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Ballast Water: Ecological and Fisheries Implications

DINOFLAGELLATE RESTING CYSTS AND BALLAST WATER DISCHARGES IN SCOTTISH PORTS

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SUMMARY

During the last 20 years, observations of the harmful impacts of non-native aquatic species being transported worldwide in ships' ballast waters and sediments appear to have increased. Until 1993, very little was known about ballast water operations in Scottish waters, but the increasing significance of problems worldwide, coupled with growing national awareness of these problems prompted the inception of a research programme to investigate non-indigenous or harmful planktonic organisms in ballast discharges to Scottish waters. The results from an earlier desk study were used to design an ongoing field programme which is primarily concerned with the ballast water transport of harmful phytoplankton. Field and laboratory work is still underway, but preliminary findings are reported here. Sediment samples have been collected from the ballast tanks of oil and gas tankers discharging ballast water in Scottish ports. Dinoflagellate cysts were found in 90% of samples examined and were most numerous during spring and autumn months. Cysts provisionally identified as *Alexandrium minutum*, a PSP toxin producing species, were found in four samples, but the possibility that these cysts may not be viable in Scottish waters due to unsuitable environmental conditions is discussed.

INTRODUCTION

Ships require ballast to adjust stability and trim when sailing without cargo or only partially laden. By 1850, vessels had begun to use sea water instead of solid materials as ballast and this was common practice by the mid 1870s. By the turn of the century, sea water ballast was tentatively suggested as a mechanism for the spreading of a large marine diatom from Asia to the North Sea (Ostenfeld, 1909). Since then, the role of ballast water as a vector for the accidental transfer of aquatic species between geographically separate areas received relatively little attention, but within the last 20 years, increasing research effort has been directed at this problem and observations of alien species being transported in ships' ballast water appear to have increased (Medcof, 1975; Carlton, 1985; Williams *et al.*, 1988; Hutchings, 1992; Kelly, 1992). Much of this research has been motivated by the variety of potentially harmful effects that may result from ballast water introductions. For example, native species may be out-competed and possibly replaced by the non-indigenous invader. Commercially important shellfish stocks may be affected by Paralytic Shellfish Poisoning (PSP) (Hallegraeff *et al.*, 1990) or fish resources may be affected by competition for food (Studenikina *et al.*, 1991). There may even be detrimental effects for human users of the marine environment (Boalch and Harbour, 1977), or human health may be at risk (McCarthy and Khambaty, 1994).

Until recently, the majority of ballast water research had been carried out in the USA and Australia (Hallegraeff, 1992; Carlton and Geller, 1993), but increasing global awareness of the potential problems has prompted worldwide proliferation of research effort in this field. In Scotland, there are three main reasons for establishing a programme of ballast water research:

1. Scotland has economically valuable fisheries for finfish and shellfish and a rapidly developing aquaculture industry involving the cultivation of both fin and shellfish. The shellfish industry, like most others worldwide, is vulnerable to the harmful effects of algal toxins, for example, PSP and Diarrhetic Shellfish Poisoning (DSP) (Hallegraeff *et al.*, 1988; Subba Rao *et al.*, 1994; Chang *et al.*, 1995). The dinoflagellates regarded as responsible for PSP in temperate waters (*Alexandrium* spp. and *Gymnodinium catenatum*) can, under certain conditions, form resting cysts allowing them to survive periods of unfavourable environmental conditions (Anderson, 1984; Anderson *et al.*, 1988). This survival strategy makes these species well suited to ballast tank transport. Whilst PSP - thought to be caused by *Alexandrium* spp - and DSP are known in Scottish waters, other forms of shellfish toxicity which cause problems elsewhere are not known.

2. Recent developments in coastal quarrying for aggregates, and further applications for licenses to develop such quarries may, if permission is granted, result in frequent movements of bulk carriers to remote areas of coastline previously unaffected by large scale shipping operations. Robust ballast water management and monitoring plans are essential to any new coastal quarrying developments, and research is required to develop those management strategies.

