BIOSECURITY NEW ZEALAND

Targeted surveillance for non-indigenous marine species in New Zealand

Design report for Dunedin

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Prepared for MAF Biosecurity New Zealand
by Kate Willis, Don Morrisey, Graeme Inglis, Olivia Johnston, Chris Woods and Lisa Peacock (NIWA Ltd)

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Requests for further copies should be directed to:

Publication Adviser
MAF Information Bureau
P O Box 2526
WELLINGTON

Telephone: 04-894 0100
Facsimile: 04-894 0300

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Objectives

The primary objective of the targeted marine surveillance programme is:

- To detect incursions of the target organisms at the identified locations.

The secondary objectives of the targeted marine surveillance programme are:

- To detect incursions of non-target non-indigenous or cryptogenic species not previously recorded in New Zealand.
- To detect incursions of established non indigenous or cryptogenic species which are exhibiting invasive characteristics (i.e. range extensions of established organisms).

The targeted marine surveillance programme must meet the primary objective. Surveillance should be designed and undertaken with the purpose of maximising the likelihood of successful "containment" of the incursion through providing sufficiently detection to maximise the range of management options available, i.e. vector management and local control etc. The secondary objectives should be considered when designing and undertaking the surveillance programme to increase the likelihood that these will be achieved within the existing design or through minor additions/modifications to this design (these will need to be clearly identified and approved).

TARGET SPECIES

MAF Biosecurity New Zealand has currently identified seven marine organisms which are listed on the unwanted organisms register. These are the:

1. Clubbed tunicate, *Styela clava*
2. Northern Pacific seastar, *Asterias amurensis*
3. European shore crab, *Carcinus maenas*
4. Aquarium weed, *Caulerpa taxifolia*
5. Mediterranean fanworm, *Sabella spallanzanii*
6. Chinese mitten crab, *Eriocheir sinensis*
7. Asian Clam, *Potamocorbula amurensis*

An additional three organisms have been identified that are not currently listed as unwanted organisms and are currently known to be established in New Zealand's coastal waters. Knowledge of changes in the distribution of these organisms is of interest for current and potential future management purposes. Within the survey design for the primary organisms, opportunities should be explored for detecting these secondary organisms. These organisms include:

8. Asian Date Mussel, *Musculista senhousia*
9. *Eudistoma elongatum*
10. *Didemnum* sp.[1]

Note: the target organism list may be subject to change by MAF BNZ during the course of the surveillance programmes. Inclusion of additional target species may be considered by MAFBNZ.

[1] Representative samples of *Didemnum* species will be collected and submitted to MITS for future reference. Further identification to species will not be undertaken as part of this programme. The samples will be made available by MAF where these are required for approved purposes.

Stakeholder engagement and governance

IDENTIFYING RESPONSIBILITIES WITHIN THE SURVEY AREA

Stakeholder groups with jurisdiction or responsibility within the surveillance location are listed in Table 1.

Table 1 List of stakeholder groups with jurisdiction or responsibility within the surveillance location.

Node/Facility	Responsible group	Contact name
Commercial trading port	Port Otago (03 4727890)	Ron Horner (General Manager Operations)
Commercial trading port	Port Otago (03 4727890)	Alan Sutherland (Manager Port Otago)
Industry	Ravensdown Fertiliser Ltd customer.centre@ravensdown.co.nz	Craig Hendry (Manager)
Fisheries regulator	Ministry of Fisheries, Otago (03 4740333)	Murray Pridham (District Compliance Officer)
Local authority harbour master	Otago Regional Council (03 4740827)	Bruce Ramsay (Harbour Master)

OBTAINING PERMITS TO CONDUCT SURVEILLANCE FIELDWORK

Contact has been made with all the organisations listed in Table 1 during previous surveys of the port and a letter (see Appendix 1) has been sent to each summarising the purpose of the surveillance programme and, where required, requesting permission to sample. To date, permission has been granted whenever requested and all stakeholders have indicated that their cooperation will continue in the future.

GOVERNANCE

MAF Biosecurity New Zealand

MAF Biosecurity New Zealand is the lead agency in New Zealand's biosecurity system. It is tasked with a "whole of system" leadership role, encompassing economic, environmental, social and cultural outcomes. It also has international trade and animal welfare responsibilities.

Biosecurity activities protect the economy, environment and people of New Zealand from the risks and consequences of the introduction of damaging risk organisms, or mitigate the effects of risk organisms that are already present. Biosecurity surveillance plays a vital role in supporting a wide range of these activities.

The targeted marine surveillance programme is administered and funded by MAF BNZ's Biosecurity Surveillance Group. Queries relating to this programme should be directed to MAFBNZ.

The MAFBNZ contact person for all marine biosecurity surveillance activity is Brendan Gould (phone 04 894 0548, fax 04 894 0736, email brendan.gould@maf.govt.nz). Alternatively, the Biosecurity Surveillance Group Manager can be contacted at the following email address: NZBiosecuritySurveillance@maf.govt.nz.

