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

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ABSTRACT

The king scallop, Pecten maximus, is a valuable economic resource in the UK. The industry relies on supplying premium "mussel"-producing 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 tissue) 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 estimate variability of ASP toxin levels over varying spatial scales (16 m to 23 km); determine the anatomical distribution of the toxin; and, identify, isolate, and culture causative Pseudo-nitzschia species. Toxic analysis was conducted using liquid chromatography-tandem mass spectroscopy (LC-MSMS) procedure. The DA content of tissues followed the predictable rank order: all other tissue—gonad—adductor. The mean levels within all other tissue (95% CI = 586–761 µg DA g−1 tissue) = 170 ng/g MSMS detected of 95% of the total individual tissue burden. DA levels in the gonad (95% CI = 83–111 µg DA g−1 tissue) = 170 ng/g were an order of magnitude below levels in all other tissue and contributed to less than 15% of the total individual tissue burden. Although levels above the regulatory limit were detected in individual gonadal samples. Adductor muscle tissue contained the lowest concentrations of DA (95% CI = 0.38–0.42 µg DA g−1 tissue) = 170 ng/g and was typically within two to three orders of magnitude below levels in all other tissue. None of the scallops examined had DA toxins in adductor muscle tissue exceeding the regulatory limit. Toxic variability among individuals and sites was high (range of coefficient of variation (CV) in all other tissue = 29%–240% and gonad = 45%–85%). The results do give an indication of the scale at which microbially different influences 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 toxicity and the occurrence of DA in the gonadal levels at above the regulatory limit clearly demystify 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 & Canetti 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; Breit & 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 & Canetti 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 neurotoxic toxin, domoic acid (DA), experienced gastrointestinal and neurological symptoms (Wright et al. 1989, Todd 1993). In this first episode, the source of the domoic acid was identified as the pinnate diatom, Pseudo-nitzschia pungens E. multiformis, which was lagged 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 (Hallegaret 1995). From laboratory studies, it is now clear that several species of Pseudo-nitzschia, and two species from separate genera (Anthane and Nitzschia), are capable of producing DA, but the levels of production are highly variable (Korabi et al. 2000, Bates 2000, Lumbroso & McMurtray 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 "mussel"-produced 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 (DETR 1999). In estimated 95% of the king scallops are pr04/51/07 as meat and roe product, of which 0% is distributed as premium chilled product and 40% frozen. Diver collected and farmed scallops are sold whole to a small, 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 established early in 1999. By July 1999, P. maximus harboring DA in the gonad at levels above the interna-

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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, *Aequipecten opercularis*, and negligible levels detected in other shellfish (FAO, 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 (Dentur, 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 area 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 >3 km), determine the anatomical distribution of ASP toxin within scallop body parts, observe muscle and gonal, and all other tissue (digestive gland, mantle, gills); 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 minimizing the economic constraints of future ASP events.

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**Figure 1.** Map of the west coast of Scotland showing the study sampling locations and substrates within locations.
Overall mean and standard error (SE) DA levels (μg DA g⁻¹ wet weight) in the adductor, gonad, and all tissue and total tissue of *P. maximus* collected from the three locations.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>n</th>
<th>Mean ± SE</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adductor</td>
<td>170</td>
<td>0.60 ± 0.14</td>
<td>0.011</td>
<td>15.42 ± 2.47</td>
</tr>
<tr>
<td>Gonad</td>
<td>170</td>
<td>9.35 ± 0.72</td>
<td>1.341</td>
<td>73.5 ± 30.08</td>
</tr>
<tr>
<td>All other</td>
<td>170</td>
<td>48.5 ± 4.7</td>
<td>0.001</td>
<td>810 ± 105.09</td>
</tr>
<tr>
<td>Total</td>
<td>170</td>
<td>245 ± 19.3</td>
<td>0.09</td>
<td>605 ± 95.85</td>
</tr>
</tbody>
</table>

Notes: Five percent confidence intervals (CI), minimum and maximum levels of DA obtained, coefficient of variation (CV %) and number of individuals with tissue burdens >20 and >1000 μg DA g⁻¹ given.