3. The UK wishes to meet the International Maritime Organisation's (IMO) request for member states to investigate ballast water introductions (MEPC, 1991). Additionally, the UK Government is responding to recommendations following the *Braer* tanker accident to encourage ballast water studies (Donaldson, 1994).

This paper discusses a field and laboratory research programme which is currently underway to study planktonic organisms transported in both ballast water and sediments. Some preliminary results concerning the transport of dinoflagellate resting cysts in ballast water and sediments are reported.

METHODS

Ballast Water and Sediment Sampling

Field sampling based on results from a previous desk study (Macdonald, 1994) began during 1994 and the programme is still underway. Water and sediment samples are collected from ships discharging ballast water in Scottish ports previously identified as important areas for ballast water discharges (Fig. 1). Net and integrated water samples are taken to examine zooplankton and motile phytoplankton present in ballast water - these data will be presented elsewhere. The methods described here concern the collection of sediment from ballast tanks to examine the presence or absence of dinoflagellate resting cysts. In all cases the samples were collected from segregated tanks of ballast water.

Sample collection

Sediment samples were collected from the deck of oil and gas tankers by deploying a weighted hose to the tank floor through a deck hatch. The hose was connected to a hand-operated pump and water and sediment from the tank floor were pumped to deck level, where approximately 20 litres was collected in polycarbonate carboys. The contents of the carboys were kept cool and dark and transported back to the laboratory where they were stored at 4°C in darkness. The sediment was allowed to settle for approximately 48 hours before the overlying water was siphoned off. The remaining sediment/water mixture was concentrated by centrifuging at 3000 rpm for 15 minutes followed by carefully removing the supernatant by syringe leaving sediment and a small volume of overlying water. Final volumes of sediment obtained ranged from 10 cm^{-3} when particulate material was very sparse, to approximately 800 cm^{-3}. The resultant sediment was split into two aliquots, one half was frozen at -20°C for heavy metal analysis, the remaining half stored in the dark at 4°C prior to microscopic examination for dinoflagellate cysts.

Sediment processing and microscopy

Sediment samples, or aliquots if material was plentiful, were sonicated for two minutes in a Kerry Instruments ultrasonic bath to break down aggregated material and remove detrital particles from cyst walls. The sonicated sediment was washed with 0.2 µm filtered sea water and fractionated through 70 µm and 20 µm sieves. The material retained on the 20 µm sieve was washed with approximately 200 ml of filtered sea water, backwashed into a beaker and made up to a known volume. Aliquots of the final suspension were examined using a Zeiss Axiovert 10 inverted microscope using brightfield, phase contrast and differential interference contrast illumination. All cysts - both full and empty/remains - observed were identified and counted.

RESULTS

Ballast Water Sampling Programme

Ballast water sampling aboard vessels docking in Scottish ports began in the summer of 1994. To date, samples have been collected from 32 ships visiting four ports (Fig. 2). Ballast water origins include ports in northern and southern Europe, USA and the UK (Table 1).

Dinoflagellate cysts in ballast tank sediments

Ten of the 24 sediment samples collected to date have been analysed for the presence of dinoflagellate cysts. Nine of these samples contained both full cysts and empty cysts or remains of cysts. The abundance of full cysts ranged from 7 to 1450 cysts cm^{-3}. The highest concentration of full cysts was found in a sample with ballast originating from Hamburg, and 55% of these cysts were round brown peridinoid dinoflagellate cysts. Five other samples had >100 full cysts cm^{-3} (Table 2). This compares with Hallegraeff and Bolch's (1992) data where they selected nine samples with high cyst abundance, where numbers ranged from 40 to 22,500 cysts cm^{-3}. Thirty-one different cyst species were identified, the genera most commonly represented being *Scrippsiella* and *Protoperidinium* (Table 3). In addition to dinoflagellate cysts, a motile *Gonyaulax* sp. was observed in one sample. Highest numbers of full cysts were found in samples taken during March and September/October (Fig. 3). Square root transformations were carried out on full cyst numbers and a two sample t-test showed a significant difference ($P=0.044$, $n=10$) between numbers of full cysts in sediment collected from September to March than in samples collected from April to August.

