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Possible cytotoxic effects of the dinoflagellate, *Gyrodinium aureolum*, on juvenile bivalve molluscs

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Juveniles of eight commercially important species of bivalve molluscs (*Spisula solidissima, Argopecten irradians, Crassostrea virginica, Mytilus edulis, Mya arenaria, Ostrea edulis, Mercenaria mercenaria, Placopecten magellanicus*) were exposed in the laboratory to the commonly occurring dinoflagellate, *Gyrodinium aureolum*. Histological analyses of gut tissues indicated that the impact of *G. aureolum* on the shellfish was species-specific. High rates of mortality were noted in the bay scallop, *A. irradians*, but not in other molluscan species. There were no pathological differences between control animals and animals fed *G. aureolum* in *S. solidissima, M. arenaria,* or *M. mercenaria*. The most severely affected molluscs were *C. virginica* and *A. irradians. C. virginica* did not exhibit differences in digestive gland parameters between control and experimental animals; however, several animals did show significant mantle and gill lesions. Bay scallops exhibited decreased height of absorptive cells and increased lumen diameter after exposure to *Gyrodinium* suggesting, at least, poor food quality of *Gyrodinium*. Evidence of toxic effects was not identified in the digestive gland. Several bay scallops also showed variable amounts of inflammation in the kidney associated with protozoal infestations and variable amounts of predominately rod-shaped bacteria within the urinary space. Aquaculturists, especially of scallop species, should monitor for the presence of *G. aureolum*. Given its large size (25–30 μm), *G. aureolum* could be filtered from incoming water to hatcheries, thus avoiding mass mortalities of spat and juvenile scallops.

KEYWORDS: Dinoflagellate (*Gyrodinium*), Molluscs, Shellfish, Toxins

INTRODUCTION

The dinoflagellate *Gyrodinium aureolum* has frequently been associated with fish kills (Jones *et al*., 1982; Roberts *et al*., 1982; Turner *et al*., 1987) and mortalities of other marine invertebrates (Partensky *et al*., 1989). While this dinoflagellate has not been indicated in any outbreaks associated with human illnesses, it has been shown to cause mortalities in a number of shellfish species (Shumway, 1990; Table 1). Recently, *G. aureolum* was associated with a massive shellfish kill in Maquoit Bay, Brunswick, Maine (Heinig and Campbell, 1992); however, the specific cause of death was not delineated.

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Previous studies on the impact of *G. aureolum* on shellfish have demonstrated mortality in juveniles (Helm *et al*., 1974; Tangen, 1977; Lassus and Berthome, 1988; Erard-LeDenn *et al*., 1990), reduced shell growth (Nielsen and Strömgren, 1991), reduced clearance rates (Shumway, unpublished results), and marked cellular damage to the gut (Widdows *et al*., 1979). *Gyrodinium aureolum* has also been shown to inhibit feeding in the postlarvae of the great scallop, *Pecten maximus*, and to cause mortalities in juvenile scallops (Lassus and Berthome, 1988). Recent unexplained mortalities of hatchery-reared, juvenile oysters, *Crassostrea virginica*, co-occurred with a bloom of another closely related species of dinoflagellate, *Gymnodinium sanguineum*. This species of dinoflagellate has not, however, been previously demonstrated to be toxic to bivalves and was not directly linked to the oyster mortalities (Bricelj *et al*., 1992). Susan Ford and co-workers (Rutgers University, personal communication) are currently investigating the hypothesis that unexplained juvenile oyster mortalities are caused by a toxin, probably of bacterial or microalgal origin, which irritates the mantle edge, causing it to retract and attempt to 'wall-off' the soft tissues by secretion of a conchiolin barrier with subsequent damage to the mantle epithelium. Recent studies showed exposure to *G. aureolum* to be lethal to several species of juvenile shellfish (Lesser and

