Vol. 63: 177–188, 1990 | MARINE ECOLOGY PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published May 29

Uptake kinetics of paralytic shellfish toxins from the dinoflagellate *Alexandrium fundyense* in the mussel *Mytilus edulis**

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ABSTRACT: A laboratory study investigated cell ingestion, absorption of organic matter, and paralytic shellfish poisoning (PSP) toxin incorporation by *Mytilus edulis* exposed to a high-toxicity isolate of the red tide dinoflagellate *Alexandrium fundyense* (strain GtCA29, toxicity = 66 pg saxitoxin equivalents (STXeq) cell^{-1}). Maximum ingestion rate was achieved at 150 to 250 cells ml^{-1}. Clearance rates on *A. fundyense* were about 48% lower than those for a non-toxic control diet of the diatom *Thalassiosira weissflogii*. Mussels with no prior history of exposure to PSP maintained a constant ingestion rate over 17 d of exposure to a *A. fundyense* (at 256 cells ml^{-1}), and absorbed ca 62% of the organic matter ingested. They experienced no mortality or sublethal adverse effects during intoxication. Maximum (saturation) toxin levels of 4.5×10^4 µg STXeq 100g^{-1} were attained after 12 to 13 d, a value comparable to maximum toxicities reported during major toxic bloom events. Mussels could exceed the quarantine toxin level (80 µg STXeq 100g^{-1}) in < 1 h of exposure to high densities of this isolate. At saturation, they incorporated 79% of the toxin ingested, primarily in the viscera. This provides the first estimate of toxin incorporation efficiency in a bivalve under steady state conditions. Dinoflagellate toxins, determined by HPLC, were dominated by carbamate derivatives. The muscle, mantle/gill and foot of *M. edulis* showed significant enrichment in STX and reduction in the gonyautoxins GTX_{2+3} and neoSTX relative to ingested cells. The toxin composition of the viscera more closely resembled that of ingested cells, reflecting the presence of numerous intact cells in gut contents. Through its potential use of *A. fundyense* as a sole food source, *M. edulis* is thus capable of remarkably efficient toxin accumulation at environmentally realistic dinoflagellate cell densities.

INTRODUCTION

Paralytic shellfish poisoning (PSP) poses a serious human health hazard and causes significant economic losses due to the closure of shellfish harvesting grounds worldwide (reviewed by Shumway in press). Suspension-feeding bivalve molluscs acquire PSP toxins by filtering vegetative cells, and possibly cysts, of dinoflagellates suspended in the water column. *Alexandrium fundyense* (Lebour) Balech (formerly known as *Protogonyaulax tamarensis* or *Gonyaulax excavata*) is an important dinoflagellate species responsible for PSP in eastern Canada and the northeastern United States (Taylor 1984). In that region the toxicity of *Alexandrium* isolates decreases along a north to south latitudinal gradient (Maranda et al. 1985). *Alexandrium* spp. produce a complex of at least 12 water-soluble, structurally related neurotoxins which vary markedly in their potency (Sullivan 1988; Fig. 1), and are subject to interconversions and changes in relative proportions as they are transferred through the food chain. They include the carbamate toxins (saxitoxin (STX), neosaxitoxin (neoSTX) and gonyautoxins (GTX_{1-4})) and the less potent N-sulfocarbamoyl or cryptic toxins (B_1, B_2, C_1 and C_2) (Hall & Reichardt 1984).

Considerable information is available on toxin dynamics in bivalves under field conditions (Dupuy 1968, Hurst & Gilfillan 1977, Shumway et al. 1988). Rates of toxin accumulation and release are species-specific, and the level of toxin accumulated has been

* Contribution no. 711 from the Marine Science Research Center, SUNY Stony Brook, NY, USA, and contribution no. 7217 from the Woods Hole Oceanographic Institution, Woods Hole, MA, USA

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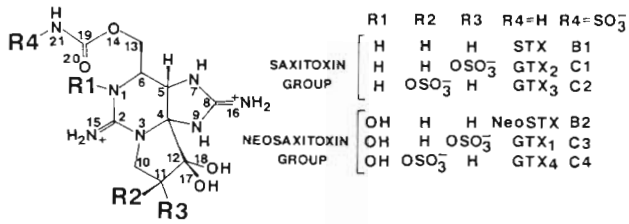

Fig. 1. Structures of saxitoxin and various carbamate (R4 = H) and N-sulfocarbamoyl (R4 = SO_3^-) derivatives. STX: saxitoxin; neoSTX: neosaxitoxin; $GTX_{1,2,3,4}$: gonyautoxins 1,2,3,4

related to the degree of sensitivity of bivalve nerve preparations to saxitoxin (Twarog & Yamaguchi 1974). The mussel *Mytilus edulis* L., commonly used as sentinel organism in monitoring PSP, is relatively unaffected by STX. *Mytilus* spp. typically become toxic earlier, show higher toxin uptake rates, and attain higher toxicities than other bivalves such as oysters *Crassostrea gigas* (Dupuy 1968, Lassus et al. 1989), soft-shelled clams *Mya arenaria* (Hurst & Gilfillan 1977), littleneck clams *Protothaca staminea* (Sullivan 1982), and butter clams *Saxidomus giganteus* (Dupuy 1968, Quayle 1969). Mussels also detoxify more rapidly than species such as *S. giganteus* (Quayle 1969, Madenwald 1985) and the surf clam *Spisula solidissima* (Blogoslawski & Stewart 1978, Shumway et al. 1988), which show prolonged toxin retention following the disappearance of dinoflagellate blooms.

The toxicity attained by a given bivalve is expected to vary as a function of dinoflagellate cell density, strain toxicity, seston composition, and environmental parameters, yet the influence of these variables is difficult to assess under field conditions. In some cases, peak toxicities in bivalve tissues can be directly related to the number of dinoflagellate cells in the water column (Needler 1949, Prakash 1963, Dupuy 1968). Few studies (Prakash 1963, Sullivan 1982, Lassus et al. 1989, Beitler & Liston 1990) have attempted to describe the kinetics of PSP toxins in shellfish under controlled conditions. Such studies are helpful in developing improved predictive capabilities on the fate of PSP toxins in nature, and in designing cost-effective and reliable monitoring programs.

Routine monitoring currently relies on bulk toxin analysis by the standard mouse bioassay (AOAC 1984) which provides no information on the behavior of individual toxins. An understanding of toxin conversions in shellfish is critically important, since toxins of low potency in dinoflagellate cells can be converted to highly potent toxins through bacterial, enzymatic or pH-mediated action (Shimizu & Yoshioka 1981, Sullivan 1982). High performance liquid chromatography (HPLC) toxin profiles are also commonly used to link the occurrence of shellfish toxicity to its causative agent in the natural environment (Oshima et al. 1987).

