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Phytoplankton species associated with imports of the Pacific oyster *Crassostrea gigas*, from France to Ireland

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Summary

Prior to January 1993 importations of shellfish into Ireland were strictly subject to licence. The implementation of EC Council Directive 91/67/EEC now permits the free movement of shellfish from other EC member states into the country. Oyster imports from 2 regions in France were examined for the presence of phytoplankton species. Sixty seven species of phytoplankton (43 diatoms, 22 dinoflagellates and 2 silicoflagellates) were recorded in addition to other microspecies such as foraminferans and tintinnids. Fifteen types of dinoflagellate cysts were recorded. There is concern that potentially harmful species of phytoplankton may be imported accidentally into Ireland with shellfish transfers.

Introduction

Ireland, as an island on the western seaboard of Europe, has had a policy of restricting the importation of non-native flora and fauna ; and up to January 1993, this was permitted only under licence. However, despite such precautions, over the last thirty years, introductions associated with shellfish transfers have occurred, including *Bonamia ostrea* (in illegal importations of *Ostrea edulis*), the slipper limpet *Crepidula fornicata*, *Calyptera chinensis* and shell disease of *Ostrea edulis*. Minchin *et al.* (1993) review these in more detail.

Little or no data are available regarding importation of non-native phytoplankton species into Ireland. However a review of world literature in recent years has shown a marked increase in publications on the frequency, intensity and geographical distributions of harmful phytoplankton in coastal and inshore waters and the enormous harm that can result from these incidents, both economically and biologically (Anderson 1989, Hallegraeff 1992, Smayda 1990). The problems, in particular of introduced toxic phytoplankton cysts in other countries, especially in Australia and Tasmania, have been well documented, (Bolch & Hallegraeff 1990, Hallegraeff & Bolch 1991,1992), principally via ballast water on ships that have come from foreign ports. When shellfish are collected/dredged for export, phytoplankton species including algal cysts may be present in the mud in and around the shells and can be a means by which new phytoplankton species are introduced into other areas.

In February 1993 half grown Pacific oysters *Crassostrea gigas* were imported into Ireland from France for relaying and ongrowing. Some of these oysters were known to have come from a region in France which around that time was contaminated with an unidentified toxin which induced symptoms similar to those of *Alexandrium* toxins in laboratory animals. Samples from these oysters were obtained and gut contents and sediment associated with the consignments were examined for the presence of phytoplankton and other organisms. Other invertebrate species found in these samples are discussed elsewhere (Minchin et al. 1993)

Methods

Approximately 24 tonnes of half-grown *C. gigas* were imported from France into Ireland between 23rd of January and 22nd of April 1993. Samples were obtained at random from several oyster bags selected from different parts of consignments from Marennes-Oleron either (i) during unloading, (ii) as samples were received from growers or (iii) from trestles on the shore (Minchin et al. 1993). From these samples, further subsamples were obtained in the laboratory for phytoplankton analysis. This was achieved in three ways : (i) samples of gut contents of live oysters were taken, (ii) sediment samples taken from closed oyster shells that did not have meat inside (these are commonly known as "clocks" in the United Kingdom) ; many contained sediment within these shells when prised open and (iii) samples of water and sediment that drained from live oysters. Samples were kept refrigerated at 5^{o}C until examined.

Sediment samples were processed by sonicating for 2 minutes and wet sieving using Instant Ocean seawater. A subsample of each fraction was examined using Phase Contrast Inverted Microscopy. Morphometric and photographic data were collected. When naming dinoflagellate cysts the name of the living dinoflagellate is used wherever possible, otherwise geological names are used. As the method of obtaining samples was not quantitative, only qualitative results are given.

Results

Sixty-seven phytoplankton species were recorded in the sediment samples, 43 diatoms, 22 dinoflagellates (including cysts) and 2 silicoflagellates and other organisms including foraminiferans, tintinnids, ciliates, bivalve larvae and mites. These are listed in Table 1. Since the oysters imported from Normandy were also sourced to Marennes-Oleron, results are presented together.