Cysts resembling descriptions of *Alexandrium* spp. (Fukuyo *et al.*, 1990; Hallegraeff *et al.*, 1991) were found in five samples. These cysts were typically smooth walled and covered with a layer of mucilage. In three samples, these cysts were hemispherical and most closely resembled *Alexandrium minutum*, but in another sample where they resembled *A. minutum* in most respects, the cysts were rather smaller (15 µm diameter) than the size range of 20-25 µm reported in the literature for this species (Erard Le-Denn *et al.*, 1993). The *Alexandrium* type cysts in the remaining sample were approximately 25 µm diameter, but were spherical and had yellow accumulation bodies as opposed to the red ones common to some species of *Alexandrium*. These cysts resembled descriptions of *Alexandrium margalefi* (Hallegraeff *et al.*, 1991), but were slightly smaller than the size range reported (28-34 µm). The highest concentration of *Alexandrium* cysts was 50 cysts cm^{-3}.

In one sample with ballast water originating from Hamburg, an empty cyst resembling descriptions of *Gymnodinium catenatum* (Anderson *et al.*, 1988) was found. The distinctive microreticulate cyst wall was observed and the archeopyle was similar to that described by other workers (Blackburn *et al.*, 1989; Carrada *et al.*, 1991). At 26 µm diameter, this specimen was much smaller than the average 50 µm diameter reported in the literature by workers in Japan (Fukuyo *et al.*, 1990), but only slightly smaller than the 30-38 µm found for specimens from German sediments (Nehring, 1995).

Other taxa in ballast tank sediments

All sediment samples examined were found to contain other taxa (Table 4), with diatoms being found in every sample. Entire diatom cells complete with contents and diatom frustules or remains were found and at least 25 species were represented. Both centric and pennate diatoms were found, but diatom resting spores were rare. Some fresh water algal species were observed, particularly the centric diatom *Melosira granulata* and the colonial alga *Pediastrum boryanum*. Living ciliates were observed in some samples. In three samples, live nematode worms were seen moving in the sediment material.

DISCUSSION

The present study has shown that a wide diversity of planktonic organisms can be transported in the segregated ballast tank sediments of oil and gas tankers. Similar species assemblages have been recorded in sediments from bulk carriers' ballast tanks

(Hallegraeff and Bolch, 1992). The range of algal species found to date is consistent with the results of other researchers - Hallegraeff and Bolch (1992) found 53 dinoflagellate cyst species and many diatom species from ballast tank sediment samples. Many of the cysts observed in the current study have been shown to represent the resting stages of temperate dinoflagellates species (Dale, 1983; Lewis *et al.*, 1984, Lewis, 1988; Lewis, 1991) many of which are already found in UK sediments (Lewis *et al.*, 1984; Higman *et al.*, 1995, Macdonald, unpubl.). The presence of full dinoflagellate cysts in the samples indicates that cysts are present in ships discharging ballast water at Scottish ports, and this may have implications for the spread of non-indigenous or potentially toxic species presently not known in this country. It is difficult to ascertain at this stage whether the cysts were entrained into ballast tanks from the water column or by resuspension of sediment during ballasting. A significant difference was found in the number of full cysts in ballast sediments sampled between September and March than from April to August, although it should be noted that the sample size was small. Cysts were found to be most numerous during spring and autumn months, when in the northern hemisphere, cyst numbers in the environment are expected to be maximal (Higman *et al.*, 1995).

