A PRELIMINARY STUDY OF THE BEHAVIORAL AND PHYSIOLOGICAL EFFECTS OF GONYAULAX TAMARENSIS ON BIVALVE MOLLUSCS

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ABSTRACT

In spite of a number of references to the contrary, it is still stated quite frequently and usually dogmatically that dinoflagellate toxins have no effect(s) on the general well-being of the host bivalve molluscs. In light of the apparently conflicting views on this subject, a number of experiments were carried out using commonly occurring bivalve species of the Gulf of Maine. In the presence of Gonyaulax tamarensis, molluscan responses are species-specific and various combinations of the following responses are seen in individual species: shell valve activity is altered; oxygen consumption rates increase/decrease; heart rates become erratic; byssus production in mussels is reduced; and filtration rates decreased/remained unchanged.

INTRODUCTION

While toxic dinoflagellates have attracted considerable numbers of research workers, little attention has been paid to the host organisms per se and it has been generally assumed that the dinoflagellates have little or no effect(s) on the general well-being of these animals [1,2].

Several authors have already reported on adverse effects of shellfish toxin on bivalve molluscs. Adams et al. [3] reported large numbers of the bivalve Venus striatula in a moribund state, gaping and lacking tonus and large numbers of the edible Cerastoderma edule, moribund and lying on the sand surface after a bloom of G. tamarensis in Britain. Later observations at the same area showed that 30% of the C. edule were dead and a further 30% moribund. About 25% of the Macoma balthica were also affected; however, Mytilus edulis, although highly toxic, showed no mortality. Sievers [4] reported similar results for bivalve molluscs exposed to Gonyaulax monilata although the toxins are different. She found that Brachidontes recurvus and Crassostrea virginica were both adversely affected by the toxins and that B. recurvus appeared to be more sensitive than C. virginica to low concentrations of poison. Oysters did not open and mussels failed to produce byssus threads. The feeding rate of some bivalves has been shown to be reduced by the presence of shellfish toxin [5,6]. DeVilliers [7] reported an entire population of Donax serra destroyed by a bloom of Gonyaulax grindleyi. Further reports of the adverse effects of shellfish toxin on bivalve molluscs include morbidity and mortality [8], increased shell valve activity and decreased pumping rate in the oyster, Crassostrea gigas [9] and sluggish, partially paralyzed Mya arenaria [5].

The statement that invertebrates are little affected needs not only verification, but clarification. The purpose of the present study was to determine, for an array of bivalve species, the behavioral and physiological functions which are affected by the presence of the toxic dinoflagellate, Gonyaulax tamarensis (clone GT429).

TOXIC DINOFLAGELLATES, Anderson, White, and Baden, Editors

MATERIALS AND METHODS

Bivalve molluscs (see text) were collected locally and held in running seawater until use in experiments. In addition, Mytilus edulis and Guekensia (Modiolus) demissa were taken from the Sakonnet River, Tiverton, R.I. These animals were assumed to have no prior exposure to Gonyaulax tamarensis. Physiological measurements were all made using standard methods: oxygen consumption (Radiometer electrode) [10], shell valve activity (strain gauge) [11]; heart rate (impedence pneumograph) [12]; and filtration rate (Coulter Counter Model ZM; Flow cytometer EPICS V) [13].

To monitor the immediate effects of GT429 on shell valve activity, animals were attached to a strain gauge and 'normal' activity patterns were obtained after which GT429 were added to the experimental chamber at a concentration of approximately 5 x 10^5 cells$\cdot l^{-1}$. Activity was monitored, as was filtration rate.

The effect of GT429 on oxygen consumption was determined after the animals had been exposed to cell concentrations of 10^5-10^6 l^{-1} for 5d. Initial rates, rates after 5d exposure, as well as the rates after a 1 week recovery period were measured. Heart rates were measured using the same concentrations of GT429. Heart rates were measured each hour after exposure for the first 8 hours and 2 or 3 times a day for the duration of the experiments (4-9 days). In 2 of the experiments, half of the animals were untreated and served as controls. Heart rates were calculated during the period when the heart was actually beating. For individuals that showed rhythmic patterns of cardiac activity followed by cardiac arrest, the rates were calculated for the times when the heart was beating, which overestimated total cardiac output. Mean heart rates were calculated for each individual before and after treatment.

