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THE EFFECT OF *PROTOGONYAULAX TAMARENSIS* ON BYSSUS PRODUCTION IN *MYTILUS EDULIS* L., *MODIOLUS MODIOLUS* LINNAEUS, 1758 AND *GEUKENSIA DEMISSA* DILLWYN

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Abstract—1. Byssus production in the presence of the toxic dinoflagellate *Protogonyaulax tamarensis* was monitored in *Mytilus edulis*, *Modiolus modiolus* and *Geukensia demissa* from two localities.
2. Byssus production was inhibited in *M. edulis* and *G. demissa* and there were geographic differences.
3. *Modiolus modiolus* was unaffected.
4. Results suggest that the observed response is more likely an indication of impaired activity by the individual animals and not to the presence of toxins *per se* or to the interruption of the byssus function in particular.

INTRODUCTION

Protogonyaulax (=*Gonyaulax*) *tamarensis* is the toxic dinoflagellate (causative organism) commonly associated with the so-called red tides in the western Atlantic. Blooms occur annually on the eastern coasts of Canada and the northeastern United States between May/June and September/October. Many commercially important filter-feeding bivalve molluscs accumulate the toxins in their tissues (predominantly the digestive gland) rendering them vectors of paralytic shellfish poisoning (PSP) and unfit for human consumption. PSP is a serious, sometimes fatal illness induced by consuming shellfish that have ingested quantities of toxic dinoflagellates and has been extensively reviewed (see Russell, 1965; Halstead, 1965; Schantz, 1975).

While the toxic dinoflagellates and the threat to the public health caused by their presence have been widely studied (see LoCicero, 1975; Taylor and Seliger, 1979; Anderson *et al.*, 1985), very little attention has been given to the effects of these organisms on their molluscan hosts. Many workers have assumed that the dinoflagellates have little or no effect(s) on the general well-being of these animals (e.g. Prakash *et al.*, 1971; Quayle, 1969). In a recent series of papers (Shumway *et al.*, 1985a,b; Cucci *et al.*, 1985; Shumway and Cucci, 1986; Shumway and Gainey, 1986), it has been shown that bivalve molluscs exhibit an array of adaptations and responses to the presence of *P. tamarensis* which range from altered shell valve activity, erratic heart beats, increased/decreased rates of oxygen consumption and filtration rates to the preferential ingestion, digestion and egestion of various phytoplankton species including *P. tamarensis*.

Production of byssal threads is an easily measured and informative index of activity in the Mytilidae. Byssus production in mussels (*Mytilus edulis*) is affected by a number of factors including temperature (Glaus, 1968; van Winkle, 1970; Allen *et al.*, 1976; Young, 1985), excision (Young, 1985), salinity (Glaus, 1968; van Winkle, 1970; Allen *et al.*, 1976; Reish and Ayers, 1968), tidal regime (Young, 1985), seasonality (Price, 1983; Young, 1985), agitation (van Winkle, 1970; Young, 1985), pollutants (Martin *et al.*, 1975; Carr and Linden, 1984; Carr and Reish, 1978), dissolved oxygen concentration (Reish and Ayers, 1968), current (Maheo, 1970; van Winkle, 1970; Priced, 1980 and Glaus, 1968). Fewer data are available for *Geukensia* (=*Modiolus*) *demissa*. Van Winkle (1970) has shown byssus production in this species to be effected by aerial exposure, agitation, salinity, body size and current.

In the present study, we have investigated the effect of *P. tamarensis* on byssus production in three species of mussels. This study is part of an integrated study of the effects of *Protogonyaulax tamarensis* on the behavior and physiology of a number of commercially important bivalve molluscs.