Of potential concern were the observations of cysts preliminarily identified as resting stages of potential PSP-producing motile dinoflagellates. Further taxonomic investigations should be attempted to confirm the thecal affinity of these cysts identified as closely resembling *Alexandrium minutum* and possibly *Alexandrium margalefi*. If these are indeed resting cysts of *Alexandrium* spp, they may represent a potential problem in terms of PSP toxin production. Toxin studies on Australian strains of *A. margalefi* showed them to be non-toxic (Hallegraeff *et al.*, 1991), but *A. minutum* has been associated with PSP intoxication in France (Erard Le-Denn *et al.*, 1993) and Australia (Hallegraeff *et al.*, 1991). Higman *et al.* (1995) and Macdonald (unpubl.) in recent studies of dinoflagellate cysts on the east coast of Britain found concentrations of a cyst closely resembling, but larger, than known specimens of *A. minutum*. Although presently unidentified, Higman *et al.* (1995) report its thecal affinity is likely to be with *A. minutum*. These cysts were most numerous in sediments of the Firth of Forth. This area has a history of PSP toxicity (SOAFD data) - usually attributed to *Alexandrium tamarense* - and also has a high density of shipping due in part to the presence of both oil and gas terminals. Cysts of *A. tamarense*, the dinoflagellate normally associated with PSP events in Scotland and abundant in parts of the Firth of Forth (Lewis *et al.*, 1995) were not found in any of the ballast sediments. Whilst cysts resembling *A. minutum* have been observed in the Firth of Forth and in ballast sediments of ships discharging there, motile cells of *A. minutum* have not been observed in the Firth of Firth, nor in the waters off Sullom Voe, the other port where these cysts were observed in ballast sediments. Efforts should be made to germinate both the cysts found in the current study and those from the Firth of Forth to ascertain their respective motile stages, and genetic studies might help establish any relationships between them.

Laboratory studies have shown that germination of *A. minutum* cysts is maximised at temperatures of around 16°C and at salinities ranging from 14-26 psu (Cannon, 1993). Maximum bottom temperature in the outer Firth of Forth occurs during August when the mean value is only 12.97°C, whilst mean bottom salinity never goes below 34 psu throughout the year (Turrell and Slesser, 1992). If *A. minutum* cysts are being transported to the Firth of Forth, conditions may be unsuitable for their germination, so the cysts may simply remain dormant in the sediment or become non-viable.

One empty cyst was observed which resembled *Gymnodinium catenatum*, but was smaller than those observed by other workers. This cyst may have been the resting stage of another similar species of *Gymnodinium*, or a very small specimen of *G. catenatum*, which perhaps occurs as smaller cells in colder waters. The distribution of this species in European waters has traditionally been associated with southern European countries (Blanco, 1989; Carrada *et al.*, 1991). However, the appearance of living *G. catenatum* cysts in recent surveys off both the German (Nehring, 1993) and Danish (Ellegaard *et al.*, 1993) coasts coupled with the findings of Dale *et al.* (1993), who presented evidence for prehistoric blooms of *G. catenatum* in the Skagerrak/Kattegat, suggest that either parts of the North Sea are being newly colonised by *G. catenetum*, or that the cysts may be a relic from prehistoric times (Nehring, 1995). Whichever is the case, it is clear that northern European countries should not discount the threat of this potential PSP producing dinoflagellate becoming endemic to their regions.

Hallegraeff and Bolch's (1992) research found cysts only in vessels originating from Japan or South Korea. In this study, cysts were observed in samples taken from ships which had loaded their ballast in a variety of northern and southern European countries. Sediments may have accumulated over a period of time, depending on when the ship was last in drydock or when the tanks were completely cleaned out, so samples might reflect an accumulation of material from many ports. For safety reasons, entry to the ballast tanks of oil and gas tankers is prohibited and samples were collected from deck. It was therefore not possible to assess the nature of the sediment, to note if large accumulations were present, or if the state of the tank suggested that sediment was flushed out regularly.

Northern European ports are the most common origins of ballast water discharges to Scottish ports (Macdonald, 1994). This might imply that introductions of non-indigenous species are unlikely to occur, as the differences in flora and fauna between origin and discharge ports may be small. However, there are harmful algal species found in northern European waters not yet observed in Scotland (Nehring, 1995). Environmental conditions in waters of relatively close proximity are likely to be more similar than those which are geographically distant, so it could be argued that the chances of survival in adjacent or nearby "new areas" may be enhanced.