Normal byssus production rates in mussels were monitored visually. Animals were cleaned and placed in filtered seawater to obtain values for normal byssus production rates. These animals were then placed in seawater containing GT429 (10^5 cells$\cdot l^{-1}$) and byssus threads produced were counted over specific time intervals.

All experiments were carried out between 5 and 10°C. After exposure to GT429, toxicity levels for the various bivalve species were determined using the standard mouse bioassay [14].

RESULTS AND DISCUSSION

Actual traces of molluscan shell-valve activity are shown in Figure 1. Responses varied between species and Mytilus edulis (local animals), Spisula solidissima, Arctica islandica and Modiolus modiolus showed no response to the addition of GT429.

Mercenaria mercenaria were initially open with the siphons fully extended. On addition of GT429, siphons became constricted and the shell valves partially closed. Both siphons then slowly retracted and finally the shell valves closed completely. The animal shown represented in Fig. 1 did not re-open until clean seawater was added to the container.

Ostrea edulis normally had the shell valves open and the mantle edges were visible. Addition of GT429 caused partial adduction of the valves, although pumping (filtration) continued. This response was seen in two animals (Fig. 1); two other animals, however, showed no adduction of the shell valves and continued to filter in the presence of GT429.

While local M. edulis showed no response to the addition of GT429, mussels from R.I. exhibited a variety of responses including shell valve closure (Fig. 1), continuance of normal activity (Fig. 1) and a partial closing in some animals. Siphons (exhalent) were seen to be closed and the mantle edges in otherwise 'open' animals were retracted. Copious

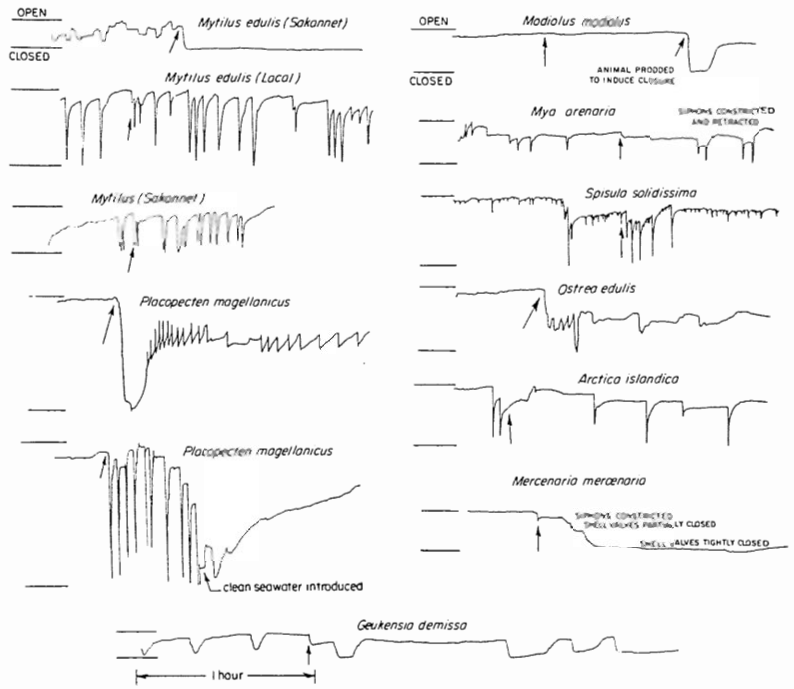

FIG. 1. Traces of shell valve activity for 9 species of bivalve molluscs exposed to GT429. Arrows indicate addition of GT429.

amounts of a white, mucus-like material were produced and 3 animals died from exposure to GT429. Filtration rates were similar to those of local Mytilus ($\sim 8 \times 10^4$ cells·h^{-1}·g dry wt^{-1}) and in 4 animals no filtration occurred.

G. demissa, also from R.I., under normal conditions showed an activity pattern indicating that the animals were 'open' most of the time, and closed for approximately 3 min every 20 min. Figure 1 shows an animal which closed on addition of GT429 and then re-opened after approximately 1h. The animal then closed and showed intermittent opening for the next 22h. This response was seen in 4 mussels. Even in mussels that were open, mantle edges were retracted in the presence of GT429 and no filtering was seen.

Mya arenaria showed more activity associated with the siphons than with the shell-valves. On addition of GT429, most specimens showed withdrawn or partially withdrawn siphons, constricted and/or retracted for extended periods (up to several days in 3 cases). The clams did filter the GT429 ($\sim 7.6 \times 10^3$ cells·h^{-1}·g dry wt^{-1}) and produced copious amounts of pseudofaeces.