Postal Address:
MAF Biosecurity New Zealand

PO Box 2526
Wellington

NIWA

NIWA has been contracted by MAF Biosecurity New Zealand to design and deliver the surveillance programme to the required specifications.

The NIWA project leaders and contact persons for the targeted surveillance programme are Don Morrisey (NIWA PO Box 893 Nelson, phone 03 548 1715, fax 03 548 1716, email d.morrisey@niwa.co.nz) and Graeme Inglis (NIWA PO Box 8602, Riccarton, Christchurch, phone 03 348 8987, fax 03 348 5548, email g.inglis@niwa.co.nz).

Graeme Inglis and Don Morrisey were also responsible for the design of the programme, with inputs from Isla Fitridge, Oliver Floerl, Nick Gust, Olivia Johnston, Marie Kospartov, Crispin Middleton, Sheryl Miller, John Oldman, Lisa Peacock, Helen Roulston, Matt Smith, Kate Willis and Chris Woods. This team also collated existing data.

Field work was carried out by a large team of NIWA staff (over 40 individuals), with additional support from commercial divers from Northern Underwater Technical Services and Southern Aqua Adventures where necessary. Field teams were led by a core of NIWA staff experienced in targeted surveillance: Niki Davey, Olivia Johnston, Crispin Middleton, Sheryl Miller, Don Morrisey, Kate Neill, Lisa Peacock, Matt Smith and Chris Woods. During fieldwork, field teams were generally divided into two groups, each in a separate boat and each including at least one person with previous experience of surveillance. All field team members are under the authority of the field team leader during field work and in communication by telephone or VHF radio. Field team leaders refer to the project leaders as required.

NIWA's Chief Scientist for Biodiversity and Biosecurity, Don Robertson, can be contacted at d.robertson@niwa.co.nz.

Existing information on the survey location

Table 2 lists individuals and groups with local knowledge of the surveillance location.

Table 2 List of individuals/groups with local knowledge of the surveillance location.

Category	Individual/group	Contact name
Commercial trading port	Port Otago, Dunedin (03-4727890) www.portotago.co.nz	Ron Horner (General Manager Operations)
Local authority	Otago Regional Council (03-4740827)	Environmental Monitoring and Investigations Department
Local authority	Otago Regional Council (03-4740827)	Bruce Ramsay (Harbour Master)
Private Wharf Operator	Ravensdown Fertiliser Ltd customer.centre@ravensdown.co.nz	Craig Hendry (Works Manager)
Fisheries regulator	Ministry of Fisheries (03-4740333)	Murray Pridham (District Compliance Officer)
National government	Department of Conservation	Otago Conservancy Office (03-4770677)
Research provider	NIWA (03-4778615)	Ian Maze (Field Team Leader)
Research provider	University of Otago www.otago.ac.nz/marinescience/pml	Portobello Marine Laboratory (03-4795810)
Research Provider	Kingett Mitchell Golder Associates	Dunedin Office (03-4790390)
Conservation NGO	Royal Forest and Bird Protection Society	Dunedin Branch (03-4893233)

The following map (Figure 1) shows natural and man-made features and structures in the survey area. Information on sediments in the harbour was obtained from Rainer (1981), and from information on sediment type collected during sled-sampling for previous monitoring surveys (NIWA, unpublished data). Information on shoreline composition (beaches, rocky shores, sea defences, etc.) and artificial structures was obtained from the navigational chart of the area (Land Information New Zealand Chart Number 6612, published July 2004), Google™ Earth, and personal knowledge. Habitat data were mapped by eye, since we are not aware of any sources of georeferenced information. Information from Google™ Earth is georeferenced and coordinates were used to map the structures in GIS.

The water area of the harbour at high tide is ca 4791 ha, the shoreline length is ca 80 km and the spring tidal prism (the volume of water entering on the flood tide) is ca 60.3 million m^3 (information from NIWA's Estuarine Environment Classification database: Hume *et al.* 2007). The ratio of the spring tidal prism to volume at high water is 0.33 (i.e. approximately a third of the water present in the harbour at high tide leaves on the subsequent ebb). The index of shoreline complexity is 0.30 (this index, calculated from the 1:50,000 topographic map as the reciprocal of the length of the perimeter of the estuary shoreline divided by the circumference of a circle that has the same area as that estuary, varies from 1.0 for a simple circular basin to <0.1 for a very complex shoreline with multiple arms), indicating the moderately simple and non-indented form of the harbour's shoreline (the value for the complex, highly-indented Waitemata Harbour, in contrast, is 0.12).

Figure 1a Map of the sampling area in Otago Harbour showing habitats (upper) and artificial structures (lower) present.

