Journal of Shellfish Research, Vol. 20, No. 1, 75–84, 2001.

AMNESIC SHELLFISH POISONING IN THE KING SCALLOP, *PECTEN MAXIMUS*, FROM THE WEST COAST OF SCOTLAND

D. A. CAMPBELL,[1] M. S. KELLY,[1] M. BUSMAN,[2] C. J. BOLCH,[1] E. WIGGINS,[2] P. D. R. MOELLER,[2] S. L. MORTON,[2] P. HESS,[3] AND S. E. SHUMWAY[4]

[1]*Scottish Association for Marine Science, Oban, Argyll, Scotland, UK, PA34 4AD;* [2]*National Ocean Service, South Carolina, 29412;* [3]*FRS Marine Laboratory, PO Box 101, Victoria Rd., Aberdeen, Scotland, UK, AB31 6BF;* [4]*Natural Science Division, Southampton College, Long Island University, Southampton, New York, 11968*

ABSTRACT The king scallop, *Pecten maximus*, is a valuable economic resource in the UK. The industry relies on supplying premium "roe-on" processed scallops to the continental market. In July 1999, king scallops harboring the amnesic shellfish poisoning (ASP) toxin, domoic acid (DA), in gonadal tissue at levels above the regulatory limit (20 μg DA g^{-1}) were detected across a wide area of northern and western Scotland. In response, a survey of the southern extent of the closed harvest areas was initiated to describe variability of ASP toxin levels over varying spatial scales (<5 m to >5 km); determine the anatomical distribution of the toxin, and identify, isolate, and culture causative *Pseudo-nitzschia* species. Toxin analysis was conducted using a liquid chromatography-tandem mass spectroscopy (LC-MS/MS) procedure. The DA content of tissues followed the predictable rank order: all other tissue → gonad → adductor. The toxin levels within all other tissue (95% CI = 580–760 μg DA g^{-1}, $n = 170$) consistently accounted for 99% of the total individual toxin burden. DA levels in the gonad (95% CI = 8.2–11.0 μg DA g^{-1}, $n = 170$) were an order of magnitude below levels in all other tissue and contributed to less than 0.5% of the total individual toxin burden, although levels above the regulatory limit were detected in individual gonad samples. Adductor muscle tissue contained the lowest concentrations of DA (95% CI = 0.38–0.82 μg DA g^{-1}, $n = 170$), and was typically within two to three orders of magnitude below levels in all other tissue. None of the scallops examined had DA toxicities in adductor muscle tissue exceeding the regulatory limit. Toxin variability among individuals and sites was high (range of coefficients of variation (CV) in all other tissue = 29%–120% and gonadal = 45%–85%). The results do give an indication of the scale on which microhabitat differences may influence ASP toxicity in *P. maximus* populations, because significant differences were found in all other and gonadal tissue toxin levels between groups of individuals only 25-m apart. In total, seven species of *Pseudo-nitzschia* were identified from west coast waters. A suspected causative species, *P. australis*, was found to produce high levels of DA, in culture. The high individual variation in toxicities and the occurrence of DA in the gonad at levels above the regulatory limit clearly demonstrate the complexity of managing the king scallop fishery during ASP events.

KEY WORDS: amnesic shellfish poisoning, domoic acid, *Pseudo-nitzschia*, *Pecten maximus*, scallop fishery

INTRODUCTION

Marine algal toxins comprise a diverse group of biologically active compounds with high acute toxicities in humans (Shumway & Cembella 1993). Scallops, opportunistic filter feeders exploiting both pelagic and benthic microorganisms as food sources, are liable to the accumulation and concentration of phycotoxins from toxic algal species present in the water column (Shumway et al. 1987, Bricelj & Shumway 1991). The risk of human illness as a result of toxic scallop consumption poses a significant threat to both public health and shellfish industries (Shumway & Cembella 1993).

Amnesic shellfish poisoning (ASP), a relatively new type of seafood toxicity, was first described from Prince Edward Island, Canada, in 1987 (Bates et al. 1989). Over 100 people who consumed mussels contaminated with a naturally occurring neuroexcitatory toxin, domoic acid (DA), experienced gastroenteritis and neurological symptoms (Wright et al. 1989, Todd 1993). In this first episode, the source of the domoic acid was identified as the pennate diatom, *Pseudo-nitzschia pugens* f. *multiseries*, which was ingested and accumulated by the mussels during normal filter feeding (Bates et al. 1989). Global awareness of ASP has since been raised, and, to date, ASP toxin-producing species of *Pseudo-nitzschia* have now been reported from the gulf of Mexico region, North America, Canada, Europe, Australia, Japan, and New Zealand (Hallegraeff 1995). From laboratory studies, it is now clear that several species of *Pseudo-nitzschia*, and two species from separate genera (*Amphora* and *Nitzschia*), are capable of producing DA, but the levels of production are highly variable (Kotaki et al. 2000, Bates 2000, Lundholm & Moestrup 2000).

The king scallop, *Pecten maximus*, is a valuable economic resource in the UK. The UK scallop industry is principally a wild fishery exploited by scallop dredgers, which account for an estimated 97% of UK landings. However, small quantities are landed by divers, and in Scotland, there is an emergent aquaculture industry. The industry is largely reliant on supplying premium "roe-on" processed scallops to the continental market. In 1998, 9,700 tons of *P. maximus* were landed in Scotland, with a first sale value of £15.5 million, equating to approximately 25% of all EU scallop landings (Denton 1999). An estimated 95% of the king scallops are processed as meat and roe product, of which 60% is distributed as premium chilled product and 40% frozen. Dive collected and farmed scallops are sold whole to a smaller, yet higher value market for live shellfish.

The incorporation of systematic ASP/domoic acid testing of shellfish into the Food Standards Agency (FSA) Scottish waters surveillance program was initiated early in 1999. By July 1999, *P. maximus* harboring DA in the gonad at levels above the internationally accepted closure limit (20 μg DA g^{-1}) were detected across a wide area of northern and western Scotland. This prompted a widespread closure of the king scallop fishery, which persisted in excess of 9 months, resulting in financial hardship for scallop dredging, diving, and cultivation industries. The nature of the ubiquitous and prolonged high toxicity seemed to be confined to the king scallop, because only sporadic, short-term toxicities were noted in the queen scallop, *Aquipecten opercularis*, and negligible levels detected in other shellfish (FSA pers. comm., 1999). The direct cost to the industry to date has been estimated at £10 million, and the loss of skilled processing staff and disruption of established supply routes to continental markets led to serious concern for its survival (Denton 1999). The restriction on all scallop landings provoked controversy, stimulating much media interest. To date, there has been no documented history of human illness caused by ASP in the UK.

In response, an opportunistic survey of the southern region of the closed harvest areas was initiated to provide fundamental information on the ASP incident. The objectives were to: describe ASP toxin variability among individual and neighboring scallop populations over varying spatial scales (<5 m to >5 km); determine the anatomical distribution of ASP toxin within scallop body parts: adductor muscle and gonad, and all other tissue (digestive gland, mantle, gill); assess any influence of size, age, and depth on scallop toxicity levels; and isolate, culture, and identify causative *Pseudo-nitzschia* species. The data collected provide basic information to assist with the development of rational management strategies to continue to protect public health while minimize the economic constraints of future ASP events.