Placopecten magellanicus showed the most striking response of any of the species studied. While 2 animals showed no response, the majority [11] exhibited an immediate closure of the shell valves followed by either violent swimming activity, partial sustained shell-valve closure or a combination of the two. On addition of clean seawater, the activity ceased (Fig. 1) and the scallops remained open with the mantle edges and tentacles freely exposed. Scallops exposed to bloom conditions of GT429 for extended periods of time produced copious amounts of a white mucus-like substance and tended to remain at least partially (if not totally)

closed for extended periods of time following the initial bursts of activity. Filtration of GT429 was $\sim 1.7 \times 10^4$ cells$\cdot h^{-1} \cdot g$ dry wt^{-1}.

Thus, we see an array of responses to the presence of Gonyaulax tamarensis ranging from no change in activity to complete isolation from the external environment and, in the case of Placopecten, a swimming/escape response. The fact that emerges here is the existence of a wide range of variable responses at initial exposure to toxic dinoflagellates. If a mollusc 'chooses' not to isolate itself from a potentially noxious external environment, the onus is then on the organism to cope with the impending stress in some other manner. Further studies were then undertaken to study such physiological functions.

Heart rate

Exposure of Mya arenaria to GT429 had no effect on cardiac activity. Traces of cardiac activity appeared normal for up to 15 days after exposure. Moreover, the mean percent original heart rate for 6 individuals was only 103% ± 5.3 which is not different from 100%, i.e. there was no change in cardiac output. The only alterations in heart rates occurred when the siphons were closed, during which time there was no measurable cardiac activity.

Upon exposure to GT429 the 12 M. edulis (local animals) tested exhibited one of 3 grades of responses: maximal, intermediate or minimal. Four individuals exhibited a maximal response (Fig. 2). Between 2 and 4h after exposure, slight irregularities in cardiac activity were noted (Fig. 2b). These irregularities were characterized by the presence of additional 'spikes' superimposed upon the normal pattern of activity. This pattern was followed by pronounced arrhythmias during which it was often impossible to discern any rhythmic activity (Fig. 2c). Within a day after the addition of GT429, a normal pattern of activity resumed, but at a lower rate. This was followed by a burst pattern, in 2 individuals, where the heart would beat for 30s to a minute and then cease for another minute, followed by another burst (Fig. 2e). Three animals died within 2-6 days after exposure. One animal showed burst activity for 9 days after exposure, when the experiment was terminated. Comparison of heart rates after addition of GT429 showed that the mean percent original heart rate was only 75% ± 5.5; which is a significant reduction in cardiac activity.

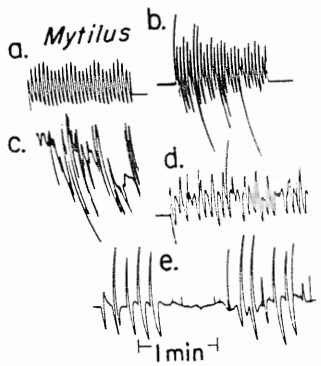

FIG. 2. Cardiac activity in Mytilus edulis exposed to GT429. A: pre-treatment; B: 3h post-treatment; C: 4h post-treatment; D: 5h post-treatment; and E: 4d post-treatment.

Three individuals showed an intermediate response. These animals showed cardiac arrhythmias and burst activity which were indistinguishable from the first group. Recovery occurred within 3 days and cardiac activity stayed normal for the duration of the experiment. There was no significant change in cardiac activity when comparing pre- and post-treatment rates, although the rates were depressed by as much as 50% during the first day following exposure.

Lastly, there were 5 individuals that showed a minimal response to GT429. These animals responded in a manner similar to that in Fig. 2b,i.e. 'spikes' superimposed upon the normal pattern of activity. However, these slight irregularities only lasted for 2-3h after their appearance which was several hours after the addition of GT429. Not surprisingly, there was no significant change in heart rates caused by GT429 in these individuals.