CONCLUSIONS

Preliminary analyses of ballast tank sediments demonstrate that dinoflagellate cysts are commonly found in tankers visiting Scottish ports. If these cysts are discharged to the environment and survive in their new location, there may potentially be problems, and shellfish resources in particular may be affected if toxin-producing species are involved. However, introduced cysts may not germinate if environmental conditions are unsuitable, but may remain dormant or be rendered non-viable. Efforts should be directed at investigating the taxonomic relationships with other cyst species known in Scottish waters and the viability of ballast tank cysts should be assessed. Further studies will be carried out to extend our knowledge of the ballast water transport of planktonic organisms to Scottish ports.

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TABLE 1

Vessels sampled in current ballast water programme, May 1994-July 1995

Sampling number	Date sampled	Port of sampling	Vessel name	Vessel DWT	Location BW loaded	Region
BW/001	05 May 94	Braefoot Bay	*Turid Knutsen*	22600	La Pallice, France	S Europe
BW/002	30 Jun 94	Hound Point	*Ambon*	285000	Le Havre/R'dam	N Europe
BW/003	15 Aug 94	Sullom Voe	*Nosurps*	135000	Le Havre	N Europe
BW/004	17 Aug 94	Sullom Voe	*Boree*	283861	Immingham	UK
BW/005	18 Aug 94	Sullom Voe	*Shinobu*	82279	Gdansk	N Europe
BW/006	18 Aug 94	Sullom Voe	*Sunetta*	90000	Shellhaven	UK
BW/007	18 Aug 94	Sullom Voe	*Sunetta*	90000	Shellhaven	UK
BW/008	20 Sep 94	Hound Point	*Castillo de Lorca*	131400	St James Mississippi	USA
BW/009	20 Sep 94	Hound Point	*Castillo de Lorca*	131400	St James Mississippi	USA
BW/010	20 Sep 94	Hound Point	*Alfa America*		Hamburg	N Europe
BW/011	21 Sep 94	Hound Point	*Shinobu*	82279	Bilbao	S Europe
BW/012	12 Oct 94	Hound Point	*Star Westminster*	88821	Pembroke	UK
BW/013	27 Oct 94	Hound Point	*Star Windsor*	88821	Milford Haven	UK
BW/014	25 Jan 95	Hound Point	*Almanama*	97002	Europort	N Europe
BW/015	08 Feb 95	Hound Point	*Autan*	278220	Fos, Marseille	S Europe
BW/016	19 Feb 95	Hound Point	*British Resource*	261913	Fos, Marseille	S Europe
BW/017	08 Mar 95	Hound Point	*Sylvia Tapias*	135271	Malaga	S Europe
BW/018	28 Mar 95	Hound Point	*Enalios Ethra*	152109	Wilhemshaven	N Europe
BW/019	21 Apr 95	Hound Point	*Golar Edinburgh*	306000	Fawley	UK
BW/020	05 May 95	Hound Point	*Berge Sigval*	306430	Rotterdam	N Europe
BW/021	11 May 95	Hound Point	*Atlantic Sun*	83107	Rotterdam	N Europe
BW/022	24 May 95	Hound Point	*Malde*	147500	Malta	S Europe
BW/023	04 Jun 95	Sullom Voe	*Hellespoint Paramo*	387858	Rotterdam	N Europe
BW/024	05 Jun 95	Sullom Voe	*Solena*	88836	Hamburg	N Europe
BW/025	07 Jun 95	Sullom Voe	*Alandia Wave*	97693	Tranmere	UK
BW/026	08 Jun 95	Sullom Voe	*Heidrun*	124502	Rotterdam	N Europe
BW/027	15 Jun 95	Hound Point	*Vesta*	97602	Livorno	S Europe
BW/028	20 Jun 95	Montrose	*Marlin 1*	960	Seaham	UK
BW/029	22 Jun 95	Braefood Bay	*Etagas*	9384	Antwerp	N Europe
BW/030	30 Jun 95	Braefoot Bay	*Lulu*	28840	Bay of Biscay	S Europe
BW/031	12 Jul 95	Hound Point	*Solena*	88836	Rotterdam	N Europe
BW/032	12 Jul 95	Hound Point	*Velimir Scorpik*	86943	Immingham	UK and Ireland