TABLE 1. Effects of *Gyrodinium* spp. on shellfish

Gyrodinium species	Shellfish species	Effect	Reference
G. aureolum	*Pecten maximum*	Cessation of feeding in postlarvae; mortality in juveniles	ICES (1988); Lassus and Berthome (1988)
G. spirale	'Shellfish species'	Mortalities	ICES (1988)
G. aureolum	Clams	Mortalities	ICES (1988)
G. aureolum	*Pecten maximus*	Mortalities of postlarvae; formation of 'stress rings' by adults and juveniles; reduced filtration rates	Erard-LeDenn *et al*. (1990)
G. aureolum	*Crassostrea gigas*	Reduced larval survival	Helm *et al*. (1974)
G. aureolum	*Mytilus edulis*	Some mussel deaths	Tangen (1977)
G. aureolum	*Pecten maximus*	Decline in numbers of larvae during bloom	Minchin (1984)
G. aureolum	*Mytilus edulis*	Reduced clearance rates; marked cellular damage to gut	Widdows *et al*. (1979)
G. aureolum	*Mytilus edulus*	Significant reduction in growth rate	Nielsen and Strömgren (1991)
G. aureolum	*Mesodesma mactroides*	Mortalities	Rosa and Buselato (1981) (see also Odebrecht *et al*., 1995)
G. aureolum	*Mercenaria mercenaria* *Argopecten irradians* *Crassostrea virginica* *Spisula solidissima*	Mortality (dependent on temperature and length of exposure)	Lesser and Shumway (1993)

Shumway, 1993). Feeding inhibition and survival of eight species of juvenile shellfish exposed to *G. aureolum* were shown to be species-specific and temperature dependent, with the bay scallop, *Argopecten irradians*, being one of the most sensitive species. There is some evidence that the harmful effects of *G. aureolum* can be reversed if animals are returned to clean seawater before permanent damage has taken place (Widdows *et al.*, 1979; Erard-LeDenn *et al.*, 1990).

Specific toxins associated with *G. aureolum* have not been clearly identified. Partensky *et al.* (1989), Gentien and Arzul (1990) and Gentien *et al.* (1991) have all shown that *G. aureolum* produces toxins and Partensky *et al.* (1989) confirmed the presence of at least one fat-soluble cytotoxin. Gentien and Arzul (1990) determined that the toxic action proceeds from two different processes which are possibly associated with two types of toxic compounds. Yasumoto *et al.* (1990) have recently isolated two compounds known to be haemolytic and ichthyotoxic, 1-acyl-3-diga-lactosylglycerol and octadecapentaenoic acid, from *Gyrodinium aureolum*.

The current survey was undertaken as a preliminary study to: (1) determine the possible cytotoxic effects of exposure to *Gyrodinium aureolum* on juvenile, commercially important shellfish from the Gulf of Maine (surfclam, *Spisula solidissima*; blue mussel, *Mytilus edulis*; sea scallop, *Placopecten magellanicus*; softshell, *Mya arenaria*; eastern oyster, *Crassostrea virginica*; and European oyster, *Ostrea edulis*); and (2) assess possible reversibility of any cellular damage. These species were chosen because they are either cultured or fished commercially in Gulf of Maine waters. Only juveniles were used in the experiments as they were expected to be the most susceptible to any toxins present and because they represent a crucial stage of the life history.

MATERIALS AND METHODS

Juvenile shellfish (all <15 mm shell length) were supplied by Mook Sea Farms, Damariscotta, Maine and Beal's Island Hatchery, Beal's Island, Maine. All animals were held for 2 days in filtered seawater (0.45 μm) prior to use in experiments to void the guts of any previously ingested materials. All experiments were carried out at 15 °C. A total of 30 animals were placed in 4 l of seawater for exposure to *G. aureolum*. Animals were exposed to bloom conditions (initial concentration of 10^5 cells l^{-1}) of *Gyrodinium aureolum* daily for 1 week. Animals were allowed to feed for 24 h. The seawater and remaining cells were siphoned out and a new supply of algae was added to the container. Control animals were maintained at bloom levels (10^5 cells l^{-1}) of both a non-toxic dinoflagellate (PLY173) and the alga T-*Isochrysis* (TISO), commonly used in culture operations. To monitor any recovery, one-half of the survivors were placed in filtered seawater (0.45 μm) and provided with the isolate TISO. The other half were killed and preserved in Davidson's solution for subsequent histological analyses of gut tissues. At the end of 1 week, recovering animals were also killed and preserved in Davidson's solution for histological analyses.

Fixed animals were transported to Woods Hole in Davidson's solution. Shellfish were sorted by treatment (3 day and 7 day controls, 3 day and 7 day dinoflagellate fed, and 7 day and 14 day post-dinoflagellate feeding) and by species. Animals were

processed in paraffin and 6 μm sections were stained with haematoxylin and eosin. Each animal was evaluated separately.

Animals were examined first by species and then by treatment group. Results from each treatment group within that species were compared. Comparisons were made using a *t*-test assuming unequal variances.

RESULTS AND DISCUSSION

Mercenaria mercenaria, Mya arenaria, Spisula solidissima

No pathological differences were noted between control (exposed to TISO or PLY173) animals and *Gyrodinium*-fed animals at any time period.