Dinoflagellates

Fifteen dinoflagellate cyst species were found and vegetative theca of one species. Two cyst forms of *Alexandrium spp.* were recorded. The first cyst forms were 32.5μm in diameter, oval in shape, contained a prominent red body and appeared to have thin clear cell wall and were found in the sediment samples. The second forms, found in the drainings, were cylindrical in shape with rounded ends, an average length of 48μm (range 44-52μm) and an average breadth of 34 um (range 31-39 um). Attempts to hatch these species are being made, and a sample has also been sent to colleagues at IFREMER in France.

Two types of *Scrippsiella* cysts were found. The first types resembled *S. lachrymosa/crystallina* and were egg shaped, with clear reticulations on the cell surface. The second types, of *Scrippsiella trochoidea/faeroense* were spherical in shape, with numerous spines.

Gonyaulacoid cysts identified included *Gonyaulax spinifera* (*Spiniferites bulloideus/mirabilis)* and cysts of *Gonyaulax grindleyi* (=*Protoceratium reticulatum*, cyst name = *Operculodinium centrocarpum*). One empty theca of what resembles a young theca *G. grindleyi* was also found.

TABLE 1 . Phytoplankton recorded in imports of Oysters to Ireland

Bacillarophyceae	41 spp.	**Dinophyceae**	18spp.
Acnanthes spp.		Alexandrium spp.	
A. longipes		Caledoninium sp.	
Actinotychus senarius		?Epidinium shagrinum	
Amphiprora sp.		Ginginodinium concretum (Protoperidinium spp.)	
A. alata		Gonyaulax grindleyi	
Auliscus sculptus		Hexasterias problematicus	
Bellarochrea horologicalis von Stosch		Operculodinium centrocarpum	
Biddulphia aurita		Polykrikos schwartzii (=Valensiella moderna)	
Biddulphia biddulphiana (=pulchella)		Protoperidinium cyst	
B. mobiliensis		P. claudicans (V. calvum)	
B. regia		P. oblongum (= Votadinium spinosum)	
B. rhombus		Round brown cysts	
B. vesiculosus		Scrippsiella cysts	
Centric diatoms indet.		S. crystallina/lachrymosa	
Coscinodiscus spp.		S. trochoidaea/faeroense cysts	
C. centralis		Selenophemplix (=Xiphodinium sp.)	
C. radiatus		Spiniferites bulloideus/mirabilis	
?Detonula cystifera		?Tristolodinium leizosterum	
Diploneis spp.		Unidentified cysts	
Fragilaria spp.		Xanthodinium xanthum	
F. cylindrus			
F. oceanica			
Gyrosigma/Pleurosigma angulatum		**Silicoflagellates**	2spp.
G. wansbeckii		Dictyocha sp.	
Melosira jurgensii		Diastephanus speculum	
M. nummuloides			
M. monoliniformis		**Other groups**	
M. sulcata		Foraminferans (11 species)	
Navicula spp.		Tintinnids	
N. lyroides		Trematode eggs	
N. ?marina		Other invertebrate eggs	
Nitzschia spp.		Pollen grains	
N. bilobata		Nematode worms	
N. closterium		Globigerina spp.	
Paediastrum sp.		Bivalve larvae	
Pennate diatoms indet.		Paramoecium type	
Podosira stelliger		Faecal pellets	
Rhabdonema sp.		Mites	
R. adriaticum			
Streptotheca thamensis			
Surrirella spp.			
S. comis			
Thalassionema nitzschoides			

Other cyst species recorded were those of *Protoperidinium spp.* including ***P. claudicans*** (*Votadinium calvum*), *P. oblongum* (*V. spinsoum*)., *Ginginodinium concretum* and other unidentified *Protoperidinium* cysts. Unidentified round brown cysts were also found. Two cyst forms of *Polykrikos schwartzii (=Valensiella moderna)* cysts were identified. Six other dinoflagellate cysts were also recorded.