Oxygen consumption

Oxygen consumption rates ($\dot{V}O_2$) (Fig. 3) increased in M. edulis (Sakonnet) and M. arenaria, decreased in P. magellanicus and S. solidissima and remained unchanged in local M. edulis after 5d exposure to GT429. After a one week recovery period in clean seawater, rates returned to normal in M. arenaria and Sakonnet M. edulis but remained low in both S. solidissima and P. magellanicus. Both of these latter species remained highly toxic during this time period. The causes for the alterations in $\dot{V}O_2$ seen in

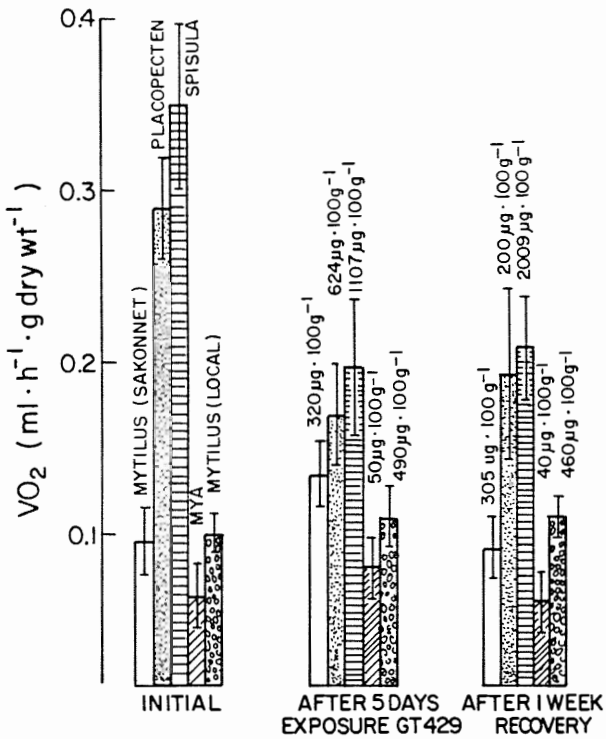

FIG. 3. Oxygen consumption rates for 4 species of bivalve molluscs exposed to GT429.

this study are not at all clear at the present and may be due not only to the presence of GT429 but also to increased/decreased activity levels, variations in feeding rates or repayment of oxygen debts.

Filtration rates

Filtration rates measured before and after the addition of GT429 remained unchanged with the exception of M. arenaria which showed a decrease of 47% after addition of GT429. The effects of GT429 on feeding rates are discussed in detail in a subsequent paper 15 .

Byssus production

Byssus production by M. edulis was reduced from a normal value of 5.67 + 1.33 per day to 1.58 + 1.21 per day in the presence of GT429. Mussels from R.I. failed to produce byssus threads in the presence of GT429. Further studies are in progress with M. edulis and G. demissa.

SUMMARY

The results reported here, although preliminary, all indicate that there is no singular 'bivalve response' to the presence of Gonyaulax tamarensis, but rather a complex array of responses that can occur individually or in combination.

LITERATURE CITED

1. A. Prakash, J.C. Medcof and A.D. Tennant. Bull. Fish. Res. Bd. Can. 168, 68 pp. (1971).
2. D.B. Quayle. Fish. Res. Bd. Can. Bull. 168, 68 pp. (1969).
3. J.A. Adams, D.D. Seaton, J.B. Buchanan and M.R. Longbottom. Nature 220, 24-25 (1968).
4. A.M. Sievers. J. Protozool. 16, 401-404 (1969).
5. E.S. Gilfillan and S.A. Hansen. Proc. 1st Internatl. Conf. Toxic Dinoflagellate Blooms, pp. 367-376 (1975).
6. L. Nishitani and K.K. Chew. Advisory Report, Washington Sea Grant Program (1982).
7. G. DeVilliers. Fish. Bull. S. Afr. 12, 69-74 (1979).
8. H.R. Ingham, J. Mason and P.C. Wood. Nature 220, 25-27 (1968).
9. J.L. Dupuy and A.K. Sparks. Proc. Natl. Shellfish Assn. 58, 2 (1967).
10. S.E. Shumway. Biol. Bull. 160, 332-347 (1981).
11. J.S. Djangmah,S.E. Shumway and J. Davenport. Mar. Biol. 50, 209-213 (1979).
12. E.R. Trueman, J.G. Blatchford, H.D. Jones and G. Lowe. Malacologia 14, 377-383 (1973).
13. T.L. Cucci, S.E. Shumway, R.C. Newell and C.M. Yentsch. Mar. Ecol. Prog. Ser. in press (1985).
14. Association of Official Analytical Chemists in: Official Methods of Analysis, 12th ed. (rev.), (AOAC 1975), pp. 312-321.
15. T.L. Cucci, S.E. Shumway, R.C. Newell and C.M. Yentsch. This volume (1985).

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