### MATERIALS AND METHODS

**Sampling**

In December 1999, 10 specimens of adult *P. maximus* (shell height >90 mm) were collected by SCUBA at three substrates (total of 30 individuals) within Loch Sibgachan (57°15'55"N 06°15'55"W) and at four substrates (total of 90 individuals) within Tobermory Bay (56°35'55"N 08°03'55"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 JS, 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 substrates (±10 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 6–24 h, for immediate dissection.

The age of each scallop was estimated by enumerating shell growth bands and shell length and bivalve height (Macdonald 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 antifungal 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, weighed 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 were rehomogenized (4 min) with 10 mL of 100% methanol, centrifuged (10 min at 5000 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 extraction and quantification.

The extracts were evaporated to dryness using vacuum centrifugation and resublimed in 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 chro-
water. All solvents had 0.01% trifluoroacetic acid added. A portion of the effluent from the HPLC system was directed into the electrospray ionization source of a mass spectrometer via a flow splitter. The mass spectrometer was operated in positive mode with [M+H]⁺ ions (≤1.2 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 quantifications 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).

Phystoplankton Sampling and Isolation

During August and October, plankton tows were taken from Okinawa, Long Island, and Jutland to rec entrepreneurs. In December, at each sub-site within Lough Gara and Tobermory Bay, surface plankton tows, 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 sub-sites within Area J5, plankton tows and quantitative water samples (NIO bottle, at 5 m) were performed. Four replicates of each sample were obtained from each site; of which two were preserved with Lugu’s solution for cell counts, and two were enriched by addition of F2 + Si growth medium. Samples were examined for actively growing chains of Pseudo-nitzschia cells and candidate chains of cells isolated by micro-pipette and re-plated washing in sterile F2 growth medium. Individual chains of cells were incubated with F2 + 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 resuspension 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 site. The crude extract or filtrate from the supernatant was analyzed using HPLC coupled to a diode-array detector (10 µL, injected). The system (Thermospectra) comprised a solvent reservoir and degasser, F4000 pump, AS1000 autosampler, and UV 6000 diode-array detector. The HPLC column was a YMC TPG columns (250 x 4.6 mm, 5 µm) with a YMC guard column 20/6KFT (10 x 4 mm, 5 µm). The mobile phase was 0.1% trifluoroacetic acid in H2O, 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 DADS-1C. Quantification was carried out at a wavelength of 242 nm.

RESULTS

Anatomical Distribution

Despite 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 (>1,000–3,600 µg DA g⁻¹) an order of magnitude greater than mean levels (669 ± 45 µg g⁻¹). Mean DA levels (µg)