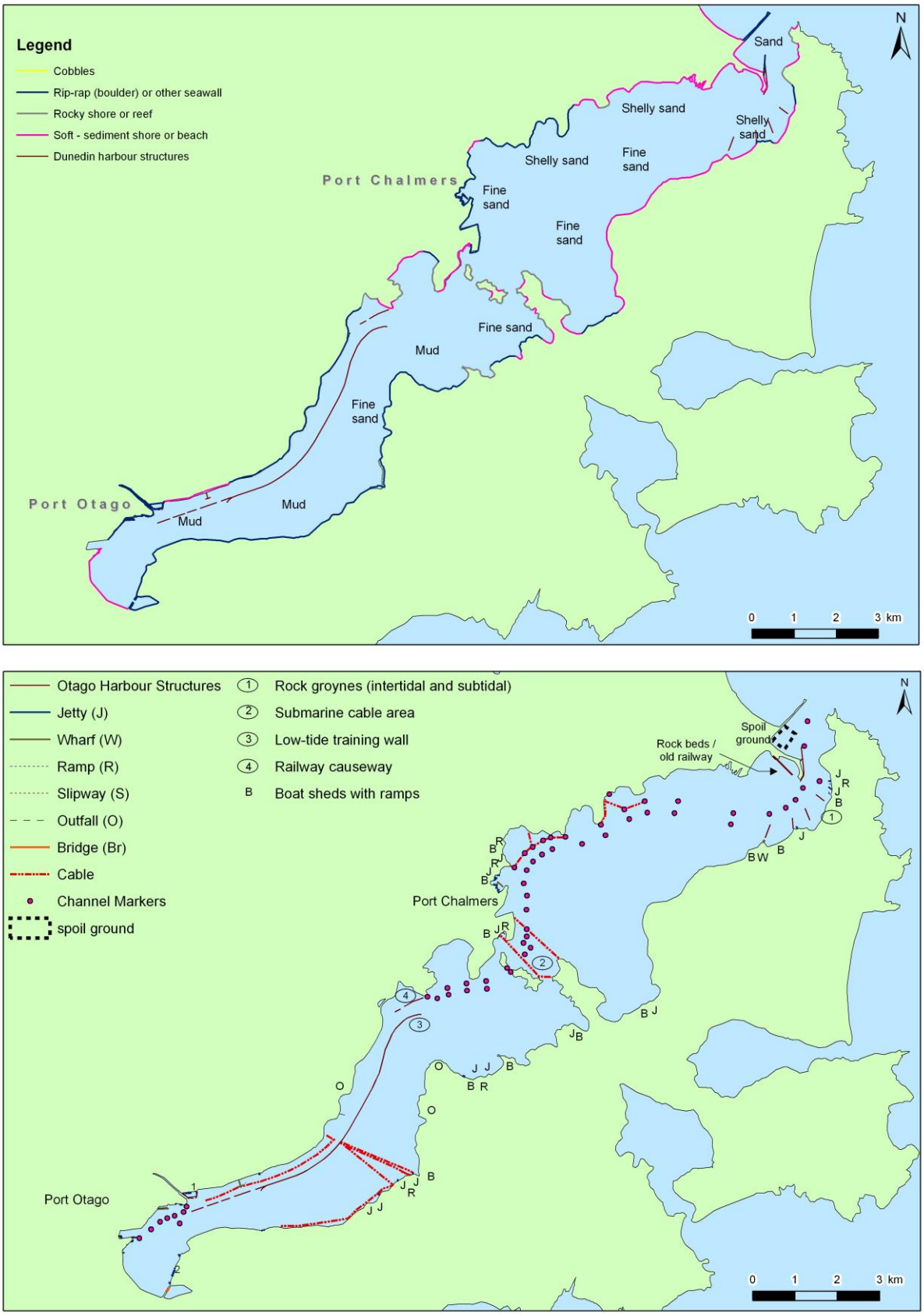
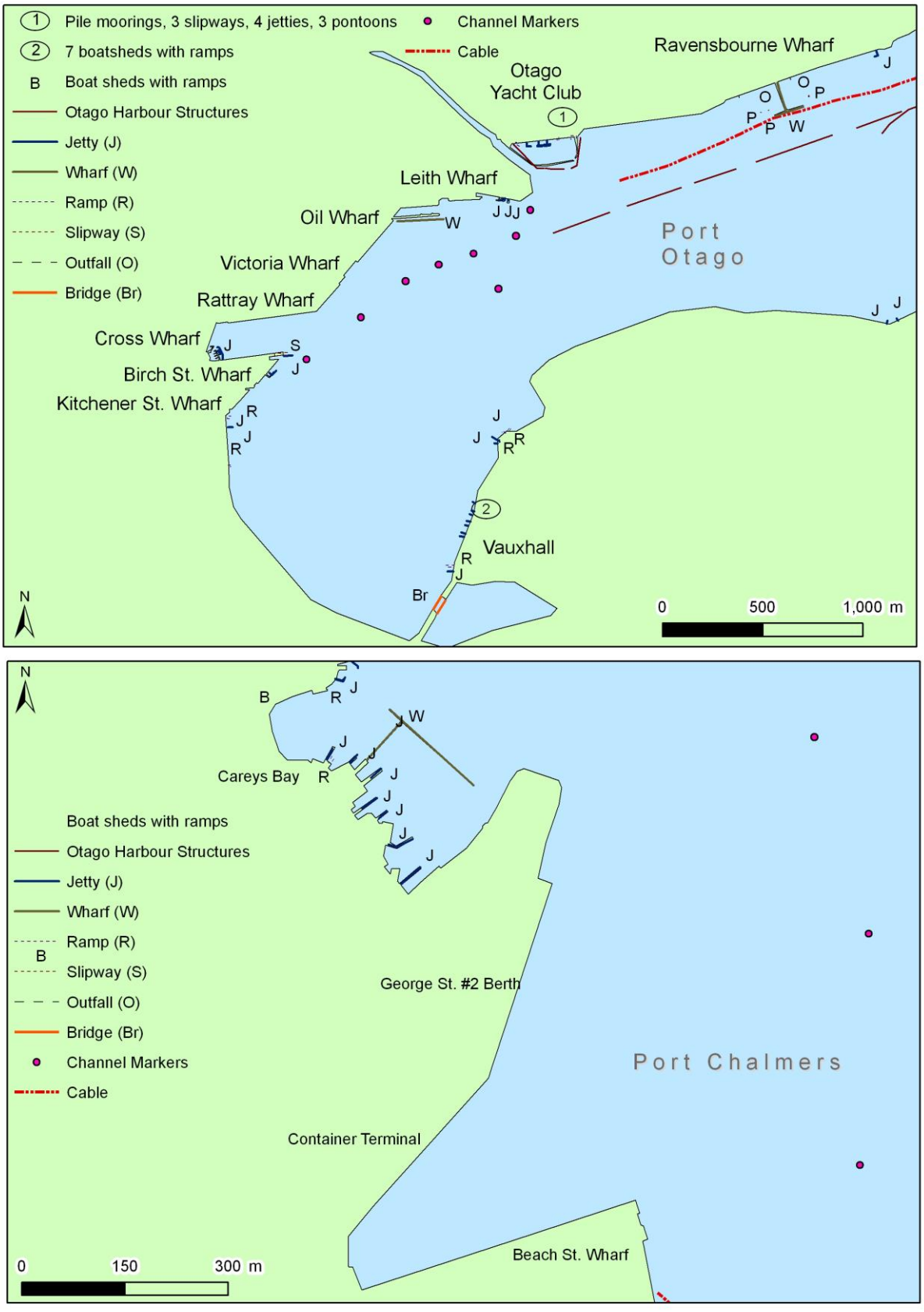