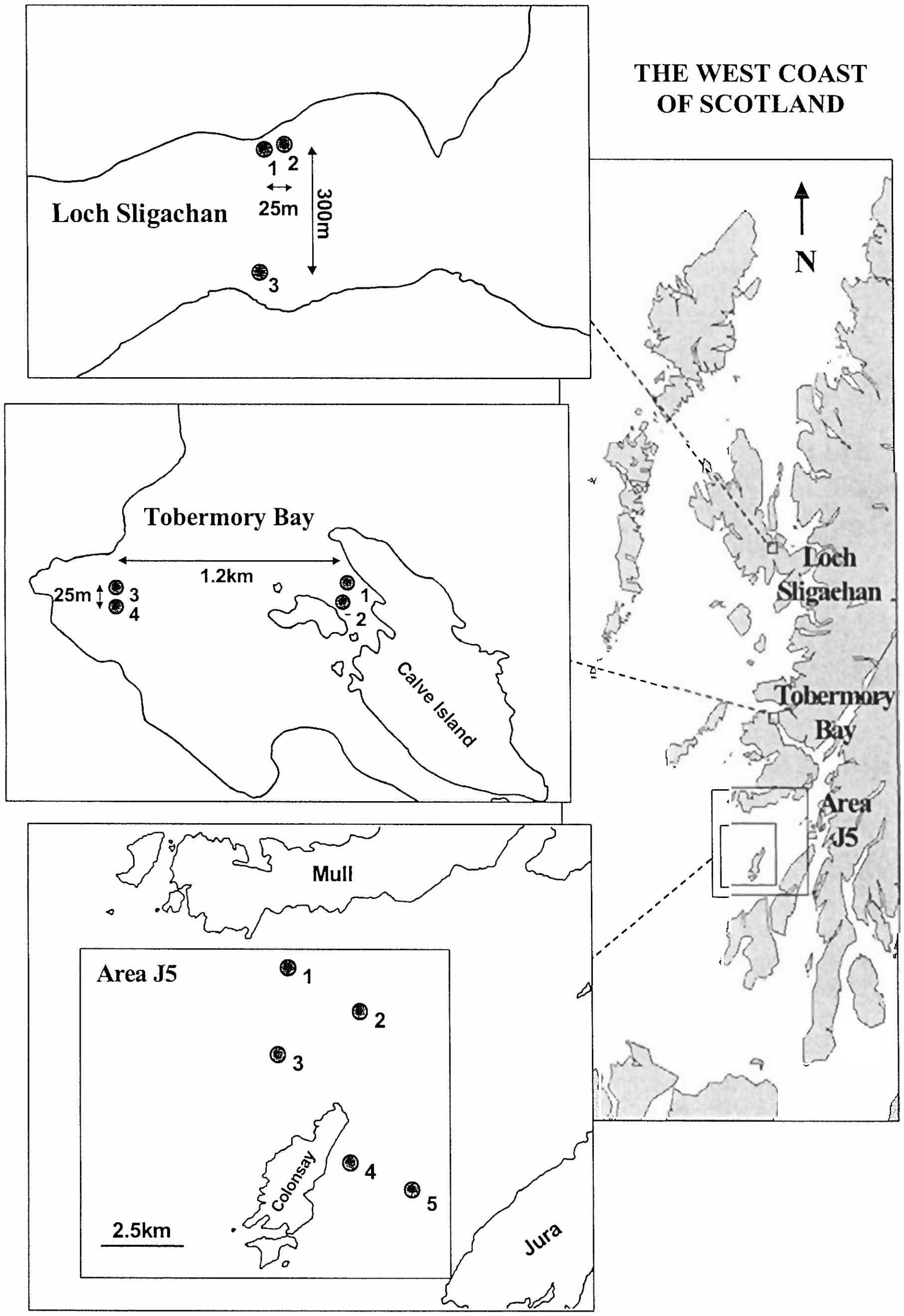

Figure 1. Map of the west coast of Scotland showing the study sampling locations and subsites within locations.

TABLE 1.

Overall mean and standard error (SE) DA levels (µg DA g^{-1} wet weight) in the adductor, gonad, all other tissue and total tissue of *P. maximus* collected from the three locations.

Tissue	*n*	Mean	SE	Minimum	Maximum	CV (%)	95% CI	no. >20 µg g^{-1}	no. >1000 µg g^{-1}
Adductor	170	0.60	0.114	0.011	15.42	247	0.38–0.82	0	——
Gonad	170	9.58	0.722	0.131	75.5	98	8.16–11.01	19	——
All other	170	669	45.7	0.2	3689	89	580–760	169	10
Total	170	295	19.3	0.9	1569	85	256–333	168	8

Ninety-five percent confidence intervals (CI), minimum and maximum levels of DA obtained, coefficient of variation (CV %) and number of individuals with toxin burdens >20 and >1000 µg DA g^{-1} are given.

MATERIALS AND METHODS

Sampling

In December 1999, 10 specimens of adult *P. maximus* (shell height >90 mm) were collected by SCUBA at three subsites (total of 30 individuals) within Loch Sligachan (57°15′5′ N 06°15′5′ W) and at four subsites (total of 40 individuals) within Tobermory Bay (56°35′5′ N 06°05′5′ W) (Fig. 1). To provide an assessment of ASP toxin levels among scallop populations on a larger spatial scale, in context with the current monitoring program; a third sampling location, the scallop fishing box-Area J5, was included in the sampling regime (Fig. 1). Twenty adult scallops (shell height >90 mm) were selected at random from the landings dredged from each of five subsites (total of 100 individuals). These locations were routinely used for the monitoring of ASP toxin under the Food Standards Agency Program and were chosen as a result of previously consistent high DA levels (above the 20 µg DA g^{-1} statutory level) within scallop gonad. Upon collection, all scallops were individually sealed in zip-lock polythene bags, placed in cool boxes, and transported to the Scottish Association for Marine Science (SAMS) Laboratory within 6–12 h, for immediate dissection.

The age of each scallop was estimated by enumerating shell growth bands and shell length and breadth (Mason 1983) measured to the nearest 0.1 mm. The scallops were dissected into the body components: adductor muscle, gonad, and all other tissue (digestive gland, mantle, and gills). Special care was taken to avoid artifactual contamination from adjacent tissues, by careful dissection, washing, and drying of individual body components. The digestive material of the intestinal loop within the gonad was physically removed. All body components were weighed to the nearest 0.001 g, sealed separately in zip-lock polythene bags, and frozen at −20°C before DA extraction (Quilliam et al. 1989).

DA Extraction and Quantification in Scallop Tissue

Tissues were homogenized in a blender (3 min), which was cleaned and rinsed with methanol and then distilled water, between each sample. Four grams of tissue homogenate was rehomogenized (4 min) with 10 mL of 100% methanol, centrifuged (10 min at 5,000 rpm), and a 5-mL subsample of the resulting supernatant filtered through a disposable 45-µm filter membrane. The extract was stored at −20°C before DA detection and quantification.