<p>| TABLE 2. | Mean and standard error (SE) of DA levels (µg DA g⁻¹ wet tissue weight) in all other tissue of P. maximus at each sample location and sub-site. |</p>
<table>
<thead>
<tr>
<th>Location and sub-site</th>
<th>µ</th>
<th>Mean</th>
<th>SE</th>
<th>Minimum</th>
<th>Maximum</th>
<th>CV (%)</th>
<th>95% CI</th>
<th>HI 1000</th>
<th>no. HI 1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lough Gara 30</td>
<td>30</td>
<td>239⁰</td>
<td>44.2</td>
<td>56.2</td>
<td>820</td>
<td>73.3</td>
<td>701-147</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Tobermory Bay 40</td>
<td>40</td>
<td>537⁰</td>
<td>93.5</td>
<td>45.2</td>
<td>3003</td>
<td>110</td>
<td>348-726</td>
<td>2</td>
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</tr>
<tr>
<td>Area J5</td>
<td>100</td>
<td>824⁰</td>
<td>62.0</td>
<td>6.2</td>
<td>3698</td>
<td>73.3</td>
<td>701-147</td>
<td>8</td>
<td></td>
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<tr>
<td>Lough Gara 10</td>
<td>1</td>
<td>108⁰</td>
<td>183</td>
<td>54.7</td>
<td>62.5</td>
<td>549</td>
<td>94.6</td>
<td>59-306</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>352⁰</td>
<td>73.9</td>
<td>59.6</td>
<td>814</td>
<td>66.2</td>
<td>185-530</td>
<td>8</td>
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<tr>
<td>3</td>
<td>10</td>
<td>455⁰</td>
<td>79.3</td>
<td>1.3</td>
<td>830</td>
<td>55.1</td>
<td>278-634</td>
<td>8</td>
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<tr>
<td>Tobermory Bay 10</td>
<td>10</td>
<td>899⁰</td>
<td>341</td>
<td>307</td>
<td>3003</td>
<td>120</td>
<td>123-1670</td>
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<tr>
<td>2</td>
<td>10</td>
<td>228⁰</td>
<td>29.6</td>
<td>5.2</td>
<td>331</td>
<td>41.5</td>
<td>296-1661</td>
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<td>3</td>
<td>10</td>
<td>407⁰</td>
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<td>665</td>
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<td>276-558</td>
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<td>4</td>
<td>10</td>
<td>618⁰</td>
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<td>984</td>
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<td>478-756</td>
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<tr>
<td>Area J5</td>
<td>1</td>
<td>20</td>
<td>1076⁰</td>
<td>224</td>
<td>379</td>
<td>3698</td>
<td>93</td>
<td>605-1545</td>
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<tr>
<td>2</td>
<td>20</td>
<td>381⁰</td>
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<td>409</td>
<td>996</td>
<td>25.8</td>
<td>518-651</td>
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<tr>
<td>3</td>
<td>20</td>
<td>586⁰</td>
<td>37.5</td>
<td>275</td>
<td>915</td>
<td>28.8</td>
<td>505-665</td>
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<tr>
<td>4</td>
<td>20</td>
<td>1016⁰</td>
<td>104</td>
<td>6.2</td>
<td>2375</td>
<td>53.8</td>
<td>645-1078</td>
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</table>

Mean ± SE from the same location with different superscripts are significantly different (P < 0.05, Kruskal-Wallis and Dunn’s method). Ninety-five percent confidence intervals (CI), minimum and maximum levels, coefficient of variation (CV %) and number of individuals with toxoburden >1000 µg DA g⁻¹ are given.
g^{-1}) in gonadal 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}, α = 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% (means) of the total individual toxin burden. Although the CV (±4%) of individual adductor muscle DA levels was observed to be considerably greater than that of gonadal tissue (98 and 85%, 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(dioxin toxicity) and log(DT), 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. AUCI 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 subunits 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 3C1 pop fishing box, Area 25, all other tissue DA toxin levels were significantly higher in scallops from subunits 4 and 5 than 2 and 3, collected 8-12 km apart. Scallop with all other tissue toxin burdens (≥1,000 μg g^{-1}) were not evenly distributed among locations or subunits. The significance of the results remained unchanged, regardless of the removal of individuals with high toxicities from the dataset.

As 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 in 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 gonadal toxin levels were found between scallops from Tobermory Bay and Loch Sligachan.

Significant differences in DA levels between subunits of the same site were observed at all threethes sampled. In Tobermory Bay, gonadal 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 tissue levels were significantly higher at subsite 5 than at 2, 3, and 4; whereas, gonadal toxicity in subsite 1 was greater than that in 3. At each location, scallops with gonadal toxicities exceeding the statutory limit (20 μg DA g^{-1} limit) were encountered. However, the frequency of these individuals was not homogeneous 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 gonadal tissue. No significant differences in adductor muscle toxin levels were found.
between scallops from Loch Sligachan and Arran JS. At all sites, an exception, large individual variation in adductor muscle toxin levels was observed (Fig. 4), indicated by the CV (Table 4).