The goal of the present study was therefore to investigate the kinetics of PSP toxin uptake in *Mytilus edulis* under experimentally defined conditions. Kinetics of detoxification will be reported in a subsequent paper Specific objectives were. (1) to determine the relationship between dinoflagellate cell density, rate of ingestion, and absorption efficiency of toxic cells; (2) to quantify the relationship between ingestion of *Alexandrium fundyense* and toxin accumulation in mussel tissues, thus allowing formulation of a toxin budget; and (3) to examine the tissue compartmentalization of individual toxins and identify differences in the relative proportions of individual toxins between dinoflagellate cells and mussel tissues during long-term intoxication.

METHODS

Mussels were collected from a subtidal population on the north shore of Long Island, Old Field, New York, USA, which has no prior history of PSP. They were acclimated to the experimental temperature, 15 to 17 °C, for 3 wk in a flow-through system provided with natural seawater supplemented with cultured *Thalassiosira weissflogii*, before being used in experiments. *Alexandrium fundyense* (isolate GtCA29 from the Gulf of Maine USA, D. M. Anderson, Woods Hole Oceanographic Institution culture collection) was grown in non-axenic batch culture in K medium (Keller et al. 1987) prepared from natural seawater (ca 28 ‰) filtered through 0.22 µm Millipore membrane filters and sterilized by autoclaving. Algae were grown on a 16:8 h light-dark cycle, with an irradiance of ca 505 µE m^{-2} s^{-1}, at 16 °C, with continuous aeration.

Short-term feeding experiments. Individual clearance rates (i.e. volume swept clear of particles per unit time) of juvenile mussels (mean height = 16.4 mm (SE = 0.3)) were determined at volume equivalent concentrations of *Alexandrium fundyense* (equivalent spherical diameter, ESD = 28.5 µm) or a control diet of *Thalassiosira weissflogii* (ESD = 11 µm), an alga known to be a good food source for bivalves. Mussels were preadapted to the experimental algal concentration for 3 to 4 h in a flow-through system. Algal cultures were incubated with ^{14}C-bicarbonate at 0.1 µCi ml^{-1} of culture for 4 d. Immediately prior to experiments dinoflagellate cells were concentrated on a 20 µm sieve, rinsed and resuspended in filtered seawater to eliminate unincorporated ^{14}C. Clearance rate (CR) was determined at 16 °C following transfer to 250 or 500 ml beakers, from the depletion of radiolabelled dinoflagellates over a 40 to 60 min period. CR was estimated from the equation: $CR = (\ln C_o - \ln C) \times V/t$ (Coughlan 1969), where C_o and C = initial and final ^{14}C activity respectively, V = volume of suspension, and t = time interval.

Algae were held in suspension with magnetic stirrers. Samples of the suspension were removed every 15 to 20 min and filtered onto Nuclepore filters. ^{14}C activity was determined with a LKB Wallac, 1217 Rackbeta scintillation counter following addition of tissue solubilizer and scintillant. Changes in ^{14}C activity of the algal suspension, monitored from a control beaker with no experimental mussel, were negligible over the experimental period. No pseudofeces were produced and no contamination by resuspended labelled feces is expected, since the experimental period was less than the mussels' minimum gut passage time (ca 1 h). Clearance rates were converted to a weight-standardized rate for a 1 g mussel using the equation: $CR_s = CR/W^{0.75}$, where CR and W = measured clearance rate and total body weight respectively, and 0.75 = exponent of the allometric equation relating CR and body weight (Bayne & Newell 1983).

Long-term toxin uptake experiments. Thirty-six adult mussels (mean height = 43.8 mm (SE = 2.05); mean total body weight = 10.0 g (SE = 0.15); mean wet tissue wt = 2.30 g (SD = 0.04), were held in a recirculating tank containing *Alexandrium fundyense* in 36 l of 0.45 um filtered seawater (salinity = ca 28‰). Algae were continuously metered into the tank from a concentrated stock with a Harvard Apparatus, Model 1203 peristaltic pump . Mussels were thus exposed to an approximately constant cell density over 17 d. The stock was replenished twice each day from cultures grown in 20 l carboys. Dinoflagellate cells were harvested in late log phase of growth about 10 d following inoculation at a final concentration of ca 8000 cells ml^{-1}, and concentrated by sieving on a 20 µm Nitex mesh. Cultures were harvested at a rate of 1 carboy d^{-1}. Dinoflagellate cells in the tank were maintained in suspension using aeration and a Supreme Superking pump and siphons mounted externally to the tank. The flow created by this pump was gentle enough to prevent disruption of dinoflagellate cells and mussel feces. Water in the tank was completely replaced every 24 to 48 h. The algal stock was mixed by means of aeration and a magnetic stirrer.

Dinoflagellate concentrations in the experimental tank were determined microscopically with a Sedwick-Rafter counting chamber. No pseudofeces were produced within the range of experimental concentrations experienced. The number of dinoflagellate cells removed (ingested) by mussels was determined twice a day by difference between the number of cells delivered from the stock and the change in concentration in the experimental tank. The absorption efficiency (AE) of organic matter (OM) from toxic dinoflagellates ([OM absorbed/OM ingested] × 100), was estimated by Conover's (1966) ash-ratio method at 3 cell densities (ca 50, 100 and 300 cells ml^{-1}). Feces were collected immediately as they were produced in the experimental tank. Algal and fecal samples were filtered onto combusted (470°C), weighed Whatman GF/C glass-fibre filters, rinsed with isotonic ammonium formate solution. Filters were dried at 85°C and weights determined to ± 1 µg with a Cahn electrobalance. Organic content was determined from weight loss on ignition.

Toxin analysis. Mussels (n=4) were removed periodically from the recirculating tank for toxin analysis and replaced with new individuals (not previously exposed to PSP toxins), which were sampled at the end of the experiment. This provided a duplicate time series (Series II) while maintaining a constant number of mussels in the tank throughout the experiment. Four tissue pools were dissected: foot; visceral mass (including the digestive gland-stomach complex); muscle (including adductors and pedal retractor muscles); and other tissues (primarily mantle plus gills). Weighed tissues were immediately frozen in liquid nitrogen, lyophilized and stored at −70°C. For analysis, toxins were extracted in 0.03 N acetic acid (1 ml HOAc to 1 g wet tissue wt), after reconstituting the tissue to the original wet weight with deionized water, using a Brinkmann Model PT d 10/35 tissue homogenizer. Slurries were centrifuged and the supernatant was filtered through ultrafiltration membranes (MPS-1, Amicon Corp.).