Figure 1b Details of the sampling areas in Port Otago (upper) and Port Chalmers (lower) showing artificial structures present.



EXISTING INFORMATION ON MARINE PESTS

Over the last three decades a number of biological surveys have been carried out in Dunedin Harbour, although they did not focus specifically on collecting and identifying non-indigenous species. The following summary detailing these studies is taken from Inglis *et al*. (2005).

Early studies were undertaken in the 1940's and 1950's, but tended to focus in the area of the harbour surrounding Portobello Marine Biological Station and the species and communities present there (e.g. Brewin 1946 on ascidians, Ralph and Yaldwyn 1956 on benthic fauna).

In the 1970's, the Otago Harbour Board commissioned the University of Otago to report on the marine environmental implications of proposed reclamation work at Port Chalmers (Probert 1975). The study sampled the bottom deposits and larger animals and plants of the seabed in the reclamation area by dredge and trawl. Soft muddy sediment was recovered at each sample station and a total species list for the proposed reclamation area generated. No non-indigenous species were recorded in the species lists.

Quinn (1978) examined the circulation and tidal flushing patterns within the harbour, recording salinity, temperature, phosphate, nitrate, nitrite, chlorophyll-a, zooplankton, and secchi depth data, with calculations made on hydraulic residence time.

Rainer (1981) examined the soft-bottom benthic communities in the harbour and Blueskin Bay, to investigate the relationship between a number of environmental variables and the structure and species composition of soft-bottom macrofaunal assemblages. His study lists a total of 397 species, with many species appearing to be restricted to a limited range of environmental conditions. Lowest species diversity was found in samples from unstable fine sand and unconsolidated silt sediments, and highest species diversity in samples from stable fine sand with an admixture of shell. Species lists compiled from the study included the non-indigenous corophiid amphipods *Corophium* (*Monocorophium*) *acherusicum* and *C.* (*Monocorophium*) *sextonae* and the ascidian, *Ascidiella aspersa*, and the cryptogenic colonial ascidian *Botrylloides leachii*.