MATERIALS AND METHODS

Specimens of three species of mussels were collected from various areas in Maine and Rhode Island: *Mytilus edulis* (Boothbay Harbor, Maine and Sakonnet River, Tiverton, RI), *Modiolus modiolus* (Damariscotta River, Maine) and *Geukensia demissa* (Sakonnet River, Tiverton, RI). Animals of a similar size range for any given species (50–60 mm length *M. edulis*; 50–80 mm *M. modiolus* and *G. demissa*) were used to eliminate the effect of body size on byssus production demonstrated previously (Allen *et al.*, 1976; van Winkle, 1970; Glaus, 1968; Reish and Ayers, 1968). Animals were cleaned of epiphytes and existing byssal threads were carefully cut to avoid injury to the byssal gland. It has been shown by van Winkle (1970) that this procedure does not affect subsequent byssus formation. Mussels were maintained in tanks supplied with seawater from Boothbay Harbor. Temperature was maintained at 15°C. Animals were used within one week of collection and no supplementary food was provided. Animals were individ-

Table 1. The production of byssus threads by three species of mussels before (control) after exposure to *Protogonyaulax tamarensis* and after a 1 week recovery period. Results are expressed as number of threads produced per mussel per day ± standard deviation

Species	*n*	Control	After exposure to GT429	After recovery
Mytilus edulis (Maine)	94	15.5 ± 2.1	7.1 ± 3.4*	13.4 ± 4.5
Mytilus edulis (RI)	91	6.2 ± 1.2	1.9 ± 0.8†	2.4 ± 1.0†
Modiolus modiolus	92	21.9 ± 4.9	23.2 ± 3.5	23.6 ± 4.8
Geukensia demissa	86	19.4 ± 4.1	10.6 ± 1.1*	16.3 ± 3.3

*Significantly different from control at $p < 0.05$.
†Significantly different from control at $p < 0.01$.

ually numbered using B-dots (Graze: Endersbach). All experiments were carried out at 15°C.

It has been shown (van Winkle, 1940) that mussels are more prone to produce byssus threads after exposure to air. Consequently, mussels in the present study were exposed to air (15°C) for 12 hr prior to beginning the experiments. Subsequent to aerial exposure, mussels were placed in seawater and byssus production was monitored over the following 24 hr period (controls). The same individuals were again exposed to air for 12 hr after which they were placed in seawater containing the toxic dinoflagellate, *Protogonyaulax tamarensis* (clone GT429) at a concentration of approximately 10^6 cells l^{-1}. This concentration approximates those encountered during natural blooms of the dinoflagellate. Cultures of GT429 were provided by the Provosolli–Guillard Culture Center for Marine Phytoplankton. Again, byssus production was monitored for 24 hr. Following exposure to GT429, some mussels were once again exposed to air for 12 hr and then returned to filtered seawater for a 1 week recovery period; others were sacrificed to obtain dry tissue weights. Byssus production was again monitored for 24 hr following the 1 week recovery period for comparison with original production values. Tanks were gently aerated during the experiments.

Previous authors have shown that a substantial number of mussels may not produce byssus threads. Preliminary experiments showed that, as in previous studies (see Young, 1985), individual mussels consistently produced a similar number of threads in a given period of time; however, there was considerable variations between individuals. In addition, some mussels failed to produce byssus. Consequently, only animals which produced byssus threads during the initial control experiments were used in subsequent experiments with GT429.

RESULTS

The data presented in Table 1 clearly indicate that byssus production is inhibited in the presence of GT429 in *M. edulis* (both RI and Maine specimens) and *G. demissa*. *M. modiolus* was unaffected. These results represent short term measurements; long term effects were not measured. Red-tide blooms are sporadic and may or may not be persistent. Both *M. edulis* (Maine) and *Geukensia demissa* showed a return to normal rates of byssus production after one week recovery period. *M. edulis* (R.I.) however, did not recover. These results are in keeping with those of previous studies using animals from the same geographic areas (see Discussion).

Numbers of threads produced per day by *Mytilus edulis* and *G. demissa* are similar to those reported in other studies (Glaus, 1968; van Winkle, 1970). No data are available on byssus production in *M. modiolus*.

DISCUSSION

Byssus threads of the Mytilacea are well developed and the threads not only secure the molluscs to the substratum, but also play an important role in locomotion and orientation (Allen *et al.*, 1976). The ability to form byssus threads is crucial to the survival of the species. While a number of authors have reported on the factors affecting byssus production in mussels (see Introduction), none have commented on the possible reasons for the observed changes.