Toxin extraction from dinoflagellate cells was conducted daily by disrupting cells with a Bronwill, Biosonik III probe sonicator in 0.03 N HOAc following previously described methods (Boyer et al. 1986, Cembella et al. 1987). Sonicated samples were centrifuged (10 min at 2000 × *g*) to remove particulate debris. The supernatant acid extract was filtered through a 13 mm syringe-mounted 0.45 µm HA-Millipore nitrocellulose membrane prior to direct analysis (10 µl injection) by HPLC.

Separation of toxin components from mussel tissue samples was achieved by reverse-phase ion-pair HPLC, followed by fluorescence detection of the post-column oxidation products, according to the method of Sullivan & Wekell (1986). Dinoflagellate extracts were similarly analyzed, with minor modifications (Boyer et al. 1986, Cembella et al. 1987). In summary, the method involved binary gradient separation on a 4.1 × 150 mm i.d. polystyrene divinylbenzene resin column (10 µm particle size; Hamilton PRP-1, Hamilton Co., Reno, NV, USA), with hexane and heptane sulfonates (Na salt) serving as ion-pair reagents. Mobile phase A consisted of 1.5 mM ammonium phosphate buffer (as PO_4), with 1.5 mM each of hexane and heptane sulfonate (pH 6.70); mobile phase B contained an identical concentration of ion-pair reagents, but differed in the concentration of ammonium phosphate buffer (6.25 mM) and by the addition of acetonitrile, to a final volume of 25 %

(pH 7.00). Following post-column oxidation (Kratos PCRS-520 post-column reaction system, Kratos Analytical Inst., Westwood, NJ, USA) with 5.0 mM alkaline periodate in 100 mM Na_3PO_4 buffer (pH 7.80) and acidification (0.75 M nitric acid), fluorescent toxin derivatives were detected by a Perkin-Elmer LS-5 spectrofluorometer equipped with a 20 µl flow cell (excitation: 340 nm; emission: 400 nm).

Toxin profiles were determined in quintuplicate at different detector sensitivities, to ensure accurate quantitation of weakly fluorescing N-1 hydroxy-derivatives, including neoSTX, GTX_1 and GTX_4. The detection limit of the HPLC method varies with individual toxins, ranging from 10 to 30 µg STXeq 100 g^{-1} (precision = ± 10%, Sullivan & Wekell 1986). Because individual components of the carbamoyl-N-sulfo group (C_1 and C_2) did not contribute greatly to the total toxicity of mussel extracts, they were not specifically quantified. However, to confirm their contribution to the toxin spectrum in the dinoflagellate isolate GtCA29, toxins C_1 and C_2, which tend to coelute with fluorescent artifacts, were converted to their respective carbamate derivatives (GTX_2 and GTX_3) by hot acid hydrolysis (5 min at 100°C in 0.1 N HCl; Boyer et al. 1986). The enhanced peak areas of the corresponding carbamates following re-chromatography of these hydrolyzed samples, allowed for the estimation of the individual C_1 and C_2 toxins. These toxins were also quantified directly using a gradient separation method (Boczar et al. 1988).

Total toxicity in µg saxitoxin equivalents (STXeq) was calculated using specific activities of individual toxins (in Mouse Units (MU) µmol^{-1} of toxin) (Boyer et al. 1986), assuming 0.18 µg STXeq MU^{-1} (Shimizu 1978). Sullivan & Wekell (1986) and Sullivan (1988) reported excellent agreement between toxin analysis of shellfish by HPLC and the standard AOAC mouse bioassay (correlation coefficient = 0.9). The overall efficiency of toxin uptake by mussels was estimated from the ratio: [(Toxin body burden at saturation)/(Cumulative toxin ingested at saturation)] × 100.

RESULTS

Concentration-dependent feeding rates and absorption efficiency

Clearance rates (CR) of juvenile mussels declined exponentially with increasing concentration of *Alexandrium fundyense* over the concentration range 50 to 700 cells ml^{-1} (Fig. 2). Cell densities plotted in Fig 2 were calculated as the geometric mean of initial and final experimental concentrations. Ingestion rate of dinoflagellate cells, calculated as the product of CR and cell density, was maximized at ca 150 to 250 cells ml^{-1}. Pseudofeces production was not observed at any of the concentrations tested. Therefore, filtration rates (number of cells removed from the suspension per unit time) are assumed to equal ingestion rates.

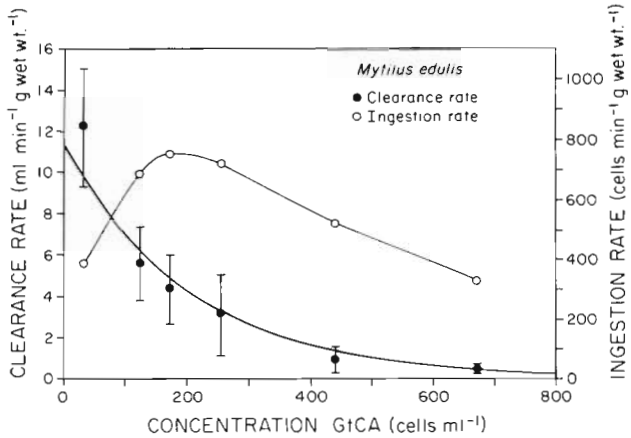

Fig 2. *Mytilus edulis*. Relationship between weight-standardized clearance rate (ml min^{-1} g total body wet wt^{-1}), ingestion rate of *Alexandrium fundyense* (cells min^{-1} g wet wt^{-1}) and cell concentration (cells min^{-1}). Error bars represent 95% confidence intervals (CI; n = 8 to 10)

Analysis of covariance (ANCOVA, Table 1) of $\log_e$-transformed values indicates that concentration-dependent CR of mussels exposed to GtCA were significantly lower than those of individuals fed equal volume rations of the *Thalassiosira weissflogii* control diet ($p < 0.01$). On average, clearance rates on GtCA were 48% of the rates on the reference diet.

Percent absorption efficiency of organic matter averaged 62.5% (SE = 8.16, n = 4), 63.7% (SE = 5.04, n = 7), and 60.3% (SE = 5.00, n = 5) at mean dinoflagellate concentrations of 56, 122 and 279 cells ml^{-1} respectively, thus showing no significant variation with cell density over the range tested.