Thrush (1988) compared macrobenthic recolonization patterns near and far away from crab burrows (*Macrophthalmus hirtipes*) on a sublittoral sand flat in the harbour. He found decreased faunal abundance nearer crab burrows, with this pattern maintained during recolonization after simulated storm disturbance.

Barnett *et al* (1989) Ltd conducted a detailed hydrodynamic study of the harbour for the Otago Harbour Board. They established basic patterns of tidal flows and level changes in the harbour, using these to predict current patterns in relations to harbour modification, and to identify sediment transport paths in the harbour and near possible dumping grounds.

The invasive kelp *Undaria pinnatifida* was identified in Dunedin Harbour in 1990, and since has spread along much of the hard shoreline. Dunedin Harbour is deemed to be in the optimal temperature zone for this macroalga (Forrest *et al* 2000; Sinner *et al* 2000).

Sediment macrobenthos of the inner harbour were surveyed by Grove & Probert (1999). In this study they examined patterns of benthic community structure and their relationship to environmental variables. They observed that a combination of percent sand, macro-algal content, water depth and chromium concentration correlated best with the observed community structure. Species lists were not produced within their review.

Taylor & MacKenzie (2001) tested Port Chalmers for the presence of the toxic blooming dinoflagellate *Gymnodinium catenatum*, and did not detect any resting cysts (sediment samples) or motile cells (phytoplankton samples).

Corfield & Hickey (2004a & b) examined the potential effects of fluoride discharge from the Ravensdown fertiliser plant on the surrounding benthic communities. They found evidence that fluoride, in combination with other sediment contaminants, had a significant effect on benthic community structure, particularly certain algae, sabellids, amphipods and bivalves.

The following summary detailing non-indigenous species in Otago Harbour is taken from the report detailing the first baseline biological survey of Otago Harbour in February 2003 (Inglis *et al*. 2005).

A total of 275 species or higher taxa were identified from the first baseline survey of Dunedin Port. This collection consisted of 18 non-indigenous species (Table 3), 38 cryptogenic[2] species (Table 4), 169 native species, and 50 species indeterminate. The biota included a diverse array of organisms from 12 phyla. Twenty-five species from the Port of Dunedin had not previously been described from New Zealand waters. These included 23 species of sponge that are thought to be new to science, a cryptogenic amphipod (*Leucothoe* sp.1), and two non-indigenous species (the polychaete worm, *Spirobranchus polytrema*, and the sponge *Leucosolenia* cf. *discoveryi*.

Eighteen non-indigenous species (NIS) were recorded from the Port of Dunedin (Table 3). NIS represented 6.5% of all identified species from this location. Two of these species - the annelid worm *Spirobranchus polytrema* and the sponge *Leucosolenia* cf. *discoveryi* - were not previously known from coastal New Zealand. The remaining NIS included one annelid, four bryozoans, three crustaceans, one mollusc, five macroalgae and two sponges.

Thirty-eight cryptogenic species were discovered in the Port of Dunedin. Cryptogenic species represented 13.8% of all species or higher taxa identified from the port. The cryptogenic organisms identified included 15 Category 1 and 23 Category 2 species. These organisms included one bryozoan, four cnidarians, one crustacean, one mollusc, 26 sponges and five ascidians (Table 4). Many of the Category 1 cryptogenic species (e.g. the ascidians *Aplidium phortax, Astereocarpa cerea, Botrylloides leachii*, and *Corella eumyota*; and the hydroids *Amphisbetia operculata, Halecium delicatum* and *Plumularia setacea*) have been present in New Zealand for more than 100 years but have distributions outside New Zealand that suggest non-native origins (Cranfield *et al* 1998).

A total of 169 native species was identified from the Port of Dunedin. Native species represented 61.5% of all species identified from this location and included highly diverse assemblages of crustaceans (33 species), molluscs (29 species), annelids (28 species), phycophyta (23 species), urochordates (13 species) and porifera (10 species). A number of other less diverse phyla including bryozoans, cnidarians, vertebrates, echinoderms, pyrrophycophyta and chelicerates were also sampled from the Port.

Fifty organisms from the Port of Dunedin were classified as species indeterminate. If each of these organisms is considered a species of unresolved identity, then together they represent

[2] A species whose status as native or introduced is unknown. Category 1 cryptogenic species are those previously recorded from New Zealand whose identity as either native or non-indigenous is unclear. Includes species that may have been introduced to New Zealand before scientific records began and those newly-described species exhibiting invasive behaviour in New Zealand but for which there are no known records outside the New Zealand region. Category 2 cryptogenic species are those newly-discovered species for which there is insufficient information to determine whether New Zealand lies within their native distribution.

18.2% of all species collected. Species indeterminate from the Port of Dunedin included 22 macroalgae, 10 annelids, five crustaceans, four vertebrates (fish), three molluscs, three cnidarians, two bryozoans and one urochordate species.