TABLE 2

Summary of dinoflagellate cysts found in ballast sediment samples

Ship name	Tank number	BW origin	Date sampled	Full cysts per cm^{-3}	Empty cysts per cm^{-3}
Shinobu	BW/005	Gdansk	18 Aug	20	120
Alfa America	BW/010	Hamburg	9 Sept	1450	500
Star Westminster	BW/012	Pembroke	12 Oct	700	1200
Star Windsor	BW/013	Pembroke	27 Oct	550	775
Almanama	BW/014	Europort	25 Jan	250	1400
Autan	BW/015	Marseille	8 Feb	0	0
Enalios Ethra	BW/018	Wilhemshaven	23 Mar	750	813
Solena	BW/024	Hamburg	6 Jun	115	198
Vesta	BW/027	Livorno	15 Jun	45	5
Lulu	BW/030	Bay of Biscay	30 Jun	7	13

TABLE 3

Occurrences of dinoflagellate cyst species in ballast sediment samples

Species	Samples with full cysts	Samples with empty cysts
Alexandrium cf. *minutum*	4	0
cf. *Alexandium margalefi*	1	0
Cyst remains	0	9
Diplopelta parva	1	0
Gymnodinium cf. *catenatum*	0	1
cf. *Gymnodinium* spp	2	0
Lingulodinium machaerophorum	2	2
Lingulodinium spp.	0	2
Multispinula sp	0	1
Operculodinium centrocarpum	3	2
Operculodinium sp	0	1
Pentapharsodinium dalei	1	0
Pheopolykrikos hartmannii	0	1
Polykrikos schwartzii	1	3
Protoperidinium avellana	0	1
Protoperidinium compressum	0	1
Protoperidinium conicum	1	7
Protoperidinium leonis	0	2
Protoperidinium minutum	1	0
Protoperidinium oblongum	1	1
Protoperidinium pentagonum	0	1
Round brown (Peridinales)	6	5
Scrippsiella crystallina	0	1
Scrippsiella lachrymosa	1	0
Scrippsiella precaria	1	0
Scrippsiella rotunda	2	0
Scrippsiella trifida	1	0
Scrippsiella trochoidea	5	1
Scrippsiella sp	1	0
Spiniferites spp.	1	4
Spiniferities bentori	1	0
Spiniferities bulloides	0	1
Spiniferities mirabilis	0	1
Unidentified cyst	5	0

TABLE 4

Other taxa found in ballast tank sediment samples

Diatoms	**Motile dinoflagellates**
Actinoptychus senarius	*Gonyaulax* sp.
Asterionella glacialis	
Asterionella kariana	
Chaetoceros spp.	**Other taxa**
Coscinodiscus spp.	a) Fresh water algae
Cyclotella spp.	*Pediastrum boryanum*
Ditylum brightwelli	*Scenedesmus quadricauda*
Grammatophora serpentina	
Leptocylindrus minimus	b) Protozoa
Melosira granulata	Ciliata
Navicula spp.	Foraminifera
Nitszchia longissima	Tintinnida
Odontella alternans	
Odontella aurita	c) Others
Odontella rhombus	Nematoda
Odontella sinensis	
Paralia sulcata	
Pleurosigma / Gyrosigma spp	
Raphoneis amphiceros	
Rhizosolenia alata	
Rhizosolenia delicatula	
Skeletonema costatum	
Stephanodiscus spp.	
Thalassionema nitzschiodes	
Thalassiosira spp.	