Table 1. *Mytilus edulis*. Regression of weight-standardized clearance rate (CR, ml min^{-1} g^{-1} total body weight) on cell concentration (C) following the equation: ln CR = a + bC. Standard error shown in brackets. F for differences among adjusted means (ANCOVA, Sokal & Rohlf 1981) = 12.24 > $F_{0.01\,[1,8]}$ (no significant differences between slopes; F = 3.81, $p > 0.05$)

Algal species	a (SE)	b (SE)	r^2
Alexandrium fundyense[a]	2.422 (0.084)	−0.0048 (0.00030)	0.98
Thalassiosira weissflogii[b]	2.676 (0.228)	−0.0033 (0.00084)	0.83

[a] C in cells GtCA29 ml^{-1}
[b] C in GtCA29 volume equivalents ml^{-1} (10^3 cells *T. weissflogii* equal in volume to 60.5 GtCA cells)

Toxin uptake kinetics

Dinoflagellate cell densities in the recirculating tank fluctuated around a time-weighted mean of 256 cells ml^{-1} (Fig. 3), a concentration shown above (Fig. 2) to maximize ingestion rate of toxic cells. The abnormally high dinoflagellate densities experienced during the first 24 h (Fig. 3) were caused by spawning activity of Series I mussels, since spawning is known to inhibit feeding activity of *Mytilus edulis* (Newell & Thompson 1984).

Over the experimental period mussels ingested GtCA29 at a constant rate of 0.81×10^6 cells d^{-1} (g wet tissue wt)$^{-1}$ (1.88×10^6 cells d^{-1} mussel^{-1}). Cumulative weight-specific ingestion rate (IR; cells [g wet tissue wt]$^{-1}$) is given by the equation:

$$IR = -211702\ (SE = 62233) + 33606.0\ (SE = 265.1)t \quad (r^2 = 0.99)$$

where t = time (h). The time course of cumulative toxin ingestion is presented in Fig. 4A. Toxicity of *Alexandrium fundyense* cultures varied considerably among carboys, averaging 65.7 pg STXeq $cell^{-1}$ (SD = 34.6) over the experimental period. Mussels experienced no mortality during the experiment, did not exhibit shell valve closure, mucus production or retraction of mantle edges, and generally did not appear to be adversely affected by the accumulation of toxins.

Based on pooled data points for Series I and II mussels, the toxin content of total tissues appeared to attain saturation levels at 12 to 13 d (Fig. 4B). For Series II mussels the following equation provided a good description of toxin uptake kinetics:

$$\text{Toxicity } (\mu g\ STXeq\ 100g^{-1}) = [K_{max}(t - t_o)]/[K_s + (t - t_o)] \quad (1)$$

where t_o = 6.95 (SE = 3.5); K_{max} = 50776 (SE = 3183.3); K_s = 70; and t = time. Series I mussels, which spawned during the first day of the experiment, attained comparable maximum toxin levels but showed somewhat lower initial toxin accumulation, which was best described by a polynomial function:

$$\text{Toxicity} = 5.3(SE{=}87.5)t + 1.03\ (SE{=}0.646)t^2 - 0.002(SE{=}0.001)t^3 \quad (r^2 = 0.95). \quad (2)$$

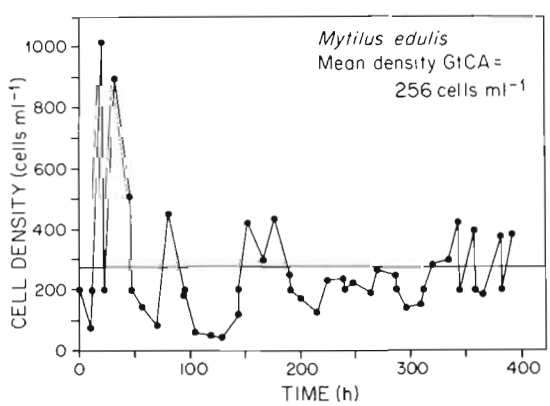

Fig. 3. Concentration of *Alexandrium fundyense* in the experimental tank. Horizontal line indicates time-weighted mean cell density over the 17 d toxin uptake experiment

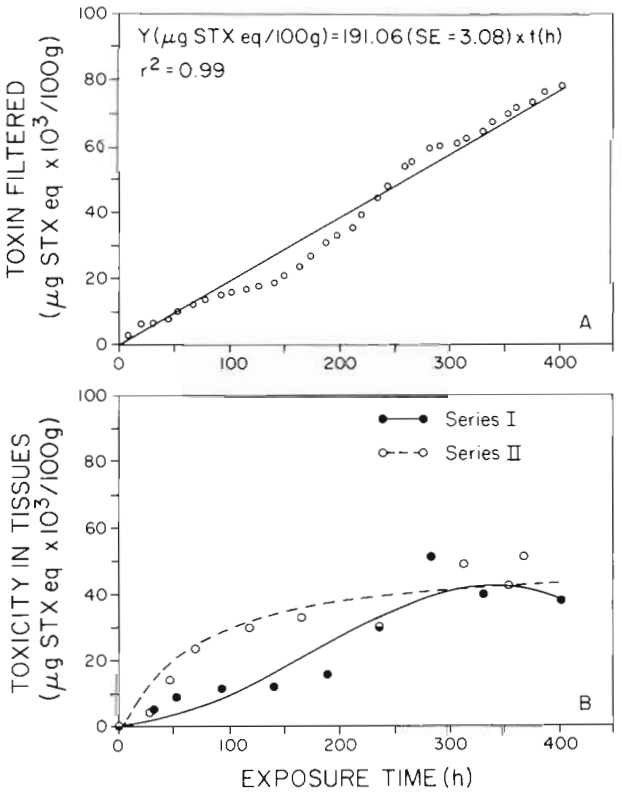

Fig. 4. *Mytilus edulis*. (A) Cumulative weight-specific ingestion rate of toxin from *Alexandrium fundyense* cells, and (B) total toxicity of mussel tissues (μg STXeq [100 g wet tissue wt]$^{-1}$) over the experimental period. Curves were fitted through the origin using SYSTAT linear (A) or non-linear (B) curve estimation

Non-linear functions provided a somewhat better fit than linear regressions. However, given the large inherent biological variability in the data set, a larger sample size would be necessary to more precisely define the shape of the intoxication curve.

Toxin body burden at saturation, calculated by averaging the last 3 data points in Fig. 4B, was 4.3×10^4 μg STXeq $100g^{-1}$ after 284 h of exposure for Series I and 4.7×10^4 μg STXeq $100g^{-1}$ after 313 h for Series II. Toxin ingestion is estimated from the regression equation in Fig. 4A. Thus, mussels incorporated 78.9% (Series I) and 79.2% (Series II) of the toxin ingested by the time they achieved maximum toxin levels. The efficiency of toxin incorporation, as defined in this study, is expected to be higher than toxin absorption efficiency, since toxin body burden includes an unknown amount of unabsorbed toxin present in gut contents. Assuming that the AE of dinoflagellate toxins

and organic matter are equal (62 %), our results suggest that undigested toxin in gut contents represents ca 17 % (79 − 62 %) of the toxin body burden at saturation.