Twenty-five species from the Port of Dunedin were previously undescribed from New Zealand waters. These species are classified either as Category 2 cryptogenic species (Table 4) or are marked as new records in the non-indigenous species list (Table 3). Previously undescribed cryptogenic species included 22 species of sponges and one species of amphipod. These specimens did not match existing species descriptions and may be new to science. The two non-indigenous species not previously recorded from New Zealand waters were the annelid *Spirobranchus polytrema* and the sponge *Leucosolenia* cf. *discoveryi.*

Sixteen locations (13 ports and three marinas) were surveyed during the summers of 2001/2002 and 2002/2003. When sample effort was adjusted for, the Port of Dunedin had a slightly above-average diversity of non-indigenous, native and Cryptogenic 1 species relative to the other ports and marinas surveyed, but the greatest number of Cryptogenic 2 species, many of which (22 species) were newly discovered species of sponge. NIS comprised 6.5% of the total sampled diversity in the Port of Dunedin, ranking it ninth highest in percentage composition of NIS from the sixteen locations surveyed.

Undaria pinnatifida was included in the list of target species in previous phases of the programme and was recorded during the first and second baseline surveys and subsequent target-species surveillance surveys. As discussed above, it has been present in the Port of Dunedin since at least 1990.

Table 3 **Non-indigenous marine species recorded from the Port of Dunedin first baseline survey in February 2003. Likely vectors of introduction are largely derived from Cranfield *et al* (1998), where H = Hull fouling and B = Ballast water transport. Novel NIS not listed in Cranfield *et al* (1998) or previously encountered by taxonomic experts in New Zealand waters are marked as New Records (NR). For these species and others for which information is scarce, dates of first detection are provided rather than probable dates of introduction.**

Phylum, Class	Order	Family	Genus and species	Probable means of introduction	Date of introduction or detection (d)	Location in Port Dunedin
Annelida						
Polychaeta	Sabellida	Serpulidae	*Spirobranchus polytrema* **(NR)**	H	Nov. 2001 [d]	Container & Cruise Ship Berth, Port Chalmers
Polychaeta	Spionida	Spionidae	*Polydora hoplura*	H	Unknown [1]	Leith Wharf, Port Otago
Bryozoa						
Gymnolaemata	Cheilostomata	Bugulidae	*Bugula flabellata*	H	Pre-1949	Container & Cruise Ship Berth; Beach St Wharf, Port Chalmers Leith Wharf; Rattray Wharf; Victoria Wharf, Port Otago
Gymnolaemata	Cheilostomata	Bugulidae	*Bugula neritina*	H	1949	Rattray Wharf, Port Otago
Gymnolaemata	Cheilostomata	Cryptosulidae	*Cryptosula pallasiana*	H	1890s	Rattray Wharf, Port Otago
Gymnolaemata	Cheilostomata	Watersiporidae	*Watersipora subtorquata*	H or B	Pre-1982	Beach St Wharf, Port Chalmers Leith Wharf; Rattray Wharf; Victoria Wharf, Port Otago

Table 3 Continued.

Phylum, Class	Order	Family	Genus and species	Probable means of introduction	Date of introduction or detection (d)	Location in Port Dunedin
Crustacea						
Malacostraca	Amphipoda	Corophiidae	*Apocorophium acutum*	H	Pre-1921	Leith Wharf; Rattray Wharf; Victoria Wharf, Port Otago
Malacostraca	Amphipoda	Corophiidae	*Monocorophium acherusicum*	H	Pre-1921	Leith Wharf; Rattray Wharf; Victoria Wharf, Port Otago
Malacostraca	Amphipoda	Ischyroceridae	*Jassa marmorata*	H	Unknown [1]	Leith Wharf; Rattray Wharf; Victoria Wharf, Port Otago
Mollusca						
Bivalvia	Ostreoida	Ostreidae	*Crassostrea gigas*	H	1961	Rattray Wharf, Port Otago
Phycophyta						
Phaeophyceae	Cutleriales	Cutleriaceae	*Cutleria multifida*	H	Pre-1870	Leith Wharf, Port Otago
Phaeophyceae	Laminariales	Alariaceae	*Undaria pinnatifida*	H or B	Pre-1987	Container & Cruise Ship Berth; Beach St Wharf, Port Chalmers Leith Wharf; Rattray Wharf; Victoria Wharf, Port Otago
Rhodophyceae	Ceramiales	Rhodomelaceae	*Polysiphonia brodiaei*	H	Pre-1940	Container & Cruise Ship Berth; Beach St Wharf, Port Chalmers Leith Wharf; Rattray Wharf; Victoria Wharf, Port Otago
Rhodophyceae	Ceramiales	Rhodomelaceae	*Polysiphonia subtilissima*	H	Pre-1974	Beach St Wharf, Port Chalmers
Rhodophyceae	Rhodymeniales	Champiaceae	*Champia affinis*	H	Pre-1855	Container & Cruise Ship Berth, Port Chalmers

Table 3 Continued.