Figure 1 Estimated ballast water discharges to Scottish ports

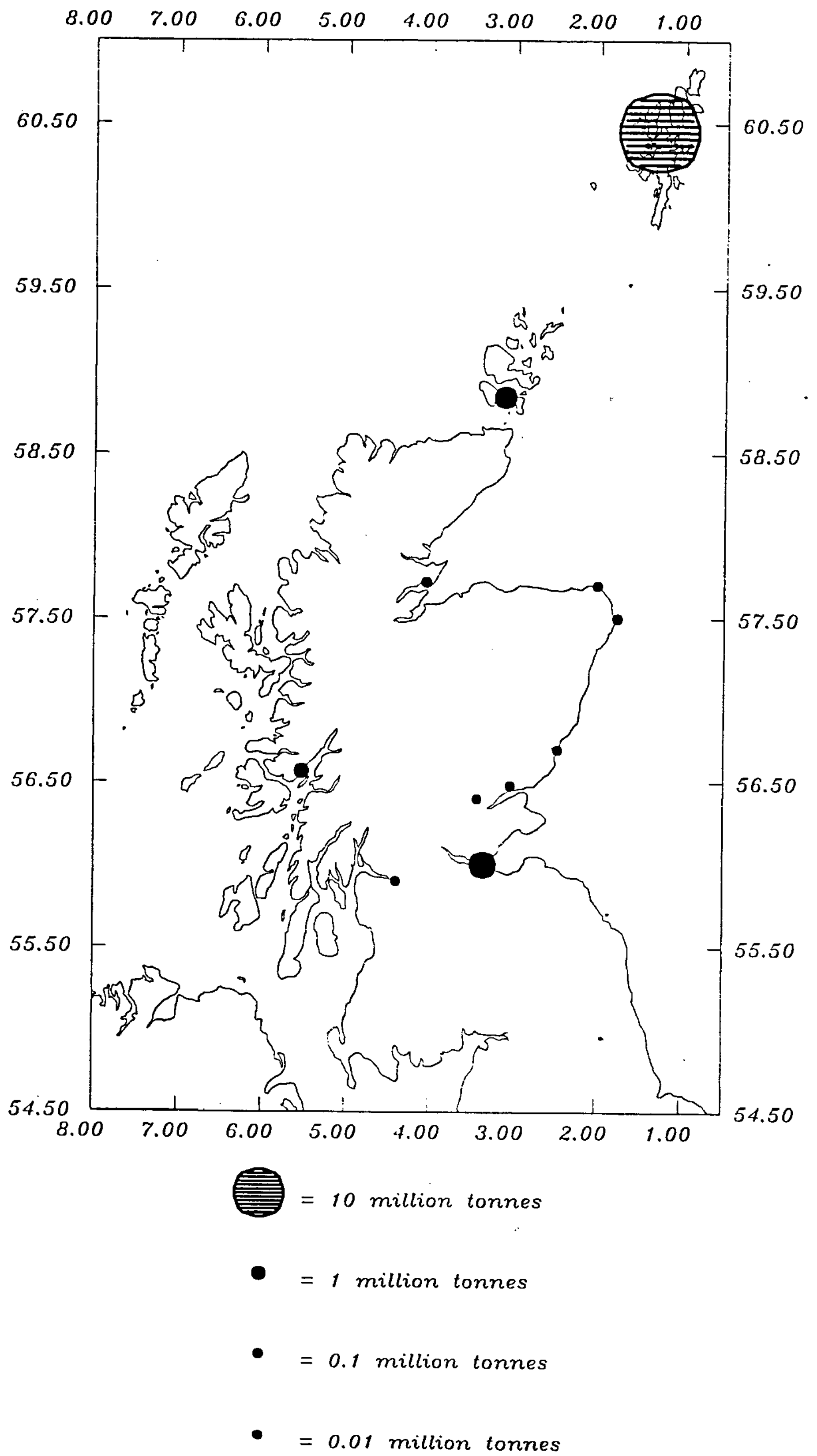

Figure 2 Ships and ports sampled to date during Scottish ballast water research programme