Argopecten irradians

Gyrodinium aureolum appears to have a marked effect on the digestive gland of these scallops (Fig. 1). Decreased height of absorptive cells and increased lumen diameter strongly suggest, at least, poor food quality of *Gyrodinium*. Evidence of toxic effects such as necrosis and sloughing of digestive gland epithelial cells is not, however, identified in the digestive gland. Average heights of absorptive cell epithelium are given in Table 2.

Several (3/5 of 3 day treated animals and 2/7 of 7 day treated animals) treated but no control animals (0/4) showed variable amounts of inflammation in the kidney associated with protozoal infestations and variable amounts of predominantly rod-shaped bacteria within the urinary space. Inflammation consisted of haemocyte infiltration into the sinusoids surrounding the kidney epithelium with attenuation of the epithelium and walling off of some urinary lumens by transformed (flattened) haemocytes. It is not clear if the kidney lesions are a result of debilitation of the scallops (resulting in secondary renal bacterial fouling), coexistent, causative for the digestive changes or caused by dinoflagellate feeding (possible renal toxin).

Crassostrea virginica

Several (3/5 of 3 day treated animals and 2/7 of 7 day treated animals treated but no control animals (0/4) showed variable amounts of inflammation in the kidney. There does not appear to be any notable difference between digestive gland parameters in controls vs. animals exposed to *Gyrodinium*.

Several treated animals did, however, show significant mantle and gill lesions (1/2 of 3 day controls, 1/6 of 3 day treated, 2/3 of 7 day controls, 3/7 of 7 day treated and 1/2 of 1 week post-treatment). Lesions within the shell epithelial portion of the mantles are characterized by focal epithelial hyperplasia associated with accumulation of inflammatory cells in the underlying sinusoids and infiltration of epithelium by moderate to significant numbers of haemocytes (Fig. 2). The occurrence of coccoid bodies is noted throughout the lesions but especially within the most severely affected epithelia. These bodies sometimes appear to be present in multiple numbers within cells (phagocytes) and may represent serial phagocytosis by haemocytes. Coccoid bodies are between 3 and 8 μm in diameter with condensed pycnotic-appearing nuclei and eosinophilic cytoplasm. Lesions affecting the water cavity epithelial surface of the mantle are characterized by similar but

less inflammation, with the presence of coccoid bodies within the epithelium and rod-shaped bacteria along the lumenal surface of the epithelium. Lesions within the