Porifera

Calcarea	Leucosolenida	Heteropiidae	*Grantessa intusarticulata*	H	Unknown [1]	Container & Cruise Ship Berth; Beach St Wharf, Port Chalmers Leith Wharf; Rattray Wharf; Victoria Wharf, Port Otago
Calcarea	Leucosolenida	Leucosoleniidae	*Leucosolenia* cf. *discoveryi* **(NR)**	H	Feb. 2003 [d]	Container & Cruise Ship Berth; Beach St Wharf, Port Chalmers Leith Wharf; Rattray Wharf; Victoria Wharf, Port Otago,
Demospongiae	Halisarcida	Halisarcidae	*Halisarca dujardini*	H or B	Pre-1973	Beach St Wharf, Port Chalmers

[1] Date of introduction not known but species had been encountered in New Zealand prior to the 2003 baseline survey.

Table 4 Cryptogenic marine species recorded from the Port of Dunedin first baseline survey in 2003. Category 1 cryptogenic species (C1); Category 2 cryptogenic species (C2). Cryptogenic species are those whose geographic origins are uncertain.

Phylum, Class	Order	Family	Genus and species	Category
Bryozoa				
Gymnolaemata	Cheilostomata	Scruparidae	*Scruparia ambigua*	C1
Cnidaria				
Hydrozoa	Hydroida	Bougainvilliidae	*Bougainvillia muscus*	C1
Hydrozoa	Hydroida	Haleciidae	*Halecium delicatulum*	C1
Hydrozoa	Hydroida	Plumulariidae	*Plumularia setacea*	C1
Hydrozoa	Hydroida	Sertulariidae	*Amphisbetia operculata*	C1
Crustacea				
Malacostraca	Amphipoda	Leucothoidae	*Leucothoe sp. 1*	C2
Mollusca				
Bivalvia	Mytiloida	Mytilidae	*Mytilus galloprovincialis*	C1
Porifera				
Demospongiae	Dendroceratida	Darwinellidae	*Darwinella cf. gardineri*	C1
Demospongiae	Dictyoceratida	Dysideidae	*Dysidea n. sp. 1*	C2
Demospongiae	Dictyoceratida	Dysideidae	*Dysidea n. sp. 4*	C2
Demospongiae	Dictyoceratida	Dysideidae	*Dysidea n. sp. 5*	C2
Demospongiae	Dictyoceratida	Dysideidae	*Euryspongia n. sp. 4*	C2
Demospongiae	Halichondrida	Halichondriidae	*Halichondria n. sp. 2*	C2
Demospongiae	Halichondrida	Halichondriidae	*Halichondria n. sp. 7*	C2
Demospongiae	Halichondrida	Halichondriidae	*Halichondria n. sp. 8*	C2
Demospongiae	Halichondrida	Halichondriidae	*Halichondria panicea*	C1
Demospongiae	Halichondrida	Halichondriidae	*Hymeniacidon n. sp. 1*	C2
Demospongiae	Halisarcida	Halisarcidae	*Halisarca n. sp. 1*	C2
Demospongiae	Haplosclerida	Callyspongiidae	*Callyspongia diffusa*	C1

Table 4 Continued.

Phylum, Class	Order	Family	Genus and species	Category
Demospongiae	Haplosclerida	Callyspongiidae	*Callyspongia n. sp. 4*	C2
Demospongiae	Haplosclerida	Chalinidae	*Haliclona n. sp. 2*	C2
Demospongiae	Haplosclerida	Chalinidae	*Haliclona n. sp. 3*	C2
Demospongiae	Haplosclerida	Chalinidae	*Haliclona n. sp. 11*	C2
Demospongiae	Haplosclerida	Chalinidae	*Haliclona n. sp. 12*	C2
Demospongiae	Haplosclerida	Chalinidae	*Haliclona n. sp. 13*	C2
Demospongiae	Haplosclerida	Chalinidae	*Haliclona n. sp. 14*	C2
Demospongiae	Haplosclerida	Chalinidae	*Haliclona n. sp. 15*	C2
Demospongiae	Poecilosclerida	Ancinoiidae	*Crella (Pytheas) incrustans*	C1
Demospongiae	Poecilosclerida	Chondropsiidae	*Chondropsis n. sp. 1*	C2
Demospongiae	Poecilosclerida	Microcionidae	*Dictyociona cf. atoxa*	C2
Demospongiae	Poecilosclerida	Mycalidae	*Mycale (Carmia) n. sp. 1*	C2
Demospongiae	Poecilosclerida	Mycalidae	*Mycale (Carmia) n. sp. 2*	C2
Demospongiae	Poecilosclerida	Mycalidae	*Paraesperella n. sp. 1*	C2
Urochordata				
Ascidiacea	Aplousobranchia	Didemnidae	*Diplosoma listerianum*	C1
Ascidiacea	Aplousobranchia	Polyclinidae	*Aplidium phortax*	C1
Ascidiacea	Phlebobranchia	Rhodosomatidae	*Corella eumyota*	C1
Ascidiacea	Stolidobranchia	Botryllinae	*Botrylloides leachii*	C1
Ascidiacea	Stolidobranchia	Styelidae	*Asterocarpa cerea*	C1