The extracts were evaporated to dryness using vacuum centrifugation and resolubilized in 50/50 methanol and water before triplicate analysis. The samples were analyzed on a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system consisting of an Agilent Model 1100 high-performance liquid chromatography (HPLC) system, coupled to either a SCIEX API-III triple quadrupole or a Finnigan LCQ ion trap mass spectrometer. The chromatography was performed on a C18 reversed phase column with a 0.2 mL/min flow of a 1–95% gradient of methanol:

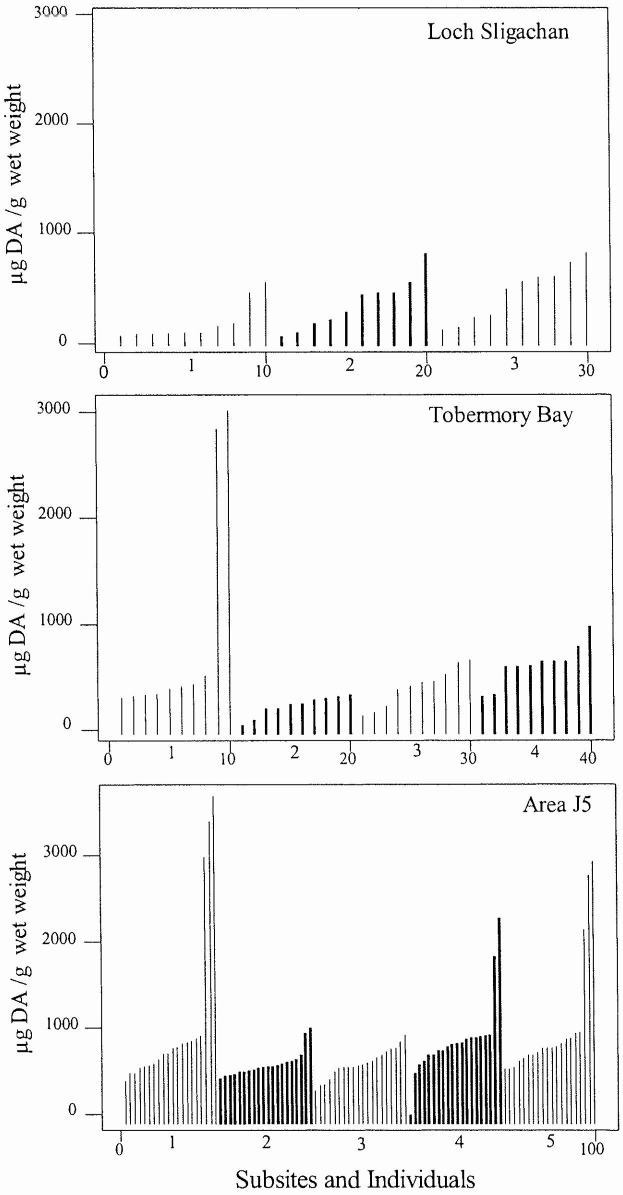

Figure 2. DA toxicity levels (µg DA g^{-1} wet weight) in all other tissue of *P. maximus* collected from subsites within locations.

water. All solvents had 0.1% trifluoroacetic acid added. A portion of the effluent from the HPLC system was directed into the electrospray ionization source of the mass spectrometer via a flow splitter. The mass spectrometer was operated in positive mode with [M+H]+ ions (~312 m/z) being isolated in a first stage of MS analysis. The isolated ions were subjected to "collision- induced dissociation" reaction conditions, which are expected to stimulate the fragmentation of the [M+H]+ ions into characteristic product ions. All quantitations were based on the integrated chromatographic intensity areas of one of the fragment ions (at 267 m/z), and the appearance of other characteristic ions was used as confirmatory evidence for the DA ion's identity (Scholin et al. 2000).

Phytoplankton Sampling and Isolation

During August and October, plankton tows were taken from Orkney, Dunstaffnage, and Jura, to record *Pseudo-nitzschia* spp. presence. In December, at each subsite within Loch Sligachan and Tobermory Bay, surface plankton trawls, quantitative water samples (NIO bottle, at 5 m), cores (water/sediment interface samples), and benthic sediment samples were taken to examine the vertical distribution of *Pseudo-nitzschia* spp. present. At the subsites within Area J5, plankton trawls and quantitative water samples with (NIO bottle, at 5 m) were performed. Four replicates of each sample were obtained from each site; of which two were preserved with Lugol's iodine for cell counts, and two were enriched by addition of f/2 + Si growth medium. Samples were examined for actively growing chains of *Pseudo-nitzschia* cells and candidate chains of cells isolated by micro-pipette and repeated washing in sterile f/2 growth medium. Individual chains of cells were incubated with f/2 + Si growth medium (Guillard & Ryther 1962) at 15°C under an approximate light intensity of 50–80 µmol PAR m^{-2} s^{-1} (12:12 light/dark cycle). Stock cultures were grown for three weeks (stationary phase) before cell harvest by gentle centrifugation (1,500 rpm), followed by removal of excess growth medium and resuspension in sterile growth medium. Samples of cell pellets and supernatant were immediately placed on ice before DA analysis.

DA Extraction and Quantification in **Pseudo-nitzschia** *spp.*

Cell pellets were subjected to ultrasonication (10 min) in 50/50 methanol and water, before filtration (Whatman filter, 0.2 µm). The supernatant samples were directly filtered using the same filter size. The crude extract or filtrate from the supernatant was analyzed using HPLC coupled to a diode-array detector (10 µL injected). The system (Thermoquest) comprised a solvent reservoir and degasser, P4000 pump, AS3000 autosampler, and UV 6000 diode-array detector. The HPLC column was a VYDAC 201TP54 (250 × 4.6 mm, 5 µm) with a VYDAC guard column 201GK54T (10 × 4 mm, 5 µm). The mobile phase was 0.1% trifluoroacetic acid in 10% aqueous acetonitrile, at 1.5 mL/min flow rate. The column temperature was kept at 40°C. Wavelengths monitored ranged from 200–360 nm, and spectral confirmation was obtained by comparison of sample spectra to those from the certified reference standard DACS-1C. Quantitation was carried out at a wavelength of 242 nm.

RESULTS

Anatomical Distribution

Despite the considerable variation in toxin levels within each body compartment, DA loading of the tissues followed a predictable rank order: all other → gonad → adductor. The toxin levels within all other tissue consistently accounted for 99% of the total DA burden. A small proportion of individuals had DA levels in all other tissue (>1,000–3,690 µg DA g^{-1}) an order of magnitude greater than mean levels (669 ± 45.7 µg g^{-1}). Mean DA levels (µg

TABLE 2.

Mean and standard error (SE) of DA levels (µg DA g^{-1} wet weight) in all other tissue of *P. maximus* at each sample location and subsite.