The structure and formation of the byssus complex in *Mytilus* has been recently reviewed by Price (1983). In its simplest terms, the process of byssus formation involves three glands, the phenol gland, the enzyme (or accessory) gland and the collagen (or white) gland. The major products associated with each of these glands are orthodiphenol, polyphenoloxidase and protein (collagen) respectively. It is possible that the presence of the toxic dinoflagellate, *P. tamarensis*, and/or its associated toxins (e.g. saxitoxin, gonyautoxin) could interfere with one or more of these processes. However, since only two of the three species studied here showed a response to the presence of GT429, the possibility of a general chemical response would seem unlikely.

Van Winkle (1970) reported that byssus thread formation, pumping and opening of valves in *M. demissus* were inhibited by red tides. The causative organism(s) of these red tides were not identified but were most likely non-toxic dinoflagellates of *Gonyaulax* spp. (Castagna, Hawes, personal communication). Thus, the observed responses in his study were most likely the result of reduced oxygen concentrations or increased levels of metabolic end products commonly associated with such blooms (van Winkle, 1970; Reish and Ayers, 1968). Populations of *M. edulis* in Los Alamitos Bay, California were reported to be periodically decimated by the outbreak of red tide blooms (*Gonyaulax polyhedra*) (Carr and Reish, 1978), but it is still not clear whether these animals were affected directly by an accumulation of toxins from the dinoflagellates or indirectly by the decrease of dissolved oxygen due to bacterial decomposition of organic material.

The results presented here clearly indicate that the presence of *P. tamarensis* inhibits byssus thread production in *M. edulis* and *G. demissa*. This reduced production is more likely an indication of impaired activity by the individual species and the results are in agreement with previous findings for these species with regard to other behavioral/physiological responses. These results also confirm preliminary

results on the production of byssus threads reported earlier (Shumway *et al.*, 1985).

In a series of previous publications (Shumway *et al.*, 1985a,b; Cucci *et al.*, 1985; Shumway and Cucci, 1986; Shumway and Gainey, 1986), it has been shown that *Modiolus modiolus* were totally unaffected by the presence of GT429. *Mytilus edulis* from Maine waters showed a reduction in heart rate in the presence of GT429, however, the significance of this finding is still not clear. *Mytilus edulis* from RI waters, however, did not fare as well as their counterparts from Maine. When exposed to GT429, the mussels from RI exhibited a variety of responses including shell valve closure, closure of the exhalent siphons, retraction of the mantle edges, production of copious amounts of a white, mucus-like material and some mortality. *G. demissa*, also from RI waters, exhibited similar responses, i.e. shell valve closure with intermittent opening, retraction of the mantle edges and a reduction or cessation of filtration rate. Again, copious amounts of pseudofeces and a white mucus-like material were produced. Thus, decrease in thread production is not simply a matter of the animal(s) being isolated from the external environment. Previous studies using *Mytilus*, *Modiolus* and *G. demissa* from these same geographic areas indicate that they remain open and exposed to the dinoflagellate during exposure periods.

It seems likely, assuming that there is no chemical interruption of the byssus production, that the responses observed here are indicative of the 'whole animal' response to the presence of the dinoflagellate cells rather than to the presence of toxins *per se* or the interruption of the byssus function in particular. Carr and Reish (1978) previously stated that byssal production is "a reasonable parameter with which to gauge the metabolic activity or physiological functioning of *M. edulis*". To this we would add "and to other byssus forming molluscs".

The observed reduction of byssus production in *M. edulis* and *G. demissa* provides a further indicator of the level of stress invoked by the presence of the toxic dinoflagellate, *P. tamarensis*, on these species.

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REFERENCES

Allen J. A., Cook M., Jackson D. J., Preston S. and Worth E. M. (1976) Observations on the rate of production and mechanical properties of the byssus threads of *Mytilus edulis* L. *J. moll. Stud.* **42**, 279–289.

Anderson D. M., White A. W. and Baden D. G. (1965) *Toxic Dinoflagellates*. Elsevier, North Holland.

Carr R. S. and Linden O. (1984) Bioenergetic responses of *Gammarus salinus* and *Mytilus edulis* to oil and oil dispersants in a model ecosystem. *Mar. Ecol. Prog. Ser.* **19**, 285–291.