BIOPHYSICAL CONDITIONS

Data collected in Dunedin Harbour from January to December 2006 by Portobello Marine Laboratory show that surface water temperatures at the Marine Laboratory jetty ranged from ca. 20°C in February to ca. 6°C in June/July (Figure 2). There is no salinity data time series for Dunedin Harbour.

Figure 2 Surface water temperature (°C) at Portobello Marine Laboratory, Dunedin Harbour, in 2006. Data courtesy of Portobello Marine Laboratory, University of Otago.

HABITAT TYPES WITHIN THE SURVEY AREA

Dunedin Harbour is located on the south-eastern seaboard of New Zealand's South Island (45° 50'S. 170° 38'E). Two peninsulas and two islands effectively divide the harbour into upper and lower basins, connected by the shipping channel with a narrow entrance situated between Heyward Point and Taiaroa head (Rainer, 1981). This physical aspect of the land has resulted in the Port of Dunedin operating two wharf systems: Port Chalmers in the lower harbour and Port of Otago in the upper (Figure 1). The lower harbour extends 9.6 kilometres from the heads to Port Chalmers, where the container facilities are located, with Dunedin city located at the far western end of the harbour a further 12 km from Port Chalmers.

Dunedin Harbour is approximately 22 km long from the eastern harbour entrance to the city of Dunedin in the west and is generally between 1 and 4.5 km wide with a minimum width of only 400 m at the entrance adjacent to Harington Point. The harbour is bounded at the entrance by an artificial mole extending seawards from the Aramoana sand spit on the western side and a basaltic headland on the east extending out to Taiaroa Head. A narrow shipping channel (maintained to 13 m) leads to Port Chalmers, enabling the largest container ships in the New Zealand trade to utilise the Port, and the channel shallows to 8 m towards the Port of Otago wharf system. The harbour was formed by the gradual build up of a low sandy isthmus that joined a volcanic island (now the Otago peninsula) to the mainland (Thompson 1981).

The harbour is generally shallow, less than 2 m deep, with extensive intertidal sand/mudflats with sea grass beds. Near the midway point of the harbour, it is restricted by two islands (Goat and Quarantine Islands) and the Portobello Peninsula.

Tides within the harbour are semi-diurnal, with mean sea level 1.1 m above chart datum. Mean low water springs are 0.1 m and high water springs 1.8 m above chart datum, resulting in a spring tide range of 1.7 m. Current speeds in the harbour are estimated to be between 50 and 75 cm/sec in the outer harbour and less than 50 cm/sec in the inner harbour (Rainer, 1981). Water in the upper harbour has a considerably longer residence time than that in the lower harbour: 4 to 14 days in the upper compared to 1.2 tidal cycles in the lower. Flushing time for the upper harbour is influenced more by freshwater input than by tidal movement. The latter merely moves the same water back and forth (Grove & Probert 1999). Sediments in the harbour vary from silt to coarse shell-sand, with the coarsest sediments found within the shipping channel, particularly near the heads (Rainer 1981).

Types of habitat present in Port Otago, Port Chalmers, and the surrounding areas, are listed in Table 5. Given that much of the available habitat for incoming non-indigenous species is represented by artificial substrata within the ports, calculation of habitat area/volume is not feasible within the present project – there are almost certainly no data on numbers of piles, marina pontoons, etc for each port and the available habitat area is structurally extremely complex. As part of the delimitation survey for *Styela clava* (Gust *et al*. 2006), an estimate was made of the length of artificial habitat (piling, pontoons, breakwalls) present in Port Otago and Port Chalmers. Values of 6,062 m for Port Otago and 3,904 m for Port Chalmers were derived from GIS maps of the ports and represent the horizontal length of structures present. Clearly they do not provide an estimate of the area of habitat available for colonisation, but allow a rough comparison among ports. For example, the equivalent value for the largest port in the targeted surveillance programme is 11,300 m for Wellington (including the Burnham Oil Wharf but not including Shelly Bay, or Seaview Wharf and Marina: note that the length of artificial habitat in the Waitemata Harbour and Lyttelton Port were not estimated by Gust *et al*. 2006).

Table 5 Types of habitat present in the survey area.

Habitat category	Habitat type	Habitat subdivision	Location
Soft-surface	Sand		Emergent sand flats along main shipping channel (often with seagrass