Introduction

Diatoms are small single cell phytoplankton (Figure 1) which play an important role in the marine food web. They harvest light energy from the sun and pass it up the food chain. Diatoms are unique in that they have a silica outer shell. Morphological variations in the size and patterns of this silica shell are used to delineate different genera and species (Fryxell and Hasle, 2003). Diatoms in Scottish waters are generally monitored using light microscopy (LM). However, in many cases, this method is not sufficiently sensitive to reveal the critical morphological pattern in the silica shell. Many species can be identified to genus level only using LM.

A study using transmission electron microscopy was performed to examine the diversity of the diatom *Pseudo-nitzschia* spp. (Figures 2 and 3) around the Shetland Isles and west coast of Scotland. This diatom is important as it can dominate the phytoplankton spring bloom. Members of this genus also produce the phycotoxin domoic acid (DA) which can concentrate in shellfish flesh and thus be a danger to human health (Fehling et al., 2004).

Methods

- Seawater samples are collected with a 10 µm mesh plankton net
- Diatom cells are concentrated using centrifugation and cleaned using HCl, H₂SO₄, KMnO₄ and oxalic acid to remove all organic material (Christiansen, 1988)
- Samples are dropped onto a TEM formvar covered mesh grid and left to air dry prior to loading into the microscope

> Cells are identified to species level using the following criteria:
> - Length/width/shape of cell (µm) (Figures 4a & b)
> - Number of fibulae per 10 µm (Figure 4c)
> - Number of interstriae per 10 µm (Figure 4c)
> - Number of poroids per 1 µm (Figure 4c)
> - Presence/absence of a central interspace (Figure 4c)
> - Poroid structure (see differences in poroids, Figures 4c & 5)

and guidelines in relevant literature (Skov et al. (1999) and Lundholm et al. (2003 & 2006))

Results

- TEM analysis identified five species of *Pseudo-nitzschia*: *P. cf. australis*, *P. cf. seriata*, *P. fraudulenta*, *P. pungens* and *P. decipiens*. A further unidentified species was also observed
- TEM micrograph images showing the central section of each species observed are shown (Figure 5)
- Morphological characters used to identify species are shown (Table 1)

![Figure 1: Diversity of phytoplankton from Scottish waters](image1)
![Figure 2: *Pseudo-nitzschia* cells as viewed with light microscopy, forming chain. X250 map.](image2)
![Figure 3: *Pseudo-nitzschia* cells as viewed with light microscopy, forming chain. X400 mag.](image3)
![Figure 5a: Micrograph of *P. cf. australis*.](image5a)
![Figure 5b: Micrograph of *P. cf. seriata*.](image5b)
![Figure 5c: Micrograph of *P. fraudulenta*.](image5c)
![Figure 5d: Micrograph of *P. pungens*.](image5d)
![Figure 5e: Micrograph of *P. decipiens*.](image5e)

Conclusions

- The use of transmission electron microscopy showed a diverse *Pseudo-nitzschia* population structure with five species being identified from Scottish waters and also one unidentified species
- This diversity could not be observed using light microscopy
- Two of these species, *P. cf. australis* and *P. cf. seriata*, are confirmed DA producers in Scottish waters (Fehling et al., 2004). This knowledge is important to provide accurate advice to the aquaculture industry
- This level of identification is essential to validate molecular techniques that are currently being developed and to assess changes in diversity that could possibly arise from eutrophication, climate change or via ballast water introductions
- Transmission electron microscopy is an essential tool in assessing diatom diversity in Scottish waters

References


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Table 1: Morphological measurements for the identification of *Pseudo-nitzschia* species

<table>
<thead>
<tr>
<th>Species</th>
<th>Central Interspace</th>
<th>Intervia</th>
<th>Intervia per 10 µm</th>
<th>Fibulae</th>
<th>Fibulae per 10 µm</th>
<th>Rosas de Poroids</th>
<th>Poroids per 1 µm</th>
<th>Width (µm)</th>
<th>Length (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. cf. australis</em></td>
<td>-</td>
<td>13-17</td>
<td>12-17</td>
<td>4-5</td>
<td>6.25-6.36</td>
<td>75.0-193.20</td>
<td>349.0-483.62</td>
<td>349.0-483.62</td>
<td></td>
</tr>
<tr>
<td><em>P. cf. seriata</em></td>
<td>-</td>
<td>15-19</td>
<td>16-20</td>
<td>3-4</td>
<td>7.96-9.50</td>
<td>139.0-195.15</td>
<td>349.0-483.62</td>
<td>349.0-483.62</td>
<td></td>
</tr>
<tr>
<td><em>P. fraudulenta</em></td>
<td>+</td>
<td>21-23</td>
<td>20-23</td>
<td>2-3</td>
<td>7.10-8.80</td>
<td>78.10-186.30</td>
<td>349.0-483.62</td>
<td>349.0-483.62</td>
<td></td>
</tr>
<tr>
<td><em>P. pungens</em></td>
<td>-</td>
<td>15-14</td>
<td>15-16</td>
<td>2</td>
<td>2.89-4.50</td>
<td>132.0-203.05</td>
<td>349.0-483.62</td>
<td>349.0-483.62</td>
<td></td>
</tr>
<tr>
<td><em>P. decipiens</em></td>
<td>+</td>
<td>30-40</td>
<td>28-36</td>
<td>2</td>
<td>8-10</td>
<td>139.0-204.04</td>
<td>349.0-483.62</td>
<td>349.0-483.62</td>
<td></td>
</tr>
<tr>
<td><em>Pseudo-nitzschia</em></td>
<td></td>
<td>30-42</td>
<td>22-24</td>
<td>4</td>
<td>5-6</td>
<td>247.0-375.0</td>
<td>349.0-483.62</td>
<td>349.0-483.62</td>
<td></td>
</tr>
</tbody>
</table>

- * = Central Interspace Present  + = Central Interspace Absent

Figure 4: Details of morphological characters used to identify species of *Pseudo-nitzschia*: (a) single cell; (b) mid-section of a single cell; (c) mid-section of a cell containing a central interspace and of a cell lacking a central interspace (TEM micrographs)