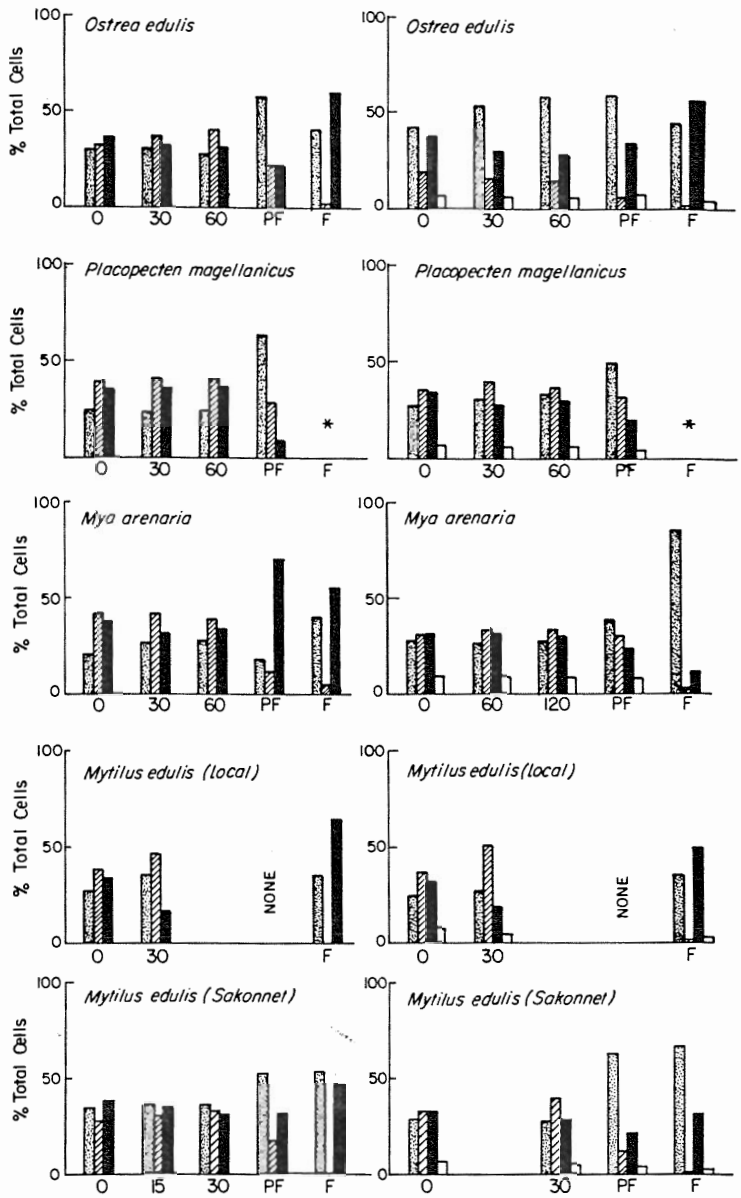

Fig. 1. Summary of the percent of the total cell count within an algal mixture of the clones 3H (stippled bars), 3C (striped bars), Exuv (solid bars) and GT429 (open bars) at time 0 min, subsequent time intervals (15,20,60 or 120 min) and within the pseudofaeces (PF) and faeces (F) during the grazing experiments. Due to the deterioration of the cells in the faeces, data for Placopecten faeces (*) are unreliable.