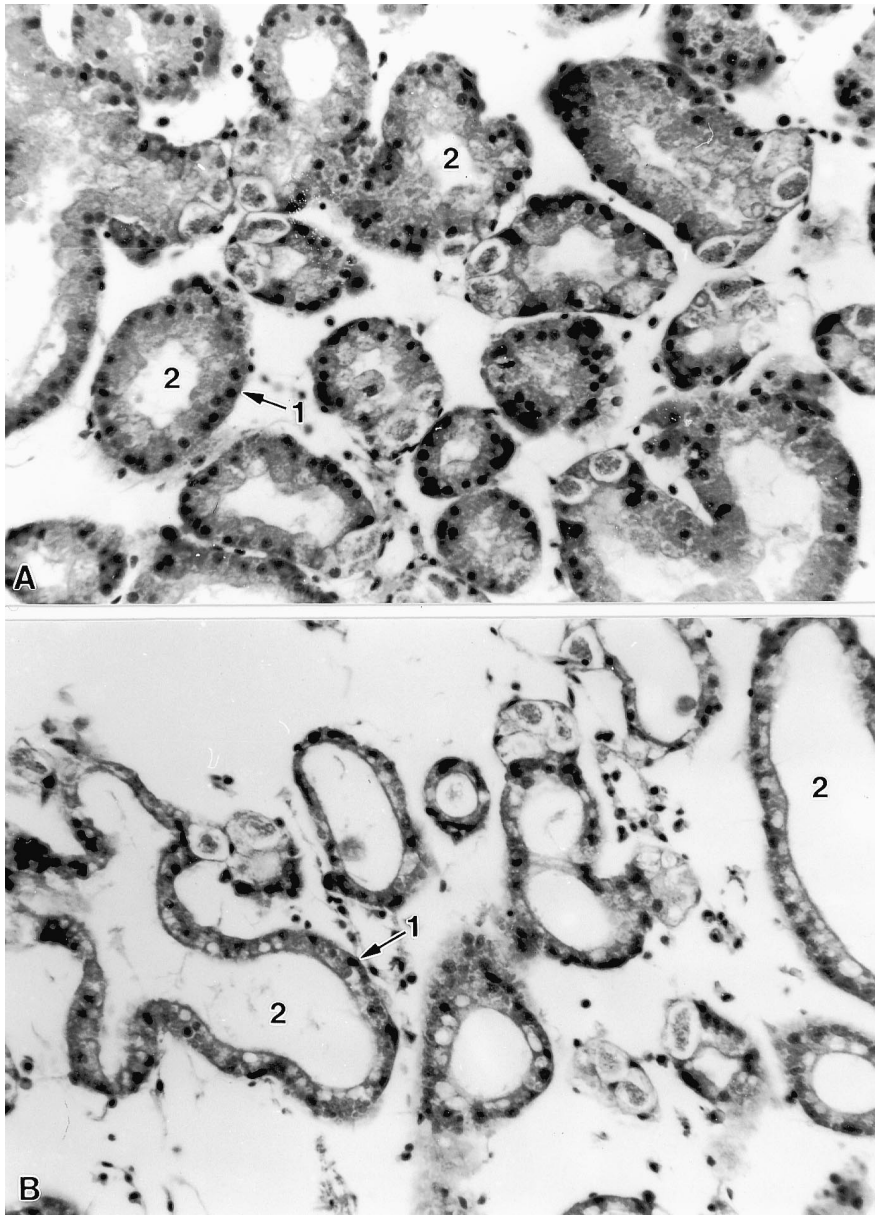

FIG. 1. *Argopecten irradians* digestive gland. (A: 3 day fed a control diet) Absorptive cells are columnar and finely vacuolated. (B: 3 day fed the dinoflagellate *G. aureolum*) Absorptive cells contain large vacuoles and are cuboidal. Lumen diameters are enlarged. (1, absorptive cells; 2, lumens; H&E, 400 ×).

TABLE 2. Average heights (μm) of absorptive cells epithelium (ACE) and average diameter (μm) of digestive tubule lumens (DTL) of mussels, *Mytilus edulis*, and scallops, *Argopecten irradians*, fed the dinoflagellate *Gyrodinium aureolum*. Asterisks (*) indicate significant differences between treated and control animals at $p < 0.05$. Number of samples (*n*) given in parentheses

	3 day control		3 day *Gyrodinium* fed		7 day control		7 day *Gyrodinium* fed		1 week recovery	
Bivalve species	ACE	DTL	ACE	DTL	ACE	DTL	ACE	DTL	ACE	DTL
Argopecten irradians	7.3 (2)	4.2 (2)	*5.6 (5)	*8.7 (5)	7.7 (3)	3.6 (3)	*4.5 (7)	*6.7 (7)	6.8 (2)	*3.4 (2)
Mytilus edulis	12.6 (3)	1.5 (3)	*6.7 (6)	*6.8 (6)	14.9 (3)	3.3 (3)	*11.0 (7)	4.0 (7)	13.8 (1)	4.0 (1)

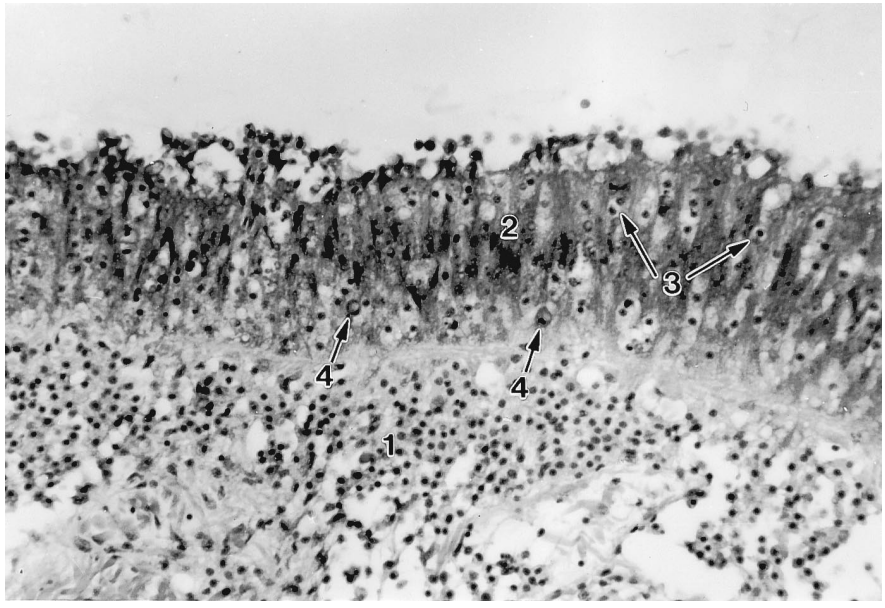

FIG. 2. *Crassostrea virginica* mantle with JOD-like disease. Within the subepithelial connective tissue (1) and the mantle epithelium (2) are numerous haemocytes (3). Coccoid bodies are also present in the epithelium (4). (H&E, 400 ×).

