Depuration of Domoic Acid from Live Blue Mussels (*Mytilus edulis*)

I. Novaczek,[1] M. S. Madhyastha, and R. F. Ablett

Prince Edward Island Food Technology Centre, Box 2000, Charlottetown, P.E.I. C1A 7N8, Canada

A. Donald

Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave., Charlottetown, P.E.I. C1A 4P3, Canada

G. Johnson

Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave., Charlottetown, P.E.I. C1A 4P3, Canada

and M. S. Nijjar and D. E. Sims

Department of Anatomy and Physiology, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave., Charlottetown, P.E.I. C1A 4P3, Canada

Novaczek, I., M. S. Madhyastha, R. F. Ablett, A. Donald, G. Johnson, M. S. Nijjar, and D. E. Sims. 1992. Depuration of domoic acid from live blue mussels (*Mytilus edulis*). Can. J. Fish. Aquat. Sci. 49: 312–318.

Industrial depuration may provide a means of removing domoic acid toxin from blue mussels (*Mytilus edulis*). Mussels containing up to 50 μg domoic acid·g^{-1} were transported from a Prince Edward Island estuary into controlled laboratory conditions to test the effects of temperature, salinity, mussel size, and feeding upon depuration. Fifty percent of toxin was eliminated within 24 h. After 72 h, mussels were either clean or contained, on average, only residual levels of toxin (<5 μg·g^{-1}), regardless of conditions. Exponential depuration curves were fitted to the domoic acid concentration data. To evaluate differences in rate of depuration under various conditions, statistical comparisons were made between slopes of the clearance curves. Rates of depuration were faster in small (45–55 mm) than in large mussels (60–70 mm) and more rapid at 11 than at 6°C. There was no significant difference in depuration rate at 18‰ salinity as opposed to 28‰ or in starved versus fed mussels. Because of their relatively large digestive glands, meats of small mussels contained more toxin per unit weight than meats of large mussels. The bulk of domoic acid appeared to reside in the gut lumen. However, the presence of small amounts of domoic acid in intracellular compartments cannot be ruled out.

La dépuration industrielle peut être un moyen d'éliminer la toxine qu'est l'acide domoïque chez la moule bleue (*Mytilus edulis*). Des moules contenant jusqu'à 50 μg d'acide domoïque·g^{-1} recueillies dans un estuaire de l'Île-du-Prince-Édouard ont été transportées dans un laboratoire pour y étudier les incidences de la température, de la salinité, de la taille des individus et de l'alimentation sur la dépuration. On a ainsi déterminé que 50 % de la concentration de toxine était éliminée en deçà de 24 h. Après 72 h, les moules étaient soit libres de toxine ou n'en contenaient, en moyenne, que des concentrations résiduelles (< 5 μg·g^{-1}), indépendamment des conditions. Des courbes exponentielles de dépuration ont été lissées en fonction des données sur les concentrations d'acide domoïque. Afin d'évaluer les différences entre les taux de dépuration dans diverses conditions, on a effectué des comparaisons statistiques entre les pentes des courbes d'élimination. Les taux de dépuration étaient plus élevés chez les petites moules (45–55 mm) que chez les gros individus (60–70 mm); de plus, la dépuration était plus rapide à 11 qu'à 6°C. Il n'y avait toutefois aucune différence significative du taux de dépuration à une salinité de 18 et de 28‰ ou entre les moules affamées et les moules nourries. À cause de leur grosse glande digestive, les petites moules contenaient plus de toxine par unité de poids que les grosses moules. La plus grande partie de l'acide domoïque semblait être accumulée dans la lumière du tube digestif. Toutefois, la présence de petites quantités d'acide domoïque dans les compartiments intracellulaires ne peut être ignorée.

Received January 29, 1991
Accepted August 21, 1991
(JA883)

Reçu le 29 janvier 1991
Accepté le 21 août 1991

Domoic acid occurring as a natural contaminant of cultivated blue mussels (*Mytilus edulis*) acts as a neuroexcitatory amino acid that has proven lethal for elderly human consumers (Todd 1989). This toxin was first found in mussels harvested from eastern Prince Edward Island (P.E.I.) in the autumn of 1987 and has recurred, to a lesser extent, in each subsequent year. In 1990, only trace levels of domoic acid were found in cultivated mussels. Domoic acid enters mussels when they ingest the diatom *Nitzschia pungens* f. *multiseries* (Bates et al. 1989), which may bloom in localized areas of P.E.I. from late September through December. Virtually all of the toxin found in mussels appears to reside in the digestive gland (Wright et al. 1989).

[1]Author to whom correspondence should be addressed.

The Canadian Federal Department of Fisheries and Oceans prohibits harvesting of shellfish that contain more than 20 μg domoic acid·g^{-1}. To date, mussel processors have been able to continue operations using mussels from uncontaminated estuaries. However, evidence from the phytoplankton monitoring program of the Department of Fisheries and Oceans suggests that the carrier organism has a much broader geographic range than originally thought, so this option may not always be available. The potential for depuration of domoic acid from live mussels was therefore of commercial as well as academic interest.