Location and subsite	*n*	Mean	SE	Minimum	Maximum	CV (%)	95% CI	no. >1000 µg g^{-1}
Loch Sligachan	30	330^{a}	44.2	58.6	820	73.4	240–420	—
Tobermory Bay	40	537^{a}	93.5	45.2	3023	110	348–726	2
Area J5	100	824^{b}	62.0	0.2	3689	75.3	701–947	8
Loch Sligachan								
1	10	183^{a}	54.7	62.5	549	94.6	59–306	—
2	10	352^{ab}	73.9	58.6	814	66.2	185–520	—
3	10	455^{b}	79.3	123	820	55.1	275–634	—
Tobermory Bay								
1	10	895^{a}	341	307	3023	120	123–1670	2
2	10	228^{b}	29.6	45.2	331	41.5	296–1601	—
3	10	407^{ab}	57.8	137	663	45.0	276–538	—
4	10	618^{a}	61.1	318	984	31.2	479–756	—
Area J5								
1	20	1076^{ab}	224	379	3689	93.0	607–1545	3
2	20	581^{a}	33.5	409	998	25.8	510.6–651	—
3	20	586^{a}	37.7	275	915	28.8	507–665	—
4	20	861^{b}	104	0.2	2270	53.8	645–1078	2
5	20	1016^{b}	159	525	2928	70.1	683–1350	3

Means from the same location with different superscripts are significantly different ($P > 0.05$, Kruskall–Wallis and Dunn's method). Ninety-five percent confidence intervals (CI), minimum and maximum levels, coefficient of variation (CV %) and number of individuals with toxinburdens >1000 µg DA g^{-1} are given.

g^{-1}) in gonad tissue were an order of magnitude below levels in all other tissue, and, on average, contributed to less than 0.5% of the total individual toxin burden. DA levels above the statutory 20 µg DA g^{-1} safety level in gonads were detected in 22% of the scallops examined, although these values were not encompassed within the 95% confidence limits (8.16–11.01 µg DA g^{-1}, $n = 170$). Adductor muscle contained the lowest concentrations of DA and was typically two to three orders of magnitude below levels in all other tissue while accounting for only 0.17% (mean) of the total individual toxin burden. Although the CV (247%) of individual adductor muscle DA levels was observed to be considerably greater than all other and gonad tissues (98 and 89%, respectively), none of the scallops examined, had adductor muscle toxicities that exceeded the statutory limit, and 95% of the samples had levels below 1.9 µg g^{-1}.

A weak, positive correlation was observed between $\log_{10}$ all other tissue toxicity and $\log_{10}$ gonadal toxicity ($r = 0.303$, $P < 0.001$, df $= 169$); whereas, no correlation could be found between DA concentrations in all other and adductor muscle tissue. No significant correlation could be made between DA toxicity in the three body compartments and scallop age and size parameters.

Spatial Distribution

At all sites, a large variation in all other tissue toxin levels between individuals was observed (Fig. 2), indicated by the CV values (Table 2). However, significant differences in all other tissue toxin levels among locations were distinguished. Scallops from Area J5 had significantly greater levels of DA in all other tissue than individuals from Tobermory Bay and Loch Sligachan; whereas, no significant differences in toxin levels were found between scallops from Tobermory Bay and Loch Sligachan.

Significant differences in toxicities between individuals from different subsites within the same location were observed at all the three locations. Within Tobermory Bay, all other tissue DA toxin levels differed significantly between neighboring scallop populations 25 and 1,200 m apart (subsite 2 DA levels were significantly lower than 1 and 4). In scallops from Loch Sligachan, all other tissue DA toxin levels differed between neighboring scallop populations 300 m apart (subsite 3 DA levels were significantly higher than 1). Within the scallop fishing box, Area J5, all other tissue DA toxin levels were significantly higher in scallops from subsites 4 and 5 than 2 and 3, collected 8–12 km apart. Scallops with high all other tissue toxin burdens (>1,000 µg g^{-1}) were not evenly distributed among locations or subsites. The significance of the results remained unchanged, regardless of the removal of individuals with high toxicities from the dataset.

At all sites, a large variation in gonadal toxin levels between individuals was observed (Fig. 2), indicated by CV (Table 3). Despite the wide variation, gonadal toxicity among locations followed the same pattern of the toxicity as all other tissue, because scallops from Area J5 had significantly greater levels of DA in the gonad than individuals from Tobermory Bay and Loch Sligachan. Similarly, no significant differences in gonad toxin levels were found between scallops from Tobermory Bay and Loch Sligachan.

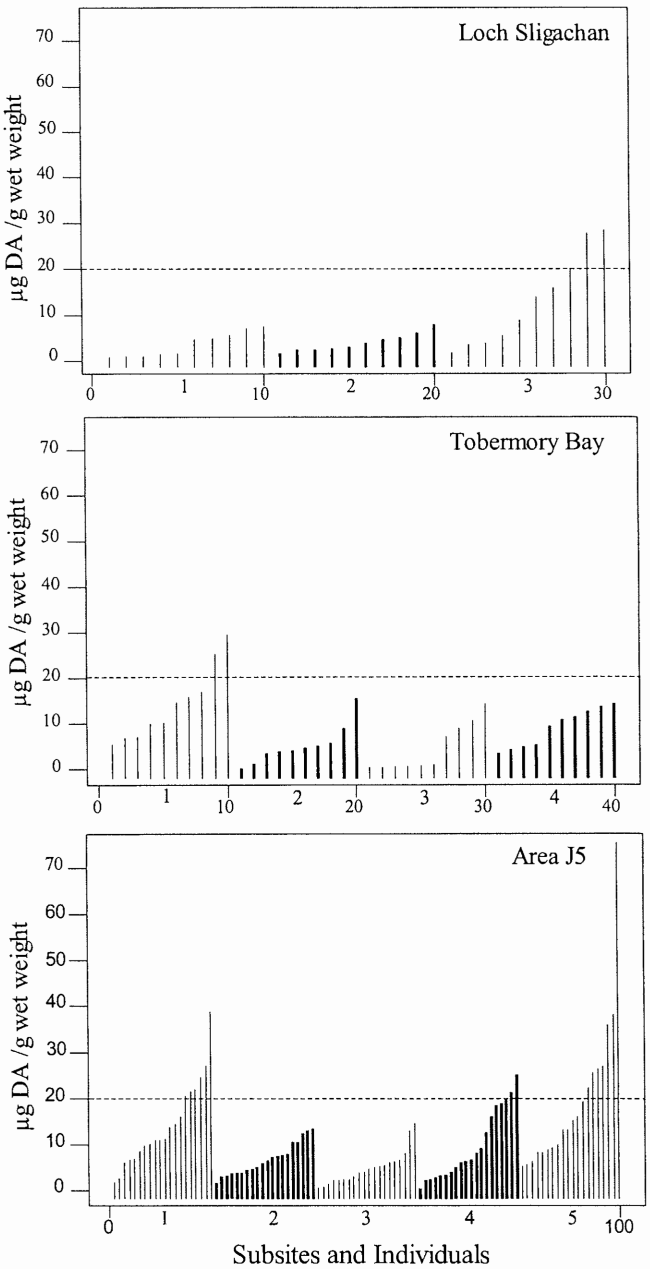

Figure 3. DA toxicity levels (µg g^{-1} wet weight) in the gonad of *P. maximus* collected from subsites within locations. Line = statutory limit (20 µg DA g^{-1} of tissue).