Within Tobermory Bay, adductor muscle DT levels differed between neighboring scallop populations 25-1,200 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 substrates. In Arran JS, 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-zeastrica sp.—Abundance and DA Production

The August to October production lows samples showed several potentially toxic Pseudo-zeastrica sp. in all seasons. At the peak of the blooms, P. australis was the dominant species followed by P. pungens. Other species were present as minor components: P. multiseries, P. sericea, P. deliciosa var. P. puvca, and P. pseudzeastrica. The blooms were observed to subside during October 1999, with low levels of P. deliciosa var. persisting through to December 1999. At the time of sc ally blooming collection (December 1999) Pseudo-zeastrica spp. cell numbers were exceedingly low with the water column (c.1 cell/mL); 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-zeastrica spp.

Three Pseudo-zeastrica culture cultures were established from samples collected in the August 1999 bloom, 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 Pseudo-zeastrica cultures (c. 3.5 ng mL−1 in cell suspensions extracts). In both P. australis cultures, DA total was partitioned with approximately one-third being intracellular and two-thirds present in the growth medium.

DISCUSSION

The trend in body compartment toxicity of P. maximus as a proportion of total scallop toxin burden (all other tissue (→ adductor), in agreement with previous studies of DA in P. maximus (Arewo et al. 1998). 3,884-fold in 1998, in Tobermory Bay. In the current study, 99%-19% of individuals had levels of DA in all other visceral tissue over the statutory 20 ng DA g−1 limit. The maximum DA concentration in all other tissue (3,884 ng DA g−1), recorded in this study was approximately 0.1 times the regulatory limit (20 μg DA g−1) and is among the highest levels recorded in bivalves. Arewo et al. (1998) found the highest levels of DA in the hepatopancreas in Pecten maximus (maximin 2,083 μg DA g−1). Similarly high levels (approximately 3,800-8,000 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 toxin 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
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 phases in minor components, may have contributed to the ASP event (e.g. *P. seriata*, *P. frustulosa*, or *P. pseudodelicatissima*). The dominance of *P. australis* observed in the w.c. coast waters, was not confirmed for other affected areas. Longhurst et al. (1998) showed *P. seriata* produced DA at low temperatures, thus it could potentially represent a source of DA in cooler, northern Scottish waters. The DA concentration of the 1999 Scottish isolates of *P. australis* (3.4 µg DA cell⁻¹) compare closely with previous studies of *Pseudo-nitzschia*, as cellular DA levels are reported to range from 0.1–10 µg 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 (12–27 µg DA cell⁻¹) (Garrett et al. 1992). However, our data are more consistent with estimates of 3.0 µg DA cell⁻¹ for New Zealand strains (Rhodes et al. 1996), and with unpublished data from Spanish and Irish *P. australis* strain (S. Botes, Fisheries and Oceans, Canada, pers. comm. 2000).

It is likely that the 1999 DA toxification, measured in December 1999, in Scottish long scallops resulted as a result of *Pseudo-nitzschia* blooms during the May to August 1999 period and not the result of continuous intake from toxic benthic sources (Boonne 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 water heated metabolites and reduced filtration activity, further influenced by colder waters and reduced cold water supply (Shumway & Cembella 1993).

The considerable degree of toxic variables observed among individual *P. maximus* and their body components was not unexpected and has been described for other shallow-water species contaminated with DA and PSP toxins (White et al. 1993; Azeredo 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 shellfish management protocols (White et al. 1993). The results do give an indication of the scale on which biological differences influence ASP toxicity in *Pecten maximus* populations; because, despite wide individual variation, significant differences were found in all other tissue and give additional 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 time, persistence, and magnitude of toxic blooms, microenvironmental 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, genetic differences among scallop populations (Brierly & Shumway 1996). However, the reasons for the few individual scallops retaining exceptionally large toxin burdens in all other tissue (t≤0.3–2.69 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 (60–120 mm shell length); i.e., legal landing size selected for use in the current study. Expanding the range of sizes used to