gill (1) are characterized by variable amounts of inflammation associated with rod-shaped bacteria in the water lumens, necrosis of associated water tubule epithelium, and the presence of variable numbers of coccoid bodies and haemocytes within epithelia. Coccoid bodies are also present in the intestinal epithelium in some animals. What the coccoid bodies represent is unknown but they could be toxic haemocytes. Electron microscopic examination is needed to identify the bodies. The shell epithelial lesions resemble those described for juvenile oyster disease syndrome as seen at the Flowers Hatchery. Other observations, such as gill inflammation and the occurrence of coccoid bodies in the intestinal epithelium, have not been described in this syndrome by other investigators but appear to be part of it, at least in this group of animals.

A second type of gill lesion (2) is characterized by proliferation of eosinophilic cells, mixed with lipocyte-like cells overlaid by epithelium. These foci protrude into the water tubule. Bacteria and coccoid bodies are not associated with this lesion. This lesion could represent a healing phase of a previous insult, or a proliferative reaction due to a chronic insult to the epithelium.

Histological evidence shows that shell epithelial lesions were present only in animals fed dinoflagellates. However, gill lesions were present in both dinoflagellate-fed and control animals. Because of the low number of control animals examined, it is possible that shell epithelial lesions in the control animals may have been missed. Therefore, the association of *Gyrodinium* food with the appearance of mantle lesions is questionable.

Mytilus edulis

There appears to be a difference in digestive gland parameters between the 3 day control and 3 day fed animals which no longer exists by the end of 7 days (Table 2). This difference may represent an adjustment by the mussels fed *Gyrodinium* to this new food type.

Additionally, several of the animals showed varying degrees of melanotic foci within the sinusoids surrounding the digestive tubules. These are characterized by brown cells intermixed with other haemocytes in clumps. The cause of these inflammatory foci is unknown but they are present in control and fed animals.

Additional lesions of interest were noted in one mussel, which appeared to be leukemic and contained multifocal brown cells (haemocytes with residual phagocytized material) in the mantle epithelium and in the sinusoids (cause undetermined).

Ostrea edulis

A possible effect of exposure to *Gyrodinium* is suggested from examination of the height of the absorptive cells in fed animals vs. that of control animals, but there are not enough control animals to establish adequately the normal height.

Both of the 7 day control animals show severe necrosis of the central area of the digestive gland associated with numerous bacteria and some ciliates (parasites in the digestive gland). Bacteria are also seen in other areas of these animals, especially in the sinusoid. Additionally, foci of mild to severe inflammation were seen in interstitial tissues around the digestive glands and in the palps in one animal. It appeared that while most of this necrosis is actually autolysis (occurred after death), a portion of it was present before death and represented a real bacterial infection of these animals. Slow fixation might account for the additional severe localized per-death necrosis/autolysis of the digestive gland epithelium. These lesions were not seen in *C. virginica*.

Placopecten magellanicus

The data suggest a difference between 3 day and 7 day control and animals fed *Gyrodinium* in values for the height of the absorptive cell epithelium and lumen size, but values are close and the difference is not significant.

CONCLUSIONS

1. Bay scallops appear to be affected significantly by feeding on the dinoflagellate *Gyrodinium aureolum*. Sea scallops may be similarly, if less severely, affected.
2. Mussels may be affected but appear to recover, perhaps due to a change in the ability of the animals to digest the dinoflagellate or to some other unidentified adaptation.
3. Numbers of animals examined were low (this work is a survey and not an in-depth evaluation) and the reaction of scallops, especially bay scallops, to feeding on dinoflagellates needs to be further evaluated.
4. The presence of shell epithelial lesions in the oysters (*C. virginica*) was unexpected. Whether the lesions occurred as a result of animals feeding on

Gyrodinium, or were unrelated and occurred coincidentally in the population in this study needs to be better examined.

5. The association of coccoid bodies with bacterial infections seen in some tissues may also be significant.

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