At the time when mussels attained maximum toxin levels, ca 96 % of the total toxin body burden was present in the viscera, although this tissue represented only 30 % of the total tissue wet weight (Table 2). Patterns of total toxin accumulation in the 4 tissue pools analyzed are shown in Fig. 5. Maximum toxin levels averaged 1648, 41.9, 36.4 and 31.9 µg STXeq g^{-1} in the viscera, mantle, muscle and foot respectively. Toxin levels in mantle and muscle declined somewhat after reaching a maximum, although mussels ingested toxic cells at a constant rate throughout the experiment.

Table 2. *Mytilus edulis*. Percent contribution of each tissue pool to total tissue wet weight and total toxicity. Values are means of Series I and II mussels at the end of exposure (12 to 17 d; standard deviation shown between brackets)

Tissue	% of tissue wet weight	% of toxin content
Viscera	29.8 (3.2)	95.6 (1.5)
Muscle	17.3 (0.7)	1.0 (0.4)
Mantle/gill	46.7 (2.3)	3.0 (1.3)
Foot	6.2 (0.2)	0.3 (0.14)

Toxin composition

The toxin composition of the *Alexandrium fundyense* cultures, expressed as a percentage of total toxicity (in pg STXeq $cell^{-1}$), yielded the following mean values (n = 16) over the experimental period: 2.7 % C_{1+2} (SE = 0.23), 5.1 % GTX_4 (SE = 0.35), 0.6 % GTX_1 (SE = 0.14), 50.1 % GTX_3 (SE = 1.39), 1.5 % GTX_2 (SE = 0.12), 13.2 % neoSTX (SE = 0.92) and 26.8 % STX (SE = 1.14). Thus, in the GtCA29 isolate used, the predominant toxins were GTX_3 and STX.

The toxin composition of each tissue pool, averaged over the experimental period, is compared to that of ingested cells in Fig. 6. The N-sulfocarbamoyl toxins, C_1 and C_2, which accounted for < 3 % of total toxin in GtCA29, were not quantified in mussel tissues, and are therefore not shown. In both Series I and II mussels, the mantle, muscle and foot showed significant enrichment in STX and the gonyautoxins GTX_{1+4}, and a significant decrease in neoSTX and GTX_{2+3} relative to dinoflagellate cells (Table 3; $p < 0.01$). The viscera of Series II mussels showed levels of GTX_{2+3} and STX intermediate between algal cells and other tissues (Fig. 6), reflecting the presence of a significant amount of intact, undigested cells in the gut. This was confirmed by microscopic observation of fecal ribbons.

Fig. 7 illustrates temporal changes in toxin composition for the mantle/gill tissue pool. Similar patterns were observed in all other tissues, including the viscera. Linear regression equations describing these time series were calculated for each individual toxin, and regression slopes (b) tested to identify significant departures from a slope of zero. Series I mussels showed a significant increase in the content of STX over time (b significantly different from 0 at $p < 0.01$), whereas no significant temporal changes were detected for any of the toxins in Series II mussels. Thus, although Series I tissues show a lower STX content and higher content of gonyautoxins than Series II tissues

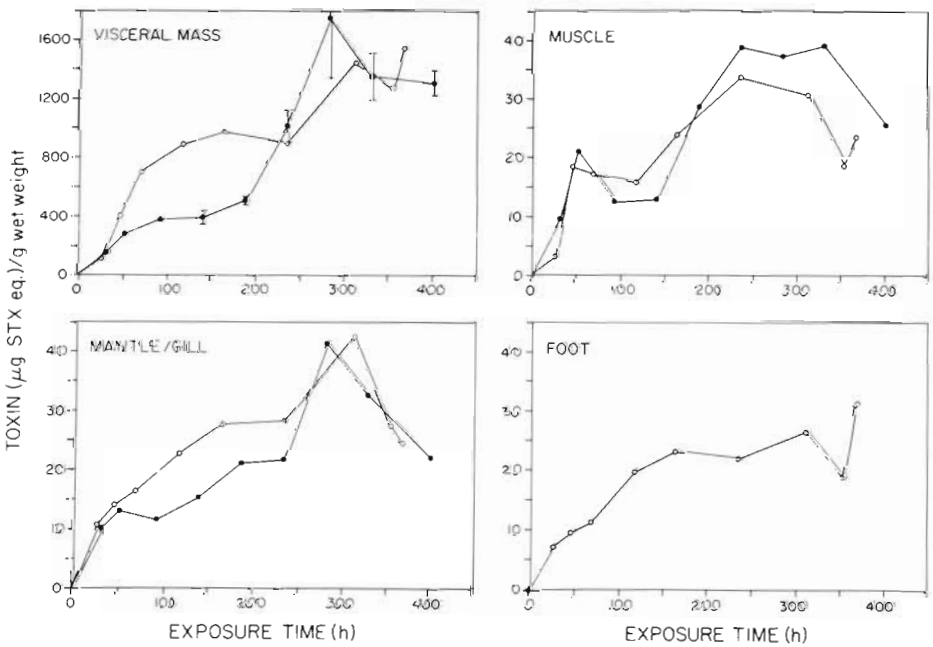

Fig. 5. *Mytilus edulis*. Patterns of PSP toxin accumulation (µg STXeq [g wet tissue wt]$^{-1}$) in mussel tissues (Series I and II as in Fig. 4). Toxin was determined from pooled samples (n = 4). Where error bars are shown, these represent 95 % CI of 2 samples, each with pooled tissues from 2 individuals

when values are averaged over the whole experimental period, both series achieved comparable toxin profiles by the end of the experiment.

Epimerization, i.e. partial conversion of the 11-β-hydroxysulfate toxins GTX_3 and GTX_4 to their corresponding α-epimers (see Fig. 1), appears to take place in mussel tissues. Molar ratios of β- to α-epimers in ingested cells and in tissues over the course of the experiment are shown in Table 4. The ratio GTX_3/GTX_2 was significantly higher in dinoflagellate cells than in mussel tissues by the end of the experiment, especially in the viscera and muscle (Table 4) ($p < 0.01$; Games and Howell method for comparison among pairs of means when variances are heterogeneous; Sokal & Rohlf 1981). The epimer ratio also consistently decreased in mussel tissues over time; this decrease, however, was significant only in the viscera and muscle (Table 4).