Carr R. S. and Reish D. J. (1978) Studies on the *Mytilus edulis* community in Alamitos Bay, California: VII: The influence of water-soluble petroleum hydrocarbons on byssal thread formation. *The Veliger* **21** 283–287.

Cucci T. L., Shumway S. E., Newell R. C. and Yentsch C. M. (1985) A preliminary study of the effects of *Gonyaulax tamarensis* on feeding in bivalve molluscs. In *Toxic Dinoflagellates* (Edited by Anderson, D. M., White A. W. and Baden D. G.), pp. 395–400. Elsevier, North Holland.

Glaus K. J. (1968) Factors inflencing the production of byssus threads in *Mytilus edulis*. *Biol. Bull.* **135**, 420.

Halstead B. W. (1965) *Poisonous and Venomous Marine Animals of the World I. Invertebrates*. Washington, D.C., U.S. Govt. Printing Office.

LoCicero V. R. (1975) *Proceedings of the First International Conference on Toxic Dinoflagellate Blooms*. Mass. Science and Technology Foundation, Wakefield.

Maheo R. (1970) Etude de la posse et de l'activite de secretion du byssus de *Mytilus edulis* L. *Cah. Biol. Mar.* **11**, 475–483.

Martin J. J., Piltz F. M. and Reish D. J. (1975) Studies on the *Mytilus edulis* community in Alamitos Bay, California V: The effects of heavy metals on byssal thread production. *The Veliger* **18**, 183–188.

Prakash A., Medcof J. C. and Tennant A. D. (1971) Paralytic shellfish poisoning in eastern Canada. *Bull. Fish. Res. Bd Can.* **177**, 1–87.

Price H. A. (1983) Structure and formation of the byssus complex in *Mytilus* (Mollusca, Bivalvia). *J. moll. Stud.* **49**, 9–17.

Quayle D. B. (1969) Paralytic shellfish poisoning in British Columbia. *Fish. Res. Bd Can. Bull.* **168**, 1–68.

Reish D. J. and Ayers J. L. (1968) Studies on the *Mytilus edulis* community in Alamitos Bay, California III. The effects of reduced dissolved oxygen and chlorinity concentrations on survival and byssus thread formation. *The Veliger* **10**, 384–388.

Russell F. E. (1965) Marine toxins and venomous and poisonous marine animals. In *Advances in Marine Biology* (Edited by Russell F. S.) Vol. 3, pp. 225–384. Academic Press, New York.

Russell F. E. (1984) Marine toxins and venomous and poisonous marine plants and animals (Invertebrates). In *Advances in Marine Biology* (Edited by Blaxter J. H. S., Sir F. S. Russell and Sir M. Yonge), pp. 59–217. Academic Press, London.

Schantz E. J. (1975) Poisonous red tide organisms. *Environmental Lett.* **9**, 225–237.

Shumway S. E., Cucci T. L., Newell R. C. and Yentsch C. M. (1985a) Particle selection ingestion and absorption in filter feeding bivalves. *J. Exp. mar. Biol. Ecol.* **91**, 77–92.

Shumway S. E., Cucci T. L., Gainey L. and Yentsch C. M. (1985b) A preliminary study of the behavioral and physiological effects of *Gonyaulax tamarensis* on bivalve molluscs. In *Toxic Dinoflagellates* (Edited by Anderson D. M., White A. W. and Bader D. G.), pp. 389–394. Elsevier, North Holland.

Shumway S. E. and Cucci T. L. (in press) The effects of the toxic dinoflagellate *Protogonyaulax tamarensis* on the feeding and behavior of bivalve molluscs. *Aquatic Toxicology*.

Shumway S. E. and Gainey L. F. (in press) A review of the physiological effects of toxic dinoflagellates on bivalve molluscs. *Proc. Unitas Malacologia*.

Taylor D. L. and Seliger H. H. (1979) *Toxic Dinoflagellate Blooms*. Elsevier, North Holland.

Young G. A. (1985) Byssus thread formation by the mussel *Mytilus edulis*: effects of environmental factors. *Mar. Ecol. Prog. Ser.* **24**, 261–271.

Van Winkle W. (1970) Effect of environmental factors on byssal thread formation. *Mar. Biol.* **7**, 143–148.