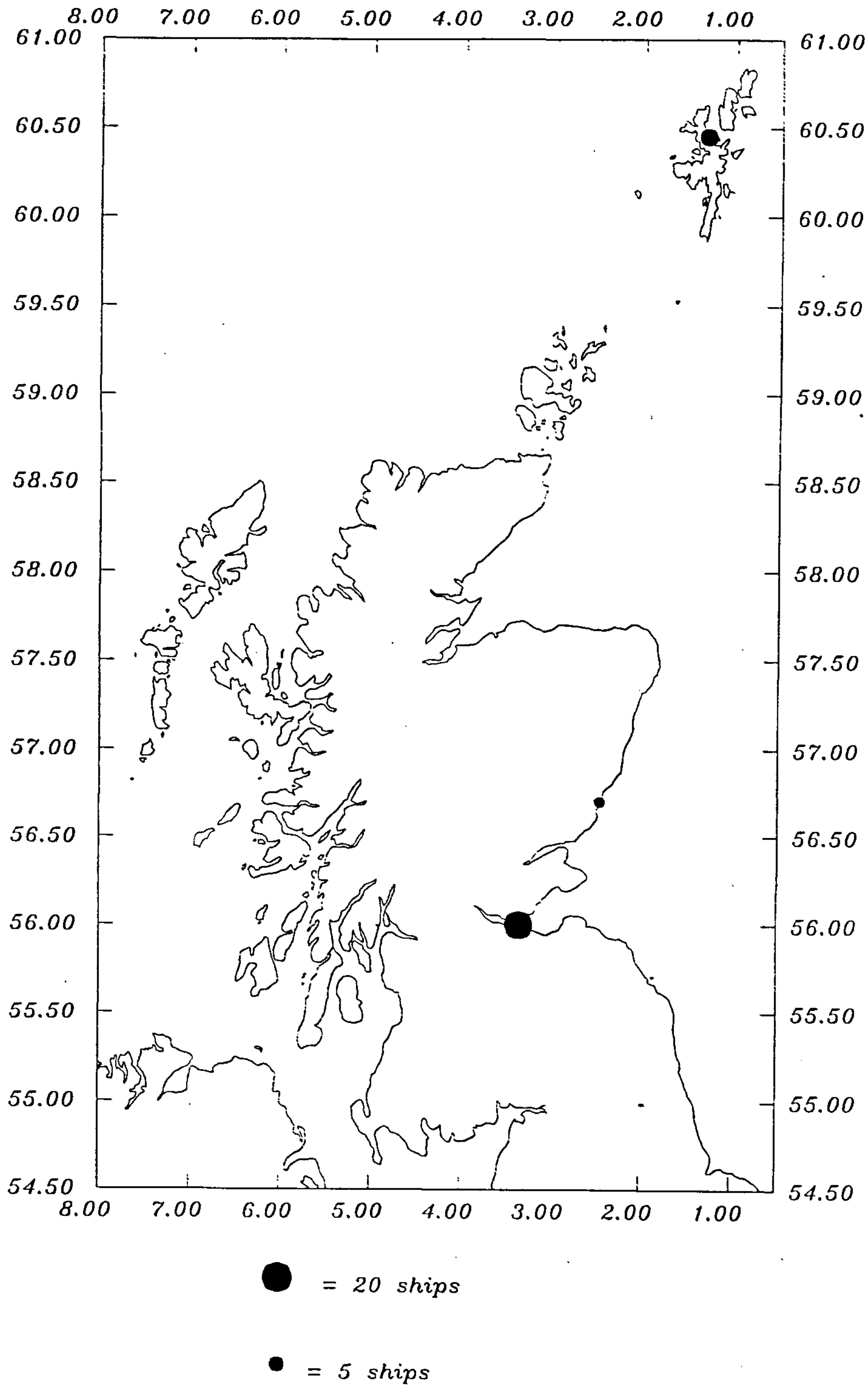

Figure 3 Full cyst counts in ballast tank sediment samples, January-December