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**Figure 4. DA toxicity levels (µg DA g⁻¹ wet weight) in the adductor muscle of *P. maximus* collected from subhabitats within locations, plotted against the ADD scale.**

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*Substitutes and Individuals*

Confimation 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.
include juveniles may indicate any allostatic 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 (Novackz et al. 1992). However, faster detritification rates per unit body mass in actively growing, smaller, or younger individuals, because of the relative difference in grazing, may mask any allostERIC relationship present (Bicelli & Shimwany 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, it is likely that gonadal toxicity is influenced by the level of digestive gland toxicity, the brain-gut loop, which passes through the gonad and may contain toxic faces. Cowbirda et al. (1993) demonstrated that PSP toxins are accumulated within gonadal follicles of P. nodiculans, even after the exclusion of the intestinal loop. However, the inherent wide individual variation precludes the ability to predict gonadal toxicity reliably from routine ASP toxin monitoring of the mussels. 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 digestion; 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 18.9%, 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 tissue 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 necessary to standardize preparation of these tissues before testing.

During ASP events, the marketing of P. maximus digestive gland, mantle, and gills, poses a high risk to public health, which has an impact primarily on diver-based and cultivation industries supplying markets for whole mussels. However, to allow the marketing of the non-toxic edible component, scallop preparation techniques should be promoted, as the immediate removal of toxic tissues and thorough washing of the edible component (having ascended 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 & Cummins 1993, Curtis et al. 2000). One results verify that strict regulatory and monitoring regimes should remain compulsory for the safe marketing of “raw-on” scallops. However, when gonad toxicities are greater than the regulatory limit, discarding of tissues that selectively separate the DA toxin may provide an effective strategy to enable the marketing of adductor muscle, in conformity with the domestic “reef out” program of the United States and Canada (Bicelli & Shimwany 1998).

The concentration of DA in gonad tissue varied by an order of magnitude (range 0.13–7.55 g DA g−1 dry), thus, if gonads with high toxicities were to be included in pooled samples, they could potentially elevate toxin levels significantly. This may explain why sciocid exposures at certain sites seemed to fluctuate throughout the winter period (IPRA 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 conclusion that monitoring tissues on an individual basis provides 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 “12” of large differ-

<table>
<thead>
<tr>
<th>Location and section</th>
<th>n</th>
<th>Mean (mg DA g−1 wet weight)</th>
<th>SE</th>
<th>Minimum</th>
<th>Maximum</th>
<th>CV (%)</th>
<th>95% CI</th>
<th>n&gt;30</th>
<th>min. g−1</th>
<th>max. g−1</th>
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<td>Lock Vilaedou</td>
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<td>1.29±0.37</td>
<td>0.15</td>
<td>0.60</td>
<td>2.42</td>
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<td>40</td>
<td>0.18±0.07</td>
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<td>0.02</td>
<td>0.35</td>
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<tr>
<td>Area 15</td>
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<td>0.20±0.06</td>
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<td>0.01</td>
<td>0.39</td>
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</table>

Values from the same location with different superscripts are significantly different (P<0.05, Kruskal-Wallis and Dunn’s method). Ninety-five percent confidence intervals of DA (mg) minimum and maximum levels of DA (mg) were determined. CV (%) and number of individuals with adductor muscle toxin burdens >20 mg DA g−1. Statistically significant limits are given.
TABLE 5.
Concentration of DA in three Pseudo-nitzschiaceae cultures established from the 1999 ASP event (µg DA g−1 of intracellular and extracellular fractions and combined total). Cultures harvested at three weeks.

<table>
<thead>
<tr>
<th>Species</th>
<th>Intracellular (µg g−1)</th>
<th>Extracellular (µg g−1)</th>
<th>Total (µg g−1)</th>
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</thead>
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<tr>
<td>P. antitisseli</td>
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</table>

nd = not detected.

ences in PSP toxicity among groupd clams, Panopea abrupta, of different depths and harvest times. Data describing individual vari-

ability 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, to human health. The use of risk assessment models should be considered to assess scalar 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.

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