In the past, depuration has been attempted for decontamination of live shellfish containing microbes (Canzonier 1971), heavy metals (Schulz-Baldes 1974; Riisgard et al. 1987; Lobel and Marshall 1988; Chassard-Bouchard and Galle 1988), algal toxins (Shumway 1990), and other poisons (Fossato and Canzonier 1976; Mattson et al. 1988; Pruell et al. 1986). The success of depuration varies with the type of chemical or microbe, the species of shellfish, the physiological condition of the shellfish, and physical factors such as temperature (Cunningham and Tripp 1975; Canzonier 1988; Shumway 1990). In some cases, agents such as UV light or ozone have been applied in an attempt to reduce contaminant levels; in other cases, manipulations of environmental factors such as salinity and temperature have been tested to determine whether natural depuration processes can be stimulated. Lipophilic contaminants are believed to depurate slowly because they are readily bound in intracellular compartments (Spacie and Hamelink 1985). Being polar and essentially hydrophilic (Wright et al. 1989), domoic acid might be relatively difficult to bioaccumulate and easy to excrete, making it a good candidate for removal through depuration techniques.

In the present study, a series of experiments was designed to test the effects of temperature, salinity, mussel size, and feeding on depuration of naturally toxic mussels held under controlled laboratory conditions. Temperatures and salinities tested were within the range found in P.E.I. estuaries or salt wells employed as water sources by mussel processors. It was hypothesized that domoic acid in contaminated mussels could reside in two general compartments: extracellular spaces, such as the lumens of the stomach and digestive gland, and intracellular spaces. If domoic acid does not cross gastrointestinal membranes to enter intracellular spaces, a single elimination curve (Spacie and Hamelink 1985) should model the depuration data well. Intracellular domoic acid would presumably clear from the system more slowly. Provided this component was large enough to be distinguished against variability in gut content, the elimination curve for mussels having domoic acid in this second compartment should be biphasic.

Materials and Methods

Mussel Depuration

Collections of cultivated mussels, naturally contaminated with domoic acid, were procured on four sampling dates during the period 2 November to 1 December 1989. Each sample was collected from a single mussel cultivation sock suspended from the surface in the Brundenell estuary, P.E.I. Ambient water temperatures ranged from 9°C on 28 October to 2°C on 6 December (J. White, Department of Fisheries and Oceans, Charlottetown, P.E.I., pers. comm.). Mussels were transported in a cooler to holding facilities in the Fish Health Unit, University of Prince Edward Island, where they were manually declumped and then acclimated overnight in air at the planned experimental temperature. Mussels were graded into two size groups, i.e. 60–70 mm long (large) or 45–55 mm long (small), with mussels outside of these grades being discarded. Size-graded mussels, in groups of nine, were aligned in Vexar No. H52 net socks (Dupont Canada Inc., Whitby, Ont.) so that the exhalent siphons pointed downwards. Each Vexar sock was randomly assigned a number. The No. 1 sock in each treatment series was taken as a sample at time 0, this being the time at which the remaining socks were immersed in freshly prepared artificial seawater (Instant Ocean, Aquarium Systems, Mentor, OH) of predetermined salinity and temperature. At intervals of 6–24 h (depending upon the experiment, see below), two socks were sampled from each treatment following the order of the previously assigned numbers.

Depuration units comprised 22-L plastic containers maintained at a constant temperature (6 or 11°C) by partial immersion in a 1000-L bath of running water at 5 or 10°C. All units were stocked with the same number of socks, to a maximum of nine socks per unit. Clean seawater of 18 or 28‰ salinity was delivered at a constant rate, without recirculation. Overflowing seawater from the depuration units was allowed to fall into the cooling bath.

Ammonia concentration was monitored using Seatest ammonia kits (Aquarium Systems, Mentor, OH) at 6-h intervals during the day. Koch rings having an established bacterial flora were placed in each depuration unit together with the mussels to control ammonia. The flow rate of incoming seawater was maintained such that the ammonia in every unit in a given trial did not exceed 0.4 μg·mL^{-1} at any time. Flow rates of 4 L·h^{-1} as well as partial water exchanges each evening were required in experiments at 11°C where 18‰ salinity was employed. Lower flow rates of 2 L·h^{-1} were used when all units were kept in 28‰ or at 6°C.

To minimize reingestion of faeces, a plastic grid with a 1-cm mesh was placed on the bottom of each container to trap solids. Each unit was continuously aerated using a 2 × 2 × 5 cm, large-pore airstone strapped to a central pipe 10 cm above the bottom. The mussel socks were suspended through holes in a plastic lid. The lids, together with suspended mussels, airstone, and water and food supply lines, were transferred to clean depuration units at 24-h intervals so that the animals were completely removed from contact with their faeces and dissolved excreta.

When the treatment was continuous feeding, food was mixed into each container at a concentration of 5.5 mg·L^{-1} and additional ration was delivered simultaneously and continuously to all treated units using a metered peristaltic pump. Spray-dried Toruteen yeast (U.F.L. Food Products Inc., Montreal, Que.) mixed in seawater was used as a food source. This food was selected for its convenience and economy so that, if effective, it could be realistically recommended for industrial application. The yeast had a particle size range of 1–5 μm. The rate of flow of food was reduced after the removal of each sample so that the water in each unit was slightly colored but never turbid, and there was minimal accumulation of food on the bottom. On average, during feeding trials the ration delivered for each mussel was 4 mg·h^{-1}.

Each sock of experimental mussels was divided into 3 subsamples of three animals each. Thus, each sample of two socks provided six data points. The three drained meats were weighed. Digestive glands (including the gut lumen content, portions of

intestine enveloped by the glands, and adjacent portions of kidney) were dissected out, weighed, and homogenized using a Polytron homogenizer.

Estimation of Domoic Acid Concentration

Extraction and quantification of domoic acid were performed according to the boiling water method of Quilliam et al. (1989). Extracts were analyzed on a Gilson HPLC system consisting of a model 302 pump, a 100-μL loop injector, a Supelcosil LC-18PAH column (15 cm × 4.6 mm I.D., 5 μm), and a variable wavelength UV detector set to 242 nm wavelength and 0.01 sensitivity. The isocratic mobile phase was 9% acetonitrile in water acidified with 0.1% trifluoroacetic acid. Parallel extractions were performed on a reference mussel homogenate (MUS-1) and using a domoic acid analytical standard (DACS), both supplied by the National Research Council of Canada, Halifax, N.S. Quantification was accomplished by comparing the areas of peaks from unknowns with those of standard solutions prepared from the DACS. Using MUS-1, 100% recovery of domoic acid was accomplished routinely.

Concentrations of domoic acid in mussel extracts were calculated in terms of micrograms of domoic acid per gram in mussel digestive glands on a wet weight basis. These values were then extrapolated to micrograms per gram in whole mussel meat, assuming that 100% of the toxin was to be found in the digestive gland as defined above.

Statistical Analysis

The data for decrease in domoate concentration in whole mussel meat over time in each treatment were transformed logarithmically and fitted to curves by linear regression technique (SAS Institute Inc. 1987). Multiple regression analysis of variance was used to determine the statistical significance of differences among depuration curves. The residuals were plotted for each data set. These plots were examined for any consistent pattern that might suggest that a better fit would be obtained using a two-compartment model.

Results

Trial 1. Effect of Salinity at 11°C (Mussels Collected 15 November)

Under conditions of 18 and 28‰ salinity at 11°C, large mussels (60–70 mm in length) with a mean (±SD) domoate concentration of 22.6 ± 12.9 $\mu g \cdot g^{-1}$ lost 50% of their toxin within 24 h and 90% within 48–72 h (Fig. 1). Two replicate units were employed for each salinity treatment. As there were no differences between replicate units, all data were pooled. Domoate concentration reached a residual level of 1 $\mu g \cdot g^{-1}$ within 72 h regardless of salinity.

In this experiment, samples were taken at 0, 6, 12, 24, 36, 48, 72, and 120 h. However, the high degree of variability in domoic acid concentration negated any benefit from sample times less than 24 h apart. There was no difference in slopes of the depuration curves and only a slight improvement in the standard errors of the slopes when the full data set was used for analysis compared with when only 24-h interval data over the 0- to 72-h period were used. The 6-h sampling program was logistically difficult. Because of the poor return for the effort involved, sampling was restricted to 24-h intervals in subsequent trials.

Trial 2. Effects of Mussel Size and Feeding at 11°C (Mussels Collected 20 November)

In this trial, toxic mussels of two size classes (45–55 and 60–70 mm) were tested in parallel. Half of the mussels were fed continuously on yeast while the others were starved. Because the ratio of weight of digestive gland to total meat weight was greater in the smaller mussels (0.24 ± 0.06) than in the larger ones (0.18 ± 0.01), the meats of the small animals were more toxic (21.9 ± 7.8 versus 14.9 ± 2.5 $\mu g \cdot g^{-1}$ in large animals) on a fresh weight basis. Toxin concentration in digestive gland tissue alone was similar in small and large mussels (88.8 ± 18.3 and 82.7 ± 11.3 $\mu g \cdot g^{-1}$, respectively).

In all treatments, the mussels, on average, lost at least 50% of their toxin in the first 24 h and 90% by 48–72 h (Fig. 2). Thus, results were similar to those of trial 1. Rates of depuration were more rapid in the smaller mussels, regardless of feeding.

Trial 3. Effects of Salinity and Feeding at 6°C (Mussels Collected 1 December)

Large mussels (60–70 mm) collected for this trial contained 13.3 ± 3.2 μg domoic acid $\cdot g^{-1}$. Small mussels (45–55 mm) from the same collection demonstrated significantly more ($p < 0.05$) toxin per unit body weight (17.4 ± 1.2 μg domoate $\cdot g^{-1}$).

Large mussels were depurated either at 18 or 28‰ salinity, and in each case, half were continuously fed and the others were starved. After 72 h, some mussels contained less than 1 μg toxin $\cdot g^{-1}$, but others still retained as much as 8.5 μg domoate $\cdot g^{-1}$ (Fig. 3). This was in contrast with the more complete clearance of toxin from mussels at 11°C. The rate of depuration was similar at low and high salinity and was consistently more rapid in the fed mussels compared with starved ones.

Multiple Regression Analysis

According to the analysis of variance (Table 1), both temperature and mussel size had significant effects upon the rate of depuration of domoic acid. Depuration was more rapid in small mussels and at the higher temperature. Salinity was marginally nonsignificant and there was no significant effect attributable to feeding.

Validity of the Single-Compartment Model

In modelling depuration with a single regression line, the working hypothesis was that the toxin resided in a single body compartment. Residuals from the linear regressions of log domoic acid concentration versus time were examined and in most cases these were randomly arranged. There was no consistent pattern suggestive of the retention of any demonstrable amount of domoic acid in a second, slowly clearing compartment.

Discussion

A depuration system for mussels was devised that had flowing seawater of suitable temperature and salinity, was well aerated, and allowed for removal of contaminated faeces. Given such favorable conditions for active filtration, one could hypothesize that mussels might depurate faster at higher temperatures (Bayne et al. 1976), as long as the temperature was

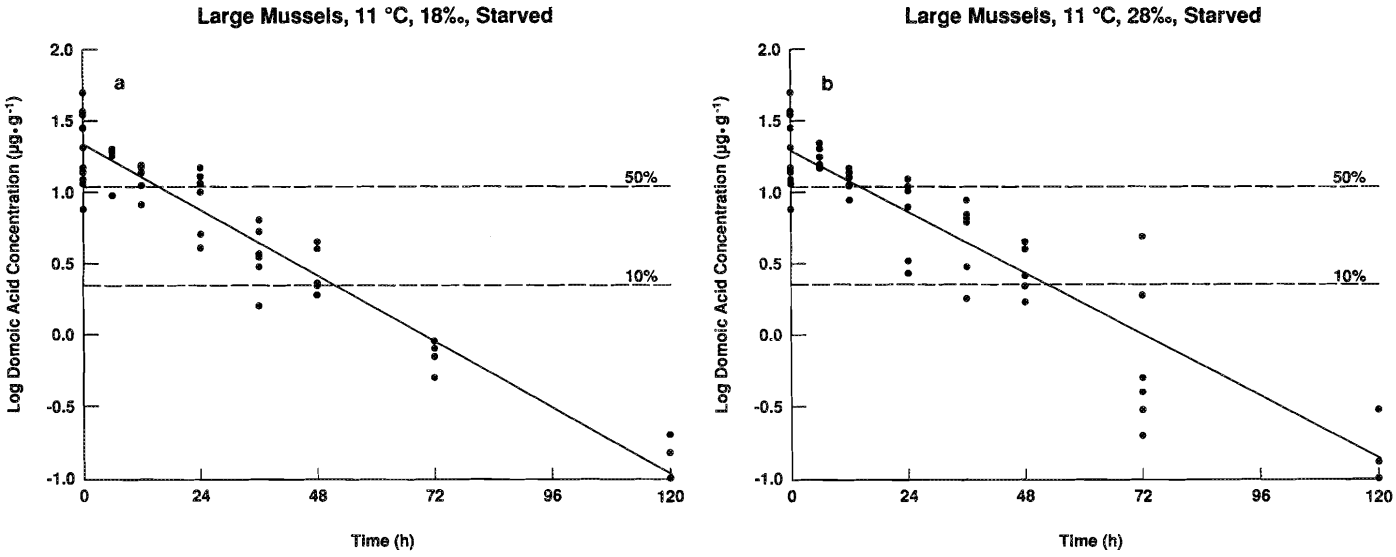

FIG. 1. Trial 1. Domoic acid concentration in whole mussel meat over time at 11°C in large, starved mussels. (a) Salinity 18‰; log DOM = $1.31 - 0.019 \cdot T$; $R^2 = 0.94$. (b) Salinity 28‰; log DOM = $1.30 - 0.018 \cdot T$; $R^2 = 0.88$. Depuration curves fitted by linear regression on log-transformed data. Broken lines indicate 50 and 10% of average initial toxin content.

not so high as to stimulate spawning (Thompson 1984) or cause heat stress. Small mussels might be anticipated to depurate faster than larger ones (Widdows et al. 1979; Tsuchiya 1980; Hawkins et al. 1990; Schulz-Baldes 1974), and continuously fed mussels might depurate faster than starved animals (Hawkins and Bayne 1984) as long as particle loads were not so high as to inhibit filtration (Thompson 1984). If domoic acid occurred as a cytosolic free amino acid in mussel cells, exposure to low salinity might effect a rapid clearance of this compartment (Gilles 1972; Wright et al. 1987).

If naturally occurring domoic acid resided only in the gut cavity of mussels, depuration should proceed in a single rapid stage coincident with gut clearance. Provided that domoic acid acted like a nontoxic food item, gut clearance should be accomplished within 24 h, depending upon temperature (Foster-Smith 1975; Hawkins and Bayne 1984). However, depuration could be prolonged if the toxin interfered with normal digestive and egestive processes. If there was also some uptake of toxin into tissues, two or more stages might be apparent, with the intracellular compartment(s) clearing more slowly.

The curve of log domoate concentration versus time (Fig. 1–3) was modelled reasonably well by a single straight line. The bulk of domoic acid was therefore associated with a rapidly clearing body compartment, probably the gut lumen. As total clearance was not accomplished within 24 h, however, this could indicate that domoic acid has an inhibitory effect on gut evacuation. There was no consistent evidence of the persistence of toxin in any more slowly clearing compartment. Other experimental evidence (Novaczek et al. 1991; Madhyastha et al. 1991) has indicated minor uptake of domoic acid into mussel cells. Clearly, the degree of intracellular incorporation of domoic acid in the naturally toxic mussels used in this study was insignificant compared with the toxin in the gut lumen, as it could not be distinguished against the mussel-to-mussel variability in gut content. However, evidence of long-term storage of residual, and presumably intracellular, toxin (about 1 $\mu g \cdot g^{-1}$ in digestive gland tissue) was noted in 10% of mussels retained in culture for up to 3 mo after exposure to toxic *N. pungens* (I. Novaczek and M. S. Madhyastha, unpubl. data).

Smaller mussels consistently carried more toxin per unit body weight than larger ones and this may be attributed to their relatively heavier digestive glands. In spite of this, smaller mussels cleared their body load of toxin more rapidly than larger mussels. This observation is consistent with previous reports of rapid metabolic rates in young mussels (Schulz-Baldes 1974; Widdows et al. 1979; Tsuchiya 1980; Hawkins et al. 1990).

Low temperature consistently depressed the rate of clearance of domoic acid. This is consistent with evidence that metabolic processes such as filtration rate and assimilation efficiency are temperature dependent (Bayne et al. 1976).

Although depuration tended to be more rapid at the lower salinity, the effect was not statistically significant. If domoic acid does occur as a cytosolic free amino acid, it is either unaffected by salinity change or it is such a small component of total toxin load that fluxes in this compartment cannot be distinguished against the background variability. The effect of feeding was not statistically significant.

On average, mussels were able to purge themselves of toxin to a level of less than 5 $\mu g \cdot g^{-1}$, and in some cases to undetectable levels, within 24–72 h. Extrapolation of the regression between domoate concentration and depuration time to 1 $\mu g \cdot g^{-1}$ indicates that there would be a doubling in depuration time for each 10-fold increase in initial toxin concentration. If this is the case, the extrapolation suggests that no more than 1 wk would be required at temperatures of 6–11°C to cleanse mussels having the highest domoate concentration yet recorded (Bates et al. 1989) of approximately 900 $\mu g \cdot g^{-1}$. However, domoic acid is toxic to other invertebrates (Maeda et al. 1987) and may be significantly toxic to mussels when present over some as yet undetermined threshold level. In this case, the extrapolation would not be valid and depuration times for highly toxic mussels could be longer. On the other hand, larger toxin loads may signify the ingestion of food that contains more concentrated toxin rather than the presence of a larger mass of toxic food. Provided that high concentrations of domoate do not retard defecation or induce unusually large intracellular uptake of toxin, then one would expect mussels to expel a given mass of gut content in the same amount of time regardless of its

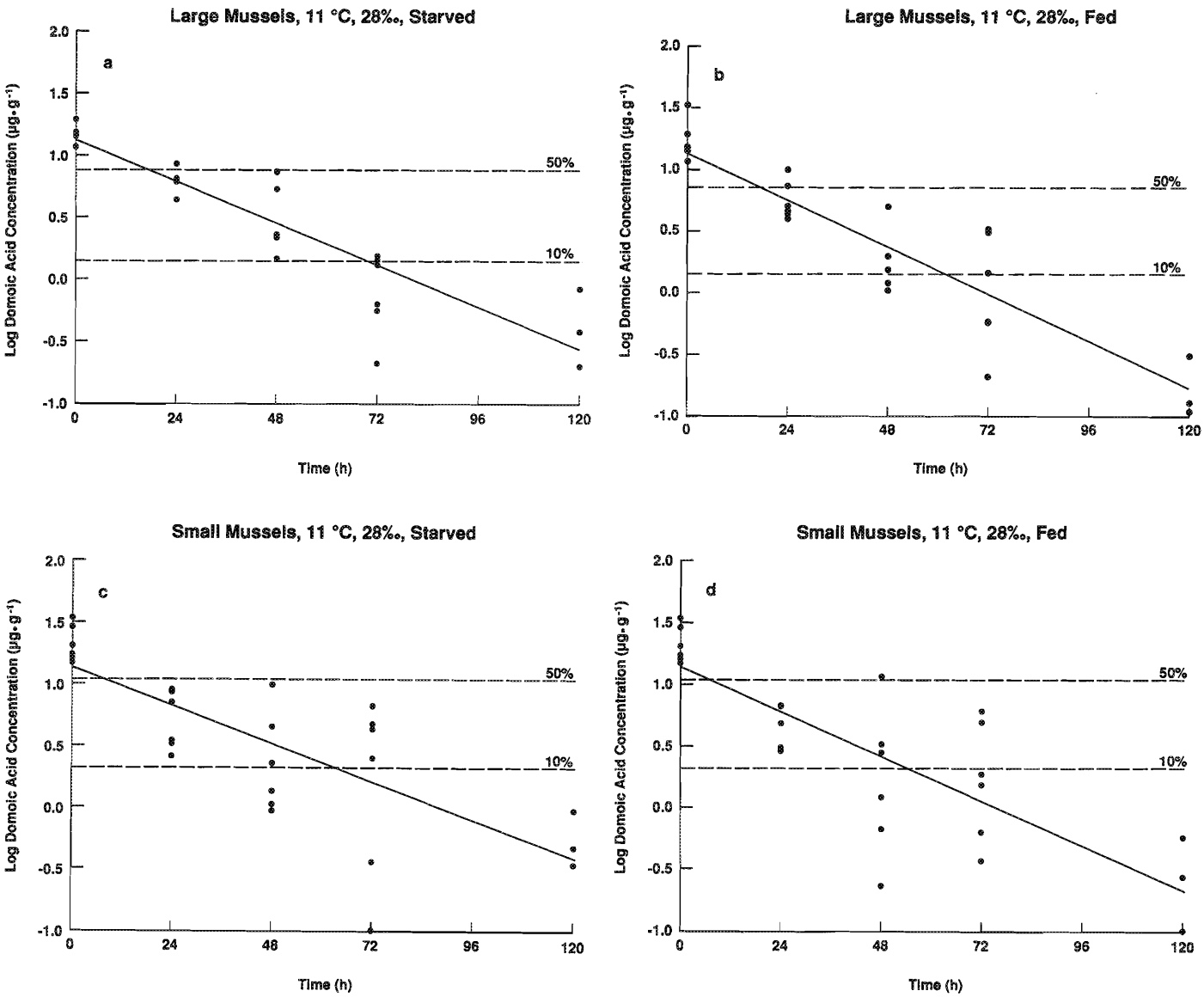

FIG. 2. Trial 2. Domoic acid concentration in whole mussel meat over time at 11°C and 28‰ salinity. (a) Large, starved mussels; log DOM = 1.15 − 0.014·T; R^2 = 0.81. (b) Large, fed mussels; log DOM = 1.13 − 0.016·T; R^2 = 0.85. (c) Small, starved mussels; log DOM = 1.14 − 0.013·T; R^2 = 0.58. (d) Small, fed mussels; log DOM = 1.14 − 0.015·T; R^2 = 0.66. Broken lines as in Fig. 1.

toxicity. Clearly, more work on mussels having a wider range of initial toxin concentrations is required.

In contrast with domoic acid, the algal toxins responsible for paralytic shellfish poisoning (PSP) have not been cleared from mussels in less than 10 d, and depuration may take a number of weeks (Shumway 1990). The rate of loss of PSP toxins appears to change with the season (Prakash et al. 1971) and may be retarded by low temperatures (Madenwald 1985). Attempts to stimulate the depuration of PSP toxins from mussels by manipulating temperature and salinity have yielded little success (Gilfillan et al. 1976; Blogoslawski and Neve 1979). PSP toxins, like domoic acid, are largely restricted to digestive gland and stomach tissue. Unlike domoic acid, PSP toxins are known to be poisonous to mussels and cause adverse effects such as reduced rates of filtration, cell damage in the gut, reduced ciliary activity, and even death (Shumway 1990).

In conclusion, the depuration trials conducted on a laboratory scale indicate the feasibility of removing roughly 90% of the domoic acid from moderately contaminated mussels within a few days. Whether this process will be effective for heavily contaminated mussels or at an industrial scale remains to be tested. The large variability observed in the toxicity of mussels in the field indicates that adequate replication of samples is essential for confident estimation of initial domoic acid concentrations. As long as a reasonable safety margin is employed, guidelines for standard depuration times for mussels of different average initial toxicities should be attainable.

For depuration on an industrial scale, a system of holding tanks for mussels should have the faeces periodically flushed out and/or trapped, and care should be taken not to resuspend faecal material during aeration. Beyond these basic precautions, our study suggests that no special conditions or equipment are needed, as depuration proceeds over a wide range of temperature, salinity, and feeding conditions and in both large and small mussels. Low-salinity salt wells should provide suitable water sources and have the advantage of being higher in temperature than ambient seawater during the latter months (November–December) of the toxic mussel season.

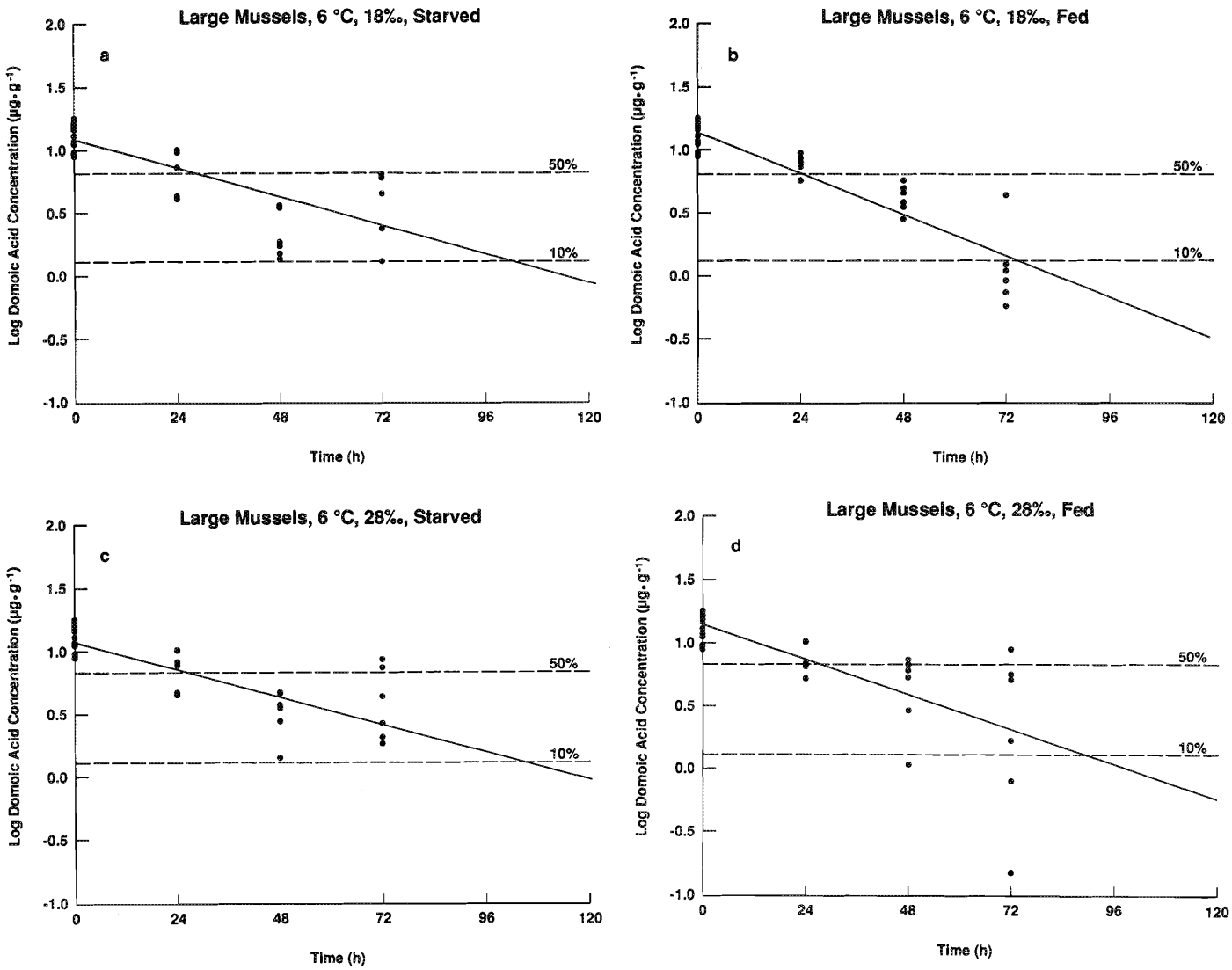

FIG. 3. Trial 3. Domoic acid concentration in whole mussel meat over time at 6°C in large mussels. (a) Salinity 18‰, starved mussels; log DOM = 1.05 − 0.009·T; R^2 = 0.55. (b) Salinity 18‰, fed mussels; log DOM = 1.16 − 0.014·T; R^2 = 0.84. (c) Salinity 28‰, starved mussels; log DOM = 1.08 − 0.009·T; R^2 = 0.61. (d) Salinity 28‰, fed mussels; log DOM = 1.12 − 0.011·T; R^2 = 0.50. Broken lines as in Fig. 1.

TABLE 1. Results of multiple regression analysis (analysis of variance) indicating the statistical significance of differences in depuration curves (log-transformed data) exhibited by toxic mussels under various conditions of mussel size, temperature, salinity, and feeding. *Significant ($p < 0.05$).

Variable	T statistic	p
Temperature (11 vs. 6°C)	−6.56	0.0001*
Mussel size (small vs. large)	−2.61	0.009*
Salinity (18 vs. 28‰)	1.86	0.06
Food (none vs. continuous)	−1.40	0.16

Finally, until government regulations are in place, depuration cannot proceed on mussels from closed harvesting areas. However, in the interest of both consumers and the industry, individual processors could perform depuration trials on legally harvested mussels containing less than 20 µg domoate·g^{-1}. The simple strategy of holding mussels in running water for 24–48 h before shipping will probably obviate any risk to consumers from these marginally contaminated mussels and thus provide mussel producers and buyers with greater confidence in the marketed product.

Acknowledgements

We thank Mr P. Lyon for designing and setting up the seawater system and the Department of Fisheries and Oceans, Charlottetown, for their help in procuring contaminated mussels. This research was conducted with financial support from the National Research Council of Canada.

References

BATES, S. S., C. J. BIRD, A. S. W. DEFREITAS, R. FOXALL, M. W. GILGAN, L. A. HANIC, G. R. JOHNSON, A. W. MCCULLOCH, P. ODENSE, R. POCKLINGTON, M. A. QUILLIAM, P. G. SIM, J. C. SMITH, D. V. SUBBA RAO, E. C. D. TODD, J. A. WALTER, AND J. C. L. WRIGHT. 1989. Pennate diatom *Nitzschia pungens* as the primary source of domoic acid toxin in shellfish from eastern Prince Edward Island, Canada. Can. J. Fish Aquat. Sci. 46: 1203–1215.

BAYNE, B. L., R. J. THOMPSON, AND J. WIDDOWS. 1976. Physiology I, p. 121–206. *In* B. L. Bayne [ed.] Marine mussels: their ecology and physiology.

International Biological Programme 10, Cambridge University Press, Cambridge, U.K.

BLOGOSLAWSKI, W., AND R. NEVE. 1979. Detoxification of shellfish, p. 473. *In* D. L. Taylor and H. H. Seliger [ed.] Toxic dinoflagellate blooms. Elsevier-North Holland, New York, NY.

CANZONIER, W. J. 1971. Accumulation and elimination of coliphage S-13 by the hard clam *Mercenaria*. Appl. Microbiol. 21(4): 1024–1031.

1988. Public health component of bivalve shellfish production and marketing. J. Shellfish Res. 7(2): 261–266.

CHASSARD-BOUCHARD, C., AND P. GALLE. 1988. Cellular and subcellular aspects of chromium concentration by the mussel *Mytilus edulis*: preliminary data. C.R. Acad. Sci. Paris 306(III): 467–473.

CUNNINGHAM, P. A., AND M. R. TRIPP. 1975. Factors affecting the accumulation and removal of mercury from tissues of the American oyster *Crassostrea virginica*. Mar. Biol. 31: 311–319.

FOSSATO, V. U., AND W. J. CANZONIER. 1976. Hydrocarbon uptake and loss by the mussel *Mytilus edulis*. Mar. Biol. 36: 243–250.

FOSTER-SMITH, R. L. 1975. The rate of concentration of suspension on the filtration rates and pseudofaecal production for *Mytilus edulis* L., *Cerastoderma edule* (L.) and *Venerupis pullastra* (Montagu). J. Exp. Mar. Biol. Ecol. 17: 1–22.

GILFILLAN, E. S., J. W. HURST JR., S. A. HANSEN, AND C. P. LEROYER III. 1976. Final report to the New England Regional Commission, p. 1–51.

GILLES, R. 1972. Osmoregulation in three molluscs: *Acanthochitona discrepans* (Brown), *Glycimeris glycimeris* (L.) and *Mytilus edulis* (L.). Biol. Bull. 142: 25–35.

HAWKINS, A. J. S., AND B. L. BAYNE. 1984. Seasonal variation in the balance between physiological mechanisms of feeding and digestion in *Mytilus edulis* (Bivalvia:Mollusca). Mar. Biol. 82: 233–240.

HAWKINS, A. J. S., E. NAVARRO, AND J. I. P. IGLESIAS. 1990. Comparative allometries of gut-passage time, gut content and metabolic faecal loss in *Mytilus edulis* and *Cerastoderma edule*. Mar. Biol. 105: 197–204.

LOBEL, P. B., AND H. D. MARSHALL. 1988. A unique low molecular weight zinc binding ligand in the kidney cytosol of the mussel *Mytilus edulis*, and its relationship to the inherent variability of zinc accumulation in this organism. Mar. Biol. 99: 101–105.

MADENWALD, N. D. 1985. Effect of water temperature on the loss of paralytic shellfish poison from the butter clam, *Saxidomus giganteus*, p. 479–484. *In* D. M. Anderson, A. W. White, and D. G. Baden [ed.] Toxic dinoflagellates. Elsevier Science Publishing, New York, NY.

MADHYASTHA, M. S., I. NOVACZEK, R. F. ABLETT, G. JOHNSON, M. S. NIJAR, AND D. E. SIMS. 1991. In vitro study of domoic acid uptake by digestive gland tissue of blue mussel (*Mytilus edulis* L). Aquat. Toxicol. 20: 73–82.

MAEDA, M., T. KODAMA, T. TANAKA, Y. OHFUNE, K. NOMOTO, K. NISHIMURA, AND T. FUJITA. 1987. Insecticidal and neuromuscular activities of domoic acid and its related compounds. J. Pestic. Sci. 9: 27.

MATTSON, N. S., E. EGIDIUS, AND J. E. SOLBAKKEN. 1988. Uptake and elimination of (methyl-^{14}C) Trichlorfon in blue mussel (*Mytilus edulis*) and European oyster (*Ostrea edulis*) — impact of neguvon disposal on mollusc farming. Aquaculture 71: 9–14.

NOVACZEK, I., M. S. MADHYASTHA, R. F. ABLETT, G. JOHNSON, M. S. NIJAR, AND D. E. SIMS. 1991. Uptake, disposition and depuration of domoic acid by mussels (*Mytilus edulis*). Aquat. Toxicol. 21: 103–118.

PRAKASH, A., J. C. MEDCOF, AND A. D. TENNANT. 1971. Paralytic shellfish poisoning in eastern Canada. Bull. Fish. Res. Board Can. 177: 87 p.

PRUELL, R. J., J. L. LAKE, W. R. DAVIS, AND J. G. QUINN. 1986. Uptake and depuration of organic contaminants by blue mussels (*Mytilus edulis*) exposed to environmentally contaminated sediment. Mar. Biol. 91: 497–507.

QUILLIAM, M. A., P. G. SIM, A. W. MCCULLOCH, AND A. G. MCINNES. 1989. High performance liquid chromatography of domoic acid, a marine neurotoxin, with application to shellfish and plankton. Intern. J. Environ. Anal. Chem. 26: 139–154.

RIISGARD, H. U., E. BJORNESTAD, AND F. MOHLENBERG. 1987. Accumulation of cadmium in the mussel *Mytilus edulis*: kinetics and importance of uptake via food and sea water. Mar. Biol. 96: 349–353.

SAS INSTITUTE INC. 1987. SAS/STAT guide for personal computers, version 6 edition. Cary, NC. 1028 p.

SCHULZ-BALDES, M. 1974. Lead uptake from sea water and food, and lead loss in the common mussel *Mytilus edulis*. Mar. Biol. 25: 177–193.

SHUMWAY, S. E. 1990. A review of the effects of algal blooms on shellfish and aquaculture. J. World Aquacult. Soc. 21(2): 65–104.

SPACIE, A., AND J. L. HAMELINK. 1985. Bioaccumulation, p. 495–525. *In* G. M. Rand and S. R. Petrocelli [ed.] Fundamentals of aquatic toxicology. Hemisphere Publ. Corp., New York, NY.

THOMPSON, R. J. 1984. The reproductive cycle and physiological ecology of the mussel *Mytilus edulis* in a subarctic, nonestuarine environment. Mar. Biol. 79: 277–288.

TODD, E. C. D. 1989. Amnesic shellfish poisoning — a new seafood toxin syndrome. *In* E. Graneli, B. Sundstrom, L. Edler, and D. M. Anderson [ed.] Toxic marine phytoplankton. Proc. 4th Int. Conf. Toxic Mar. Phytoplankton. Elsevier, New York, NY.

TSUCHIYA, M. 1980. Biodeposit production by the mussel *Mytilus edulis* L. on rocky shores. J. Exp. Mar. Biol. Ecol. 47: 203–222.

WIDDOWS, J., P. FIETH, AND C. M. WORRALL. 1979. Relationships between seston, available food and feeding activity in the common mussel *Mytilus edulis*. Mar. Biol. 50: 195–207.

WRIGHT, J. L. C., R. K. BOYD, A. S. W. DEFREITAS, M. FALK, R. A. FOXALL, W. D. JAMIESON, M. V. LAYCOCK, A. W. MCCULLOCH, A. G. MCINNES, P. ODENSE, V. P. PATHAK, M. A. QUILLIAM, M. A. RAGAN, P. G. SIM, P. THIBAULT, J. A. WALTER, M. GILGAN, D. J. A. RICHARD, AND D. DEWAR. 1989. Identification of domoic acid, a neuroexcitatory amino acid, in toxic mussels from eastern Prince Edward Island. Can. J. Chem. 67(3): 481–490.

WRIGHT, S. H., T. W. SECOMB, AND T. J. BRADLEY. 1987. Apical membrane permeability of *Mytilus* gill: influence of ultrastructure, salinity and competitive inhibitors on amino acid fluxes. J. Exp. Biol. 129: 205–230.