Significant differences in DA levels between subsites of the same site were observed at all threesites sampled. In Tobermory Bay, gonad DA toxin levels differed between neighboring scallop populations 25-and 1,200-m apart (subsite 2 and 3 DA levels were significantly lower than 1). In Loch Sligachan, gonadal DA toxin levels differed between neighboring scallop populations 300 m apart (subsite 1 DA levels were significantly higher than 3). Within Area J5, gonadal DA toxin levels were significantly higher at subsite 5 than at 2, 3, and 4; whereas, gonad toxicity in subsite 1 was greater than that in 3. At each location, scallops with gonadal toxicities exceeding the 20 µg DA g^{-1} limit were encountered. However, the frequency of these individuals was not homogenous among the subsites, ranging from 0 out of 20 individuals (Area J5, subsites 2 and 3) to 7 out of 20 (Area J5, subsite 5).

Scallops from Tobermory Bay had significantly lower levels of DA in the adductor muscle than individuals from Loch Sligachan and Area J5. Levels of toxicity among locations did not correspond to the pattern of toxicity seen in all other and gonad tissue. No significant differences in adductor muscle toxin levels were found

TABLE 3.

Mean and standard error (SE) of DA levels (μg DA g^{-1} wet weight) in the gonad of *P. maximus* at each sample location and subsite.

Location and subsite	n	Mean	SE	Minimum	Maximum	CV (%)	95% CI	no. >20 μg g^{-1}
Loch Sligachan	30	6.82^{a}	1.35	0.77	28.62	108	4.06–9.58	3
Tobermory Bay	40	8.18^{a}	1.06	0.13	29.49	81.9	6.04–10.32	2
Area J5	100	10.97^{b}	1.06	0.36	75.47	96.8	8.87–13.08	15
Loch Sligachan								
1	10	3.52^{a}	0.847	0.775	7.517	76.1	1.6–5.4	—
2	10	3.93^{a}	0.622	1.513	7.939	50.1	2.5–5.3	—
3	10	13.02^{b}	3.17	1.72	28.62	77.0	59–20.2	3
Tobermory Bay								
1	10	14.14^{b}	2.54	5.48	29.49	56.8	8.4–19.9	2
2	10	5.21^{a}	1.38	0.13	15.55	83.5	2.1–8.3	—
3	10	4.41^{a}	1.66	0.32	14.18	119	0.7–8.2	—
4	10	8.95^{ab}	1.32	3.33	14.28	46.7	6.0–11.9	—
Area J5								
1	20	14.17^{ac}	2.05	1.80	38.84	64.9	9.9–18.5	6
2	20	6.77^{ab}	0.792	1.593	13.28	52.3	5.1–8.4	—
3	20	4.86^{b}	0.823	0.594	14.59	75.7	3.1–6.6	—
4	20	9.57^{ab}	1.71	0.36	25.15	79.7	6.0–13.1	2
5	20	19.50^{c}	3.68	5.21	75.47	84.5	11.8–27.2	7

Means from the same location with different superscripts are significantly different ($P > 0.05$, Kruskall–Wallis and Dunn's method). Ninety-five percent confidence intervals (CI), minimum and maximum levels of DA obtained, coefficient of variation (CV %) and number of individuals with gonadal toxin burdens >20 μg DA g^{-1} statutory limit are given.

between scallops from Loch Sligachan and Area J5. At all sites, an exceptionally large individual variation in adductor muscle toxin levels was observed (Fig. 4), indicated by the CV (Table 4).

Within Tobermory Bay, adductor muscle DA toxin levels differed between neighboring scallop populations 25- and 1,200-m apart (subsite 3 DA levels were significantly lower than 1). In Loch Sligachan, no significant differences in adductor muscle toxin levels were observed between subsites. In Area J5, adductor muscle toxin levels were significantly higher in scallops in subsite 5 than in 2 and 3, and toxicity in subsite 1 was greater than in 2. Again, the significance of the results remained unchanged, regardless of the removal of individuals with a comparatively high toxin loading.

Pseudo-nitzschia spp. Abundance and DA Production

The August to October plankton tows samples showed several potentially toxic *Pseudo-nitzschia* species were present. At the peak of the blooms, *P. australis* was the dominant species followed by *P. pungens*. Several other species were present as minor components: *P. multiseries*, *P. seriata*, *P. delicatissima*, *P. fraudulenta*, and *P. pseudodelicatissima*. The blooms were observed to subside during October 1999, with low levels of *P. delicatissima* persisting through to December 1999. At the time of scallop collection (December 1999) *Pseudo-nitzschia spp.* cell numbers were exceptionally low throughout the water column (<1 cell/mL) at the sites sampled (water temperature range 7–9.5°C). These cell concentrations are well below that usually associated with reported ASP events. Examination of surface sediments at Loch Sligachan and Tobermory Bay also failed to detect significant quantities of living or dormant cells *Pseudo-nitzschia spp.*

Three *Pseudo-nitzschia* cultures were established from samples collected in the August 1999 blooms, two strains of *P. australis*, and one of *P. pungens*. Stationary growth-phase cultures of both *P. australis* strains produced detectable levels of DA in intracellular and extracellular fractions (Table 5). However, the presence of DA could not be detected in the *P. pungens* cultures (detection limit = 0.1 μg mL^{-1} in cell/supernatant extracts). In both *P. australis* cultures, total DA was partitioned with approximately one- third being intracellular and two-thirds present in the growth medium.

DISCUSSION

The trend in body component toxicity of *P. maximus* as a proportion of total scallop toxin burden (all other tissue → gonad → adductor), is in agreement with previous studies of DA in *P. maximus* (Arevalo et al. 1998), DA in *Placopecten magellanicus* (Douglas et al. 1996), and paralytic shellfish poisoning (PSP) in *P. magellanicus* (Cembella et al. 1994). In the current study, 99.4% of individuals had levels of DA in all other visceral tissue over the statutory 20 μg DA g^{-1} limit. The maximum DA concentration in all other tissue (3,689 μg DA g^{-1}), recorded in this study was approximately 180 times the regulatory limit (20 μg DA g^{-1}) and is among the highest levels recorded in bivalves. Arevalo et al. (1998) found the highest levels of DA in the hepatopancreas in *Pecten maximus* (maximum = 2,083 μg DA g^{-1}). Similar high levels (approximately 3,000–4,000 μg DA g^{-1}) were found in the digestive gland of *Placopecten magellanicus* (cited in Douglas et al. 1996). Thus, the levels of DA found in all other tissue in the present study are consistent with previous findings, confirming that DA is predominantly sequestered within the digestive gland in *P. maximus*.