Table 3. Results of a posteriori pairwise multiple comparisons of mean arc sine transformed levels of individual toxins (as % of total toxin averaged over the experimental period for Series I and II) based on the T' statistic (Sokal & Rholf 1981), in mussel tissues and dinoflagellate cells. Abbreviations as in Figs. 1 and 6. Means connected by a line are not significantly different, those not connected are significantly different at $p < 0.01$ (except comparisons of STX between gill and viscera, and foot and viscera in which $p < 0.05$)

Toxin	GtCA29	V	G	M	F
STX	27.6	29.6	35.2	35.6	34.9
neoSTX	13.6	8.9	9.8	10.4	10.3
GTX_{1+4}	5.9	12.4	13.7	13.1	14.3
GTX_{2+3}	53.0	49.5	41.2	40.9	40.5

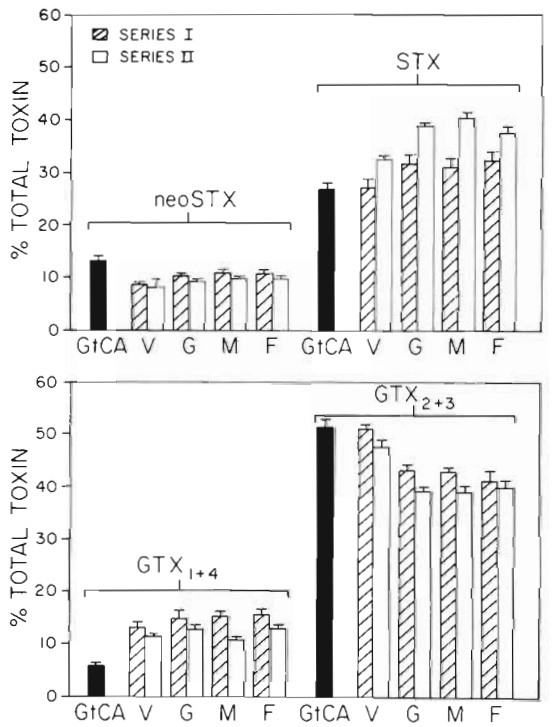

Fig. 6. *Alexandrium fundyense* and *Mytilus edulis*. Composition of individual PSP toxins (as % of total toxin) in dinoflagellate cells (GtCA29) and mussel tissues (V = viscera; G = gill/mantle; M = muscle; F = foot; means of 9 samples over the 17 d experimental period). Error bars represent standard errors. Values were determined by averaging all toxin determinations over the experimental period (toxin abbreviations as in Fig. 1)

DISCUSSION

Feeding rates and absorption efficiency

Shumway & Cucci (1987) described differences in the response of mussels to toxic dinoflagellates of the genus *Alexandrium* depending on the bivalves' prior exposure history. *Mytilus edulis* from areas free of PSP exhibited 75% mortality, copious production of white mucus, shell valve closure and mantle retraction when

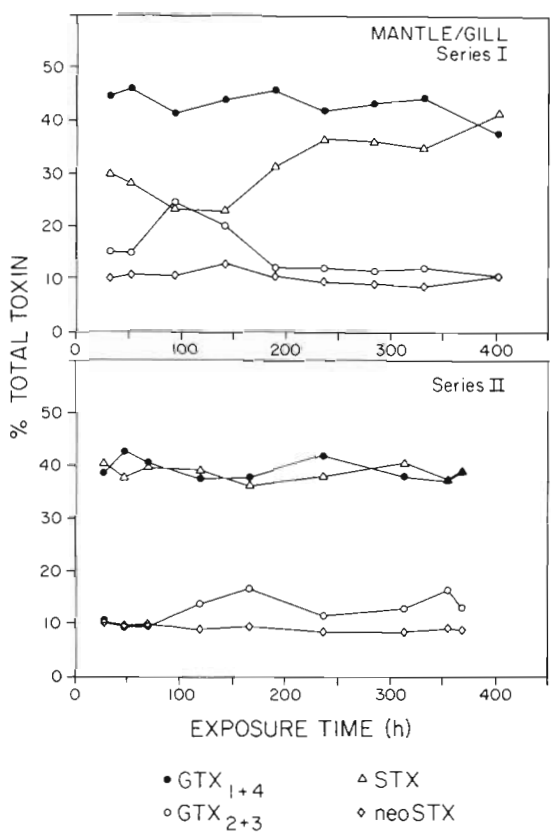

Fig. 7. *Mytilus edulis* Changes in toxin composition in the mantle/gill tissue pool during the course of experimental PSP contamination (toxins as in Fig. 1)

Table 4. Mean molar ratio (standard error) of β to α-11-hydroxysaxitoxin sulfate toxins in dinoflagellates and mussel tissues. Tissue values were averaged for the initial phase (I = Days 1 to 3) and final phase (F = Days 12 to 17) of intoxication of Series I and II mussels

Toxin ratio	GtCA29	Viscera		Mantle/gill		Muscle		Foot	
		I	F	I	F	I	F	I	F
GTX_3/GTX_2	20.9	9.6	2.0	10.4	4.4	3.8	1.6	5.1	3.4
	(1.3)	(3.9)	(0.2)	(2.4)	(0.4)	(0.4)	(0.2)	(0.8)	(1.4)
Significance[a]			•		ns		•••		ns

[a] ANOVA comparisons between the initial and final mean epimer ratio in each tissue pool, using the transformation arc sine $[((GTX_3/GTX_2) + 1)^{-1}]$; • $p < 0.05$, ••• $p < 0.001$, ns = not significant

exposed to *Alexandrium* (formerly *Protogonyaulax tamarensis* clone Gt429), whereas mussels from Maine, which experience recurrent red tides, were not visibly affected by toxic cells. These authors also reported that clearance rates of *M. edulis* were not significantly inhibited by addition of toxic dinoflagellates (at 500 cells ml^{-1}) to a mixed algal suspension. Both Shumway & Cucci (1987) and Twarog & Yamaguchi (1974) hypothesized that bivalve populations from areas prone to red tide outbreaks may have acquired resistance to the presence of toxic cells. In contrast, in the present study, *M. edulis* from Long Island waters known to be free of *Alexandrium* blooms experienced none of the detrimental effects listed above, during 17 d exposure to highly toxic cells. They did, however, exhibit reduced clearance rates on *A. fundyense* relative to the control diet. Reduced grazing in the presence of toxic dinoflagellate cells has been previously shown in copepods (Huntley et al. 1986) and several bivalve species, including *Mya arenaria*, *Crassostrea virginica* (Shumway & Cucci 1987) and *Mytilus edulis* (Widdows et al. 1979). This effect is usually attributed to endo- or epicellular toxins rather than to dissolved metabolites present in cell-free culture filtrates (Dupuy 1968, Widdows et al. 1979). Inhibition of bivalve filtration rates has also been demonstrated in the presence of microalgae that do not contain PSP toxins (Tracey 1988), or in response to their dissolved ectocrines (Ward & Targett 1989).