Diatoms

43 diatoms were recorded and were a combination of planktonic and benthic species, with representatives from both freshwater and marine habitats. Diatom auxospores were also found as were empty frustules. The most common diatom species recorded were from the genus *Melosira* such as *Melosira nummuloides, M. jurgensii & M. sulcata.*

Discussion and Conclusions

Man-induced vectors in the introduction of non-indigenous species have been suggested since early this century, when Ostenfeld (1908), indicated that cargo vessel ballast water and sediment may be the means by which exotic marine species are dispersed. Shellfish transfers are another route by which introductions occur. Whereas the introduction of some phytoplankton species are apparently without harmful/nuisance effects (for example the introduction of *Odontella* (*Biddulphia*) *sinensis* into European waters in the early 1900's from the Indo-Pacific region), problems have been caused by species such as *Coscinodiscus wailesii* which produces a mucus that clogs fishing nets (Hallegraeff & Bolch 1992). The probability of introducing Paralytic Shellfish Poison (PSP) producing dinoflagellates into Ireland is of concern, particularly in view of the importance of the aquaculture industry to local enterprises in Ireland, and the real potential for compromising our shellfish exports.

Despite concerns expressed beforehand, with the advent of the EC Council Directive 91/67/EEC, the free movement of trade in shellfish began in Ireland in January 1993. The transfers received from France were certified to be free from *Bonamia* and *Martelia* and other molluscs. However, some of the phytoplankton species found in the transfers are potentially harmful. The finding of 2 types of what resemble *Alexandrium* cysts (currently under investigation) and a theca and cysts of *Gonyaulax grindleyi* are of concern, Many members of the *Alexandrium* family cause PSP, and toxic red tides caused by *G. grindleyi* are known. Minchin *et al.* (1993) also document 5 exotic invertebtrates (*Myticola orientalis, Myicola ostrea, Crepidula fornicata, Terebella lapidaria,* and a *Pomatoceros* spp.), together with specimens of *Ostera edulis* and *Mytilus edulis* in the same consigments.

While the current EC Directive aims to prevent the introduction of oyster diseases with shellfish transfers, the prevention of the importation of other organisms particularly micro- and macro-algae, which can equally have a devastating negative effect on human health, the Irish shellfish industry and native flora and fauna has not been considered. The examination of the samples in this study (a minute proportion of the estimated total importation of 23,705 kgs), has revealed the presence of 67 species of phytoplankton of which 3 species could be harmful. Ireland, whilst having almost annual closures of the mussel areas due to the presence of Diarrhetic Shellfish Toxin caused by *Dinophysis* species, has not had a serious PSP incident to date, despite the occurrence of *Alexandrium* blooms. The last suspected poisoning of people in the Republic occurred in the southwest of the country in 1897..

Dijkema (1992), calculated that, in the Netherlands, 2.5 million viable cysts of toxic dinoflagellates can be present in one tonne of mussels imported from "red tide areas", and he calculated, using import figures, that 1.05 x 10^{10} dinoflagellate cysts can be introduced into Dutch coastal waters annually. This number is of the same order of magnitude as the amounts of cysts annually introduced with ballast water into Australia, which have caused toxic dinoflagellate blooms in Tasmania. In the Netherlands, there is a ban on immersion into the coastal waters of any imported bivalves. If the imports from France are considered to be from a "red tide area", and if 23.71 tonnes of non native oysters have been imported in to Ireland since January 1993; then using Dijkema's calculations, we have potentially imported approximately 5.93 x 10^{6} viable cysts of toxic dinoflagellates into Ireland in the above consignments.

It is not just the introduction of microalgae that may be a problem, as the potential for introducing other novel algal species are also very real. Macroalgal species such as *Sargassum muticum* and *Caulerpa taxifolia* are currently causing serious problems in France and there is the potential for introducing spores and plantlets of these species with shellfish importations. *Caulerpa taxifolia* is native to the shores of all tropical oceans, and when originally found colonising the shore below the Oceanographic Museum in Monaco, (it had "escaped" from aquaria), it was presumed that it would not tolerate the lower temperatures of a Mediterranean winter. In fact it has successfully survived and a greater cause concern is that *C. taxifolia* is producing more toxic substances and is growing to a larger size than in its native habitat (Jenkinson 1993). The probability of introductions of these and related species to Ireland is high.

This study has demonstrated that phytoplankton species, including potentially harmful ones, have been imported in consignments of shellfish, and that vigilance must be maintained against further introductions. On the basis of the findings from this study and that of Minchin *et al.* (1993), the Irish Department of the Marine has introduced a voluntary ban on shellfish transfers. Growers have been advised to consider carefully the risks involved in importing shellfish into Ireland, and should not sacrifice long terms results for the promise of short term economic gain.

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