Toxin levels of gonadal tissue were generally lower than the statutory 20 μg DA g^{-1} limit; however, toxicities above this level were encountered. Adductor muscle toxicity contributed negligible amounts to the total body burden, and levels never exceeded the statutory limit, even when toxin levels were extremely high in all other tissue. The occurrence of PSP toxins in adductor muscle is

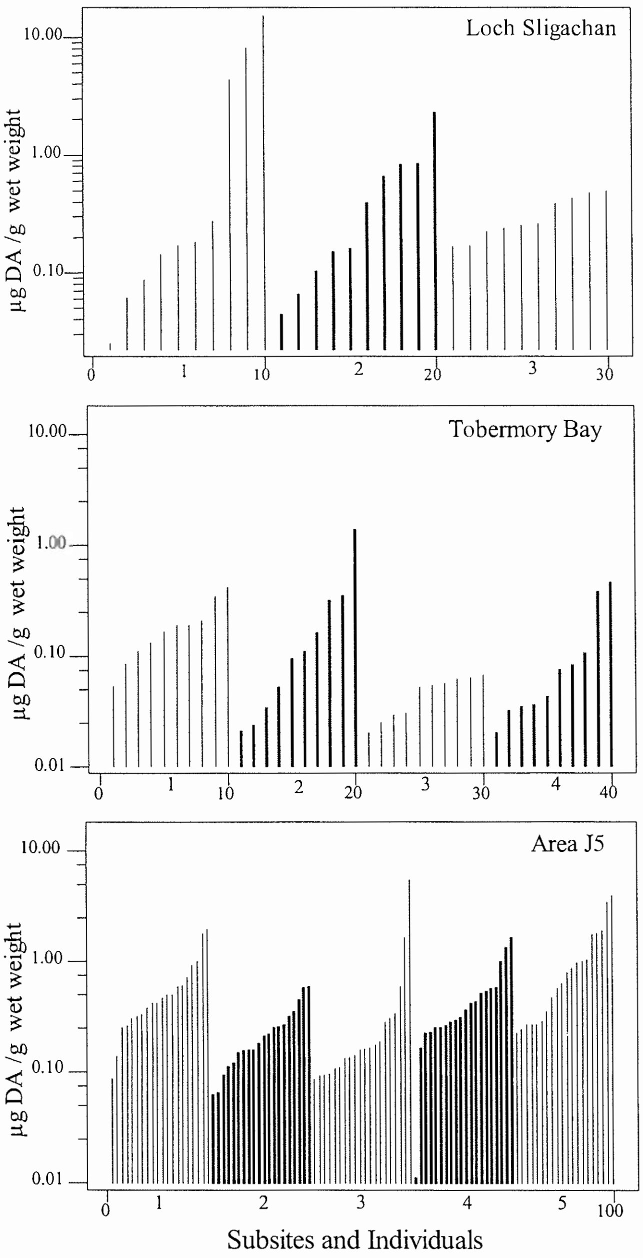

Figure 4. DA toxicity levels (μg DA g^{-1} wet weight) in the adductor muscle of *P. maximus* collected from subsites within locations, plotted against a $\log_{10}$ scale.

rare; and in the scallops *P. magellanicus*, *Patinopecten yessoeniss*, and *Crassoderma gigantea*, adductor muscle PSP toxicity is always at least 10-fold less than in corresponding digestive tissue (cited in Shumway & Cembella 1993). Bricelj and Shumway (1998) report that, during PSP events, such tissues involved in locomotion as the muscular foot, adductor muscle, and pallial muscles, invariably attain toxicities two to three orders of magnitude below those in the viscera, and contain a minimal proportion, typically < 1% of the total toxin loading, despite their relatively large mass in some species. However, Pacific razor clams, *Siliqua patula*, are reported to sequester DA principally within muscular tissue (Drum et al. 1993).

Confirmation of DA production by *Pseudo-nitzschia australis* and its dominance during the blooms indicate this species was one of the primary sources of scallop ASP contamination in 1999. Although a co-dominant species, *Pseudo-nitzschia pungens*, did not produce detectable concentrations of DA in culture, it is possible that other *Pseudo-nitzschia* species, with known DA production capabilities and present as minor components, may have contributed to the ASP event (e.g., *P. seriata*, *P. fraudulenta*, or *P. pseudodelicatissima*). The dominance of P. australis observed in the west coast waters, was not confirmed for other affected areas. Lundholm et al. (1994) showed *P. seriata* produced DA at low temperatures, thus it could potentially represent a source of DA in colder, northern Scottish waters. The DA concentration of the 1999 Scottish isolates of *P. australis* (3–4 pg total DA $cell^{-1}$) compare closely with previous studies of *Pseudo-nitzschia*, as cellular DA levels are reported to range from 0.1–10 pg DA $cell^{-1}$ for most species studied to date (cited in Bates 1998). Previous studies of western North American *P. australis* have indicated comparatively high DA production capabilities (12–37 pg DA $cell^{-1}$, Garrison et al. 1992). However, our data are more consistent with estimates of 2.0 pg DA $cell^{-1}$ for New Zealand strains (Rhodes et al. 1996), and with unpublished data from Spanish and Irish *P. australis* strains (S. Bates, Fisheries and Oceans, Canada, pers. comm. 2000).

It is likely that the 1999 DA toxification, measured in December 1999, in Scottish king scallops occurred as a result of *Pseudo-nitzschia* blooms during the May to August 1990 period and not the result of continuous intake from toxic benthic sources (Bourne 1965), because *Pseudo-nitzschia spp.* concentrations were very low during October and December, and no significant quantities of living or dormant *Pseudo-nitzschia* cells were detected within the locations at time of sampling. Therefore, the current results support the hypothesis that high DA levels in scallops are a consequence of low rates of toxin catabolism as a result of low winter basal metabolic rates and reduced filtration activity, further influenced by colder waters and reduced food supply (Shumway & Cembella 1993).

The considerable degree of toxin variability observed among individual *P. maximus* and their body components was not unexpected and has been described for other shellfish species contaminated with DA and PSP toxins (White et al. 1993, Arevalo et al. 1998). Characterizing variation in toxin levels among individual species of the same area is necessary both for ecological considerations and for development of sound management protocols (White et al. 1993). The results do give an indication of the scale on which microhabitat differences influence ASP toxicity in *Pecten maximus* populations, because, despite wide individual variation, significant differences were found in all other tissue and gonadal toxin levels between groups of individuals only 25 m apart. Variation in bivalve toxicity is reported to result from an interaction of such factors as timing, persistence, and magnitude of toxic blooms, microgeographic variation in exposure to toxic cells because of bloom patchiness, the specific toxicity per cell, and toxin composition of the contaminating organism, environmental effects on scallop metabolism, and, perhaps, genotypic differences among scallop populations (Bricelj & Shumway 1998). However, the reasons for the few individual scallops retaining exceptionally large toxin burdens in all other tissue (>1,000–3,689 μg DA g^{-1}) are not known.

The ability to detect influences of scallop size parameters on DA accumulation may have been restricted by the limited size class (90–120 mm shell length; i.e., legal landing size) selected for use in the current study. Expanding the range of sizes used to

TABLE 4.

Mean and standard error (SE) of DA levels (μg DA g^{-1} wet weight) in the adductor muscle of *P. maximus* at each sample location and subsite.