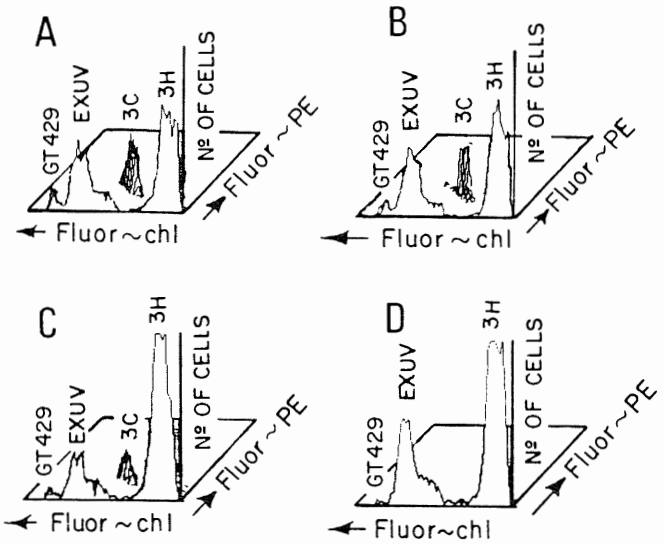

Fig. 2. Bivariate histogram plots of number of cells (Z) and X = fluorescence (approximating phyocerythrin, PE) vs. Y = fluorescence (approximating chlorophyll) comparing relative cell numbers within an algal mixture of 3H, 3C, Exuv and GT429 due to grazing by Ostrea edulis at time 0 min (A), 60 min (B) and within the pseudofaeces (C) and faeces (D) after 60 minutes.

time zero suggesting that these cells were not utilized despite being ingested. Second, Mya showed a drastic change in the proportion of cells in the faeces and pseudofaeces with a marked decrease in dinoflagellates (Exuv and GT429). Of particular note is the absence of GT429 in the faeces. This may be due to either G. tamarensis being (1) selectively rejected during pre-ingestion or (2) that the cells are digested and utilized. Subsequent experiments are being conducted to determine the actual uptake and/or rejection of G. tamarensis by Mya arenaria.

SUMMARY

Preliminary results suggest that the presence of effects of Gonyaulax tamarensis on feeding in bivalve molluscs are species-specific. Feeding rates remained unchanged or slightly increased when G. tamarensis was introduced into the food source of Ostrea edulis, Placopecten magellanicus, and Mytilus edulis. When exposed to G. tamarensis, Mya arenaria showed (1) significant decreases in feeding rates, (2) a reduction in dinoflagellate pre- and post-ingestive selectivity, and (3) a complete absence of G. tamarensis in the faeces.

ACKNOWLEDGEMENTS

This is Bigelow Laboratory contribution number 85014. This research was made possible by ONR grant N00014-81-C-0043, NSF grant OCE-82-13567 and the State of Maine.

LITERATURE CITED

1. H.M. Buley. Bull. Scripps Inst. Oceanogr. Tech. Ser. 4, 19-27 (1936).
2. D.L. Gox and W.R. Coe. J. Exp. Zool. 93, 205-249 (1943).
3. J. Widdows, M.N. Moore, D.M. Lowe and P.N. Salkeld. J. mar. biol. Ass. U.K. 59, 515-528 (1979).
4. J.L. Dupuy and A.K. Sparks. Proc. Nat. Shellfish Assoc. 58, (abstract) (1968).
5. R.J. Smith. Proc. Natl. Shellfish Assoc. 48, 115-124 (1958).
6. D. Ballantine and J.E. Morton. J. mar. biol. Ass. U.K. 35, 241-274 (1956).
7. A.M. Sievers, J. Protozool. 16, 401-404 (1969).
8. S.M. Ray and D.V. Aldrich in: Animal Toxins, Russell and Saunders, eds. (Pergamon Press, New York 1967) pp. 75-83.
9. E.S. Gilfillan and S.A. Hanson. Proc. 1st Internatl. Conf. Toxic Dinoflagellate Blooms, pp. 367-376 (1975).
10. J. Hurst (pers. comm.)
11. S.E. Shumway, T.L. Cucci, L. Gainey and C.M. Yentsch. This volume (1985).
12. T.L. Cucci, S.E. Shumway, R.C. Newell, R. Selvin, R.R.L. Guillard and C.M. Yentsch. Mar. Ecol. Prog. Ser. in press (1985).
13. S.E. Shumway, T.L. Cucci, R.C. Newell and C.M. Yentsch. J. exp. mar. Biol. Ecol. in press (1985).
14. C.M. Yentsch, T.L. Cucci and D.A. Phinney in: Tidal Mixing and Plankton Dynamics, M. Bowman, W. Peterson and C.M. Yentsch, eds. (Springer-Verlag 1985) in press.
15. J. Coughlan. Mar. Biol. 2, 356-358 (1969).
16. R.C. Newell, Biology of Intertidal Animals (Mar. Ecol. Surveys Ltd., Faversham, Kent 1979).