Feeding experiments described in this study were not designed to identify the mechanism responsible for grazing inhibition. Ives (1987) postulated progressive physiological incapacitation in response to toxin loading as the causative mechanism for lowered ingestion rates of copepods on toxic dinoflagellates. In the present study, mussels maintained a constant rate of ingestion during long-term exposure to GtCA. In short-term feeding experiments, mussels exhibited reduced ingestion relative to controls after only 3 to 4 h of exposure to GtCA. Therefore, if physiological incapacitation is operating in *Mytilus edulis*, it must be experienced within a very short time frame, i.e. within a few hours of exposure to toxic cells.

Twarog & Yamaguchi (1974) suggested that bivalve species which are periodically exposed to dinoflagellate blooms may have evolved mechanisms allowing them to exploit toxic cells as food. No prior information is available on the nutritional value of toxic *Alexandrium* spp. Microalgae known to support good growth of bivalves are typically absorbed with high efficiencies, ranging between 78 and 90% (Pierson 1983). In contrast, low absorption efficiencies, of 13 to 20%, are often associated with algae of poor nutritional value, such as *Chlorella autotrophica* (Pierson 1983) and *Nannochloris atomus* (Bass 1983). Utilization of GtCA29 was moderately high, averaging 60 to 64%. Thus, although absorption efficiency is not always indicative of food value, these results suggest that a monospecific diet of toxic dinoflagellates could potentially support growth of *Mytilus edulis*. Definitive growth trials are required to test this hypothesis.

Toxin uptake kinetics

The toxicity of *Alexandrium* isolates from the NW Atlantic coast ranges from undetectable to ca 52 pg STXeq $cell^{-1}$ (Maranda et al. 1985), and can vary 2 to 8-fold with culture conditions and growth stages (Prakash 1967, White & Maranda 1978, Boyer et al. 1986, Cembella et al. 1987). Thus the isolate used in our study is one of relatively high toxicity. The experimental concentration used (256 cells ml^{-1}) was selected to maximize ingestion of toxic cells and to simulate major *Alexandrium* blooms. Dinoflagellate concentrations during red tides typically lie in the range of 1 to 3×10^5 cells l^{-1} (Needler 1949, Larocque & Cembella 1990), although densities as high as 1.85×10^6 cells l^{-1} (Carreto et al. 1986) and 1.8×10^7 cells l^{-1} (Martin & White 1988) have been reported. Furthermore, ingestion rate of dinoflagellates is expected to be relatively independent of seasonal temperature between 10 and 20°C, since *Mytilus edulis* can fully acclimate its filtration

rate to changes in temperature within this range (Bayne et al. 1977). Thus peak toxicities attained by mussels in this study (4.5×10^4 µg STXeq $100g^{-1}$) are expected to reflect near maximal intoxication rates. They are indeed comparable to those reported during massive red tides. For example, *M. edulis* attained 3.8×10^4 µg STXeq $100g^{-1}$ in the Bay of Fundy (Needler 1949), up to 1.0×10^4 µg STXeq $100g^{-1}$ during the 1972 New England red tide (Twarog & Yamaguchi 1974), and 5.0×10^4 µg STXeq $100g^{-1}$ in the Argentine Sea in 1980 (Carreto et al. 1986).

Our results further suggest that, under optimal conditions, mussels exposed to a highly toxic strain such as GtCA29 could exceed the regulatory level (80 µg STXeq $100g^{-1}$) in less than 1 h. This rate of increase in toxicity (calculated by linear extrapolation from toxicities measured after 1 d of exposure), is comparable to maximum values measured for *M. edulis* under field conditions. For example, toxicity of mussels in the Gulf of Maine during 1980 rose from undetectable levels (as determined by mouse bioassay) to > 8000 µg STXeq $100g^{-1}$ in 2 d (Shumway et al. 1988). Assuming a linear rise in toxicity, the regulatory level would have been exceeded in ca 0.5 h. These experimental and field results underscore the difficulty in providing adequate warning through routine monitoring programs conducted on a weekly basis and justify establishing a regulatory level that is well below that likely to cause human illness.

Lassus & Berthome (1986) experimentally contaminated *Mytilus edulis* with a low toxicity Japanese strain of *Alexandrium tamarense* (MOG835, 8.8 pg STXeq $cell^{-1}$), which contained predominantly acid-labile N-sulfocarbamoyl toxins (Lassus et al. 1989). Toxin tissue compartmentalization was not determined, and toxin profiles are difficult to interpret from these data, since toxins for HPLC analysis were extracted in strong acid (0.1 N HCl) following the AOAC protocol. Furthermore, no attempt was made to supply mussels with an ad libitum ration or a constant cell density. To our knowledge, however, this study provides the only other data on long-term (13 d) PSP contamination of mussels under defined conditions (16°C, mussels averaging 1.76 g in wet tissue wt). Daily weight-specific ingestion rates of mussels in the present study were 0.81×10^6 cells g^{-1}, compared to 0.55×10^6 cells g^{-1}, an estimate based on a cumulative ingestion rate of 11.54×10^6 cells $mussel^{-1}$ by Day 12 in Lassus & Berthome's (1986) study.

Equilibrium toxin levels in the present study were attained in 12 to 13 d, which agrees well with a saturation time of 12 d reported by Lassus & Berthome (1986). Since GtCA29 is ca 7.5 times more toxic than MOG835, these results suggest that the time required to attain saturation toxin levels may be independent of dinoflagellate strain toxicity. Further work is required to verify this hypothesis.

Maximum toxin levels achieved by *Mytilus edulis* in our study (4.5×10^4 µg STXeq $100g^{-1}$) are however, 40 times higher than those obtained by Lassus & Berthome (1986) (1.14×10^3 µg STXeq $100g^{-1}$). This difference cannot be fully explained by differences in either ingestion or overall strain toxicity.

Toxin uptake efficiency estimated in the current study (78 to 79%) is also markedly higher than the 20% efficiency calculated using Lassus & Berthome's data on Day 12 of contamination ([1142 µg STXeq $100g^{-1}$ incorporated in tissues/5770 µg STXeq $100g^{-1}$ ingested] × 100). A toxin uptake efficiency of 75% was estimated by Sullivan (1982) following 48 h exposure of *Mytilus edulis* and littleneck clams *Protothaca staminea*, combined in the same experimental system, to a Pacific isolate of *Alexandrium* (*Protogonyaulax catenella* at ca 25 cells ml^{-1}. Similarly, Dupuy (1968) reported values between 72 and 96% for *Mytilus californianus* fed *A. catenella* at 20 cells ml^{-1} for 5 d.

Toxin anatomical distribution and composition

In *Mytilus edulis*, the muscles, mantle/gill and foot accumulated similar toxin levels on a per unit weight basis. In contrast, surf clams *Spisula solidissima* (Blogoslawski & Stewart 1978) and scallops *Placopecten magellanicus* (Shimizu & Yoshioka 1981, Shumway et al. 1988) do not accumulate significant toxin levels in the adductor muscle. In the Alaska butter clam *Saxidomus giganteus*, PSP toxins are concentrated in pigmented, melanin-containing tissues such as the siphons (Price & Lee 1972, Beitler & Liston 1990). Although pH-dependent binding of PSP to melanin was suggested as a mechanism of toxin retention in this species (Price & Lee 1971), no evidence was obtained in our study of higher toxin concentration in the mussel's pigmented foot.

Several prior studies have shown that in bivalves other than *Mytilus edulis*, the toxin profile of tissues may differ from that of the cells upon which they feed (Sullivan 1982, Sullivan et al. 1983). Such differences may be attributed to differential uptake and loss of various toxins or metabolic toxin interconversions. Littleneck clams *Protothaca staminea* are able to enzymatically convert both carbamate and N-sulfocarbamoyl toxins to their corresponding decarbamoyl derivatives (Sullivan et al. 1983), compounds which have not been detected in *Mytilus edulis*. Tissues of both the clam *Mya arenaria* (Oshima et al. 1977) and scallop *Placopecten magellanicus* (Wichmann et al. 1981) showed an increase in STX and reduction in neoSTX relative to dinoflagellate cells. In vitro studies

(Shimizu & Yoshioka 1981) demonstrated that PSP toxins may undergo reductive enzymatic conversions (removal of the N-1-hydroxyl and C-11-sulfate group; see Fig. 1) in *P. magellanicus* tissues (foot, viscera and adductor muscle), resulting in an increase in STX, and decrease in neoSTX and $GTX_{1,2,3}$. Only the adductor muscle was capable of toxin inactivation, i.e. reduction in total toxicity. Thus, the increase in STX, and reduction in neoSTX and GTX_{2+3}, observed in our study for *M. edulis* is consistent with toxin conversions reported in other bivalve species. The apparent increase in GTX_{1+4} in mussel tissues relative to dinoflagellate cells (Fig. 6) may be an artifact resulting from the lower sensitivity of the spectrofluorometer to these poorly fluorescing N-1-hydroxy toxins, and thus greater error in their detection. Sullivan (1982) exposed *M. edulis* to an *Alexandrium catenella* isolate rich in the N-sulfocarbamoyl toxins, C_2 and B_1. Although minor in vivo conversion of C_2 to GTX_3, and B_1 to STX, occurred, mussels largely accumulated toxins in similar proportions to their dinoflagellate diet. This led Sullivan to suggest that metabolic toxin conversions were probably not significant in this bivalve species. Results of our study with a strain dominant in the less labile carbamate toxins, however, show evidence of significant differences between mussel tissues and dinoflagellate cells in the relative proportions of various toxins.

Dinoflagellates of the genus *Alexandrium* produce mainly the β-epimer of a given toxin epimeric pair, while the more stable α-configuration arises by epimerization (Wichmann et al. 1981, Sullivan 1982, Hall & Reichardt 1984). In the present study, toxins were shown to undergo a significant amount of epimerization (conversion of GTX_3 to GTX_2) in mussel tissues. Since the toxin extraction protocols were identical for all samples, such progressive conversion cannot be explained as an artifact of acid extraction. Epimerization of GTX_3 to GTX_2, and C_2 to C_1, were also described in field-collected mussels (Sullivan 1982).

In the Alaska butter clam accumulation of saxitoxin, primarily in the siphons, occurs several weeks after initiation of feeding, even when STX is absent in the clams' dinoflagellate diet (Sullivan 1982, Beitler & Liston 1990). In this study, STX enrichment and concomitant reduction in GTX_{2+3} relative to dinoflagellate cells was detected in all mussel tissue pools within 24 h of initiation of feeding. Thus changes in the relative composition of toxins occurred rapidly following ingestion, suggesting that extracellular digestion in the stomach or intracellular digestion in the digestive gland may be the primary mechanism responsible. Nevertheless, selective uptake and release rates of individual toxins cannot be entirely ruled out as an explanation. Toxin conversion could be enzymatically mediated, and/or the product of acid hydrolysis in the bivalve's gut, where the pH can attain values as low as 5.2 to 6.0 (reviewed by Morton 1983).

Mussels with no prior history of PSP are thus shown to be capable of extremely rapid and efficient toxin incorporation in all tissue pools, when exposed to moderate concentrations of highly toxic dinoflagellates, such as are typically encountered during red tides in nature. It is further shown that toxin uptake by mussel tissues is accompanied by rapid and significant changes in the relative proportions of ingested carbamate toxins. Subsequent work will test whether the parameters measured in this study, namely cell ingestion rate, absorption efficiency of organic matter, efficiency of toxin incorporation, and time required to achieve steady state toxin levels, can vary with the potency and toxin composition of the dinoflagellate strain present in the water column.

Acknowledgements. We thank J. Christie (SUNY Stony Brook) for culturing of microalgae, and R. Larocque and I. St. Pierre (Maurice Lamontagne Institute) for HPLC analysis of toxins. J. Sullivan, formerly of the U.S. Food and Drug Administration, Seafood Products Division, Seattle, Washington, USA and Y Oshima, Tohoku University, Sendai, Japan, graciously provided reference toxins for HPLC calibration. This work is the result of research sponsored by the NOAA Office of Sea Grant, U.S. Department of Commerce, under Grant #NA86AADSG045 to the New York Sea Grant Institute, and Grant #NA86AADSG090 (R/B-92) to the Woods Hole Oceanographic Institution, and by the Biological Oceanography Division, Maurice Lamontagne Institute, Dept. of Fisheries and Oceans (Canada).

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This article was presented by Dr B. L. Bayne, Plymouth, United Kingdom

Manuscript first received: October 10, 1989
Revised version accepted: March 8, 1990