Location and subsite	*n*	Mean	SE	Minimum	Maximum	CV (%)	95% CI	no. >20 μg g^{-1}
Loch Sligachan	30	1.254^{b}	0.573	0.025	15.415	250	0.083–2.425	—
Tobermory Bay	40	0.156^{a}	0.037	0.020	1.395	151	0.081–0.231	—
Area J5	100	0.581^{b}	0.081	0.011	5.487	139	0.420–0.741	—
Loch Sligachan								
1	10	2.890^{a}	1.630	0.030	15.42	179	−0.80–6.60	—
2	10	0.559^{a}	0.219	0.045	2.310	124	0.06–1.05	—
3	10	0.313^{a}	0.040	0.168	0.501	40.5	0.22–0.40	—
Tobermory								
1	10	0.192^{a}	0.036	0.537	0.425	60.3	0.11–0.27	—
2	10	0.258^{ab}	0.132	0.021	1.395	162	−0.04–0.56	—
3	10	0.046^{b}	0.005	0.020	0.067	38	0.03–0.06	—
4	10	0.129^{ab}	0.051	0.021	0.466	124	0.01–0.24	—
Area J5								
1	20	0.600^{ac}	0.111	0.086	1.962	82.6	0.37–0.83	—
2	20	0.238^{b}	0.0345	0.063	0.592	64.7	0.16–0.31	—
3	20	0.526^{bc}	0.273	0.085	5.487	231	−0.05–1.10	—
4	20	0.484^{ab}	0.091	0.112	1.652	83.6	0.30–0.67	—
5	20	1.056^{a}	0.235	0.225	3.962	99.7	0.60–1.50	—

Means from the same location with different superscripts are significantly different ($P > 0.05$, Kruskall–Wallis and Dunn's method). Ninety-five percent confidence intervals (CI), minimum and maximum levels of DA obtained, coefficeint of variation (CV %) and number of individuals with adductor muscle toxin burdens >20 μg DA g^{-1} statutory limit are given.

include juveniles may indicate any allometric influences on DA toxin accumulation in *P. maximus*. Under controlled conditions, weight-specific DA toxicity has been demonstrated to be inversely proportionate to body size in mussels *Mytilus edulis* (Novaczek et al. 1992). However, faster detoxification rates per unit body mass in actively growing, smaller, or younger individuals, because of toxin dilution through growth, may mask any allometric relationships present (Bricelj & Shumway 1998).

A significant positive correlation was observed between toxicity of the gonad and that of all other visceral tissue. Although little is known about transfer of DA among tissues, its likely that gonadal toxicity is influenced by the level of digestive gland toxicity, via the intestinal loop, which passes through the gonad and may contain toxic feces. Cembella et al. (1993) demonstrated that PSP toxins are accumulated within gonadal follicles of *P. magellanicus*, even after the exclusion of the intestinal loop. However, the inherent wide individual variation precludes the ability to predict gonad toxicities reliably from routine ASP toxin monitoring of the viscera. Compared with other body components, the variation in adductor muscle toxicity was proportionately larger, and no correlation could be found between toxicity of the adductor muscle and that of all other tissue. The variance in toxicity values in adductor tissue may be attributed to one of, or a combination of, three sources: (1) natural variation in adductor muscle toxicity; (2) variable contamination of the tissue from digestive fluid, during dissection; and (3) analytical error close to the limits of detection. The mean CV accounted for by the detection method for all other tissue, gonad, and adductor muscle was ±11.8, 4.6, and 18.6%, respectively, indicating that the variability observed between individual scallops was not a result of analytical error. The extent to which toxic digestive fluid and exudates contaminates edible tissues should be established to ascertain the potential to reduce the ASP toxin burden by appropriate preparation of adductor muscle and gonad tissue and realize the necessity to standardize preparation of these tissues before testing.

During ASP events, the marketing of *P. maximus* digestive gland, mantles, and gills, poses a high risk to public health, which has an impact primarily on diver-based and cultivation industries supplying markets for whole scallops. However, to allow the marketing of the nontoxic edible component, scallop preparation techniques should be promoted, such as the immediate removal of toxic tissues and thorough washing of the edible component (having ascertained the gonad is safe to consume), and this practice should be regulated and conducted by skilled processing staff before the product reaches the consumer (Shumway & Cembella 1993; Curtis et al. 2000). Our results verify that strict regulatory and monitoring regimes should remain compulsory for the safe marketing of "roe-on" scallops. However, when gonad toxicities are greater than the regulatory limit, discarding of tissues that selectively sequester the DA toxin may provide an effective strategy to enable the marketing of adductor muscle, in conformity with the domestic "roe off" market of the United States and Canada (Bricelj & Shumway 1998).

The concentration of DA in gonad tissue varied by an order of magnitude (range 0.13–75.5 μg DA g^{-1}). Thus, if gonads with high toxicities were to be included in pooled samples, they could potentially elevate toxin levels significantly. This may explain why monitored toxicity at certain sites seemed to oscillate throughout the winter period (FSA pers. comm. 1999). Consequently, a large number of individuals should be included in composite samples to reflect mean population toxicity accurately. However, in species where toxicities are extremely variable, it is the consensus that monitoring tissues on an individual basis proves more informative in developing mitigating strategies for harmful algal bloom management. Curtis et al. (2000) were able to propose site-specific recommendations for management, on the basis of large differ-

TABLE 5.

Concentration of DA in three *Pseudo-nitzschia* cultures established from the 1999 ASP event (pg DA cell^{-1} of intracellular and extracellular fractions and combined total). Cultures harvested at three weeks.

	Domoic acid content (pg cell^{-1})		
Species	Intracellular	Extracellular (supernatant)	Total (combined)
P. australis (isolate 1)	1.32	2.95	4.27
P. australis (isolate 2)	1.20	2.19	3.39
P. pungens	nd	nd	0.00

nd = not detected.

ences in PSP toxicity among geoduck clams, *Panope abrupta*, of different depths and harvest tracts. Data describing individual variability of gonad toxicity within localities allow subpopulations with a low frequency of individuals of elevated gonadal toxicity to be distinguished (as seen in the current study); therefore, they permit evaluation of the level of risk, gonad tissue from specific locations with respect to its rate of consumption, poses to human health. The use of risk assessment models should be considered to assess scallop toxicity with respect to rate of consumption by humans, to continue to maintain public safety standards while at the same time ensuring optimum utilization of the high-quality king scallop resource.

ACKNOWLEDGMENTS

We thank Ms N. Lundholm (University of Copenhagen, Denmark) for the initial identification of *Pseudo-nitzschia* species and the members of the Scallop Association for their invaluable support and assistance. This study was funded by The Scallop Association, The Highlands and Islands Enterprise, PESCA, and The Highland Council.

LITERATURE CITED

Arevalo, F. F., M. Bermudez de la Puente, & C. Salgado.1998. ASP toxicity in scallops: individual variability and tissue distribution. In: B. Reguera, J. Blanco, M.L. Fernandez, & T. Wyatt, editors. Harmful algae. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO. pp. 499–502.

Bates, S. S. 2000. Domoic acid producing diatoms: another genus added! *J. Phycol* 36:978–85.

Bates, S. S., A. S. W. De Freitas R. A. Foxall,M. Gilgan,L. A. Hanic,G. R. Johnson,A. W. McCullock,P. Odense,R. Pocklington,M. A. Quilliam,P. G. Sim,J. C. Smith,D. V. Subba Rao,E. C. D. Todd, J. A. Walter, & J. L. C. Wright. 1989. The pennate diatom *Nitzschia pungens* as the primary source of domoic acid, a toxin in shellfish from eastern Prince Edward Island. *Can. J. Fish. Aquat. Sci.* 46:1203–1215.

Bates, S. S., D. L. Garrison & R. A. Horner. 1998. Bloom dynamics and physiology of domoic acid producing *Pseudo-nitzschia* species. In: D. Anderson, A.Cembella, & G. Hallegraeff, editors. Physiological ecology of harmful algal blooms. NATO-ASI series G: Ecological sciences, vol. 41. Berlin: Springer.

Bourne, N. 1965. Paralytic shellfish poisoning in sea scallops (*Placopecten magellanicus*, Gmelin). *J. Fish. Res. Board Can.* 22:1137–1149.

Bricelj, V. M. & S. E. Shumway. 1991. Physiology: energy acquisition and utilization. In: S. E. Shumway, editor. Scallops: biology, ecology, and aquaculture. New York: Elsevier/North Holland, pp. 305–346.

Bricelj, V. M. & S. E. Shumway. 1998. Paralytic toxins in bivalve molluscs: occurrence, transfer kinetics, and biotranformation. *Rev. Fish. Sci.* 6:315–383.

Cembella, A. D., S. E. Shumway & R. Larocque. 1994. Sequestering and putative biotransformation of Paralytic shellfish toxins by the scallop *Placopecten magellanicus*: seasonal and spatial scales in natural populations. *J. Exp. Mar. Biol. Ecol.* 180:1–22.

Cembella, A. D., S. E. Shumway & N. I. Lewis. 1993. Anatomic and spatio-temporal variation in paralytic shellfish toxin in two bivalve species from the Gulf of Maine. *J. Shellfish Res.* 12:389–403.

Curtis, K. M., V. L. Trainer & S. E. Shumway. 2000. Paralytic shellfish toxins in geoduck clams (*Panope abrupta*): variability, anatomical distribution, and comparison of two toxin detection methods. *J. Shellfish Res.* 19:313–319.

Denton, W. 1999. Proposals for dealing with the algal toxin problem that has hit the scallop industry. Sea Fish Executive Report. Hull: Sea Fish Industry Authority.

Douglas, D. J., E. R. Kenchington, C. J. Bird, R. Pocklington, B. Bradford & W. Silvert. 1996. Accumulation of domoic acid by the sea scallop (*Placopecten magellanicus*) fed cultured cells of toxic *Pseudo-nitzschia* multiseries. *Can. J. Fish. Aquat. Sci.* 54:907–913.

Drum, A. S., T. L. Siebens, E. A. Crecelius & R. A. Elston. 1993. Domoic acid in the Pacific razor clam, *Siliqua patula* (Dixon, 1789). *J. Shellfish Res* 12:443–450.

Garrison, D. L., S. M. Conrad, P. P. Eilers & E. M. Waldron. 1992. Confirmation of domoic acid production by *Pseudonitzschia australis* (Bacillariophyceae) cultures. *J. Phycol.* 29:418–426.

Guillard, R. R. L. & J. H. Ryther. 1962. Studies of marine planktonic diatoms. 1. *Cyclotella nana* Hustedt, and *Detonula confervalea* (Cleve) Gran. *Can. J. Microbiol.* 8:229–239.

Hallegraeff, G. M. 1995. Harmful algal blooms: a global overview. Manual on harmful marine microalgae. Intergov. Oceanogr. Comm. New York: UNESCO.

Kotaki, Y., K. Koike, M. Yoshida, C. V. Thuoc, N. T. Huyden, N. C. Hoi, Y. Fukuyo & M. Kodama. 2000. Domoic acid production in Nitzschia isolated from a shrimp-culture pond in Do Son Vietnam. *J. Phycol.* 36:1057–1060.

Lundholm, N. & Ø. Moestrup. 2000. Morphology of the marine diatom Nitzschia navis-varingica sp. nov., another producer of the neurotoxin domic acid. *J. Phycol.* 36:1162–1174.

Lundholm, N., J. Skov, R. Pocklington & Ø. Moestrup. 1994. Domoic acid, the toxic amino acid responsible for Amnesic shellfish poisoning, now in *Pseudo-nitzschia seriata* (Bacillariophyceae) in Europe. *Phycologia* 33:457–478.

Mason, J. 1983. Scallop and queen fisheries in the British Isles. Buckland Foundation. Fishing News Books.

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

Quilliam, M. A., P. G. Sim, A. W. McCulloch & A. G. McInnes. 1989. High performance liquid chromatography of domoic acid, a marine neurotoxin, with application to shell-fish and plankton. *Int. J. Environ. Anal. Chem.* 36:139–154.

Rhodes, L., D. White, M. Syhre & M. Atkinson. 1996. *Pseudo-nitzschia* species isolated from New Zealand coastal waters: domoic acid production in vivo and links with shellfish toxicity. In: Yasumoto,T., Oshima, Y., & Fukuyo, Y., editors. Harmful and toxic algal blooms. Intergov. Oceanogr. Comm. New York: UNESCO, pp. 631–636.

Scholin, C. A., F. Gulland, G. J. Doucette, S. Benson, M. Busman, F. P. Chavez, J. Cordaro, R. Delong, A. De Vogelaere, J. Harvey, M. Haulena, K. Lefebvre, T. Lipscomb, S. Loscutoff, L. J. Lowenstine, R.

Marin III, P. E. Miller, W. A. McLellan, P. D. R. Moeller, C. L. Powell, T. Rowles, P. Silvagni, M. Silver, T. Spraker, V. Trainer & F. M. Van Dolah. 2000. Mortality of sea lions along the central California coast linked to a toxic diatom bloom. *Nature* 403:80–84.

Shumway, S. E., R. Selvin & D. F. Schick. 1987. Food resources related to habitat in the scallop *Placopecten magellinicus* (Gmelin, 1791): a qualitative study. *J. Shellfish Res.* 6:89–95.

Shumway, S. E. & A. D. Cembella. 1993. The impact of toxic algae on scallop culture and fisheries. *Rev. Fish. Sci.* 1(2):121–150.

Todd, E. C. D. 1993. Domoic acid and amnesic shellfish poisoning: a review. *J. Food Prot.* 56:574–576.

White, A. W., S. E. Shumway, J. Nassif & D. K. Whittaker. 1993. Variation in levels of paralytic shellfish toxins among individual shellfish. In: T. J. Smayda & Y. Shimizu, editors. Toxic phytoplankton blooms in the sea. New York: Elsevier/North Holland, pp. 353–358.

Wright, J. C. L., R. K. Boyd, A. S. W. De Freitas, M. Falk, R. A. Foxall, W. D. Jamieson, M. V. Laycock, A. W. McCullock, A. G. McInnes, P. Odense, V. P. Pathak, M. A. Quilliam, A. Ragan, P. G. Sim, P. Thibault, J. A. Walter, M. Gilgan, D. J. A. Richard & D. Dewar. 1989. Identification of domoic acid, a neuroexcitatory amnio acid, in toxic mussels from eastern Prince Edward Island. *Can. J. Chem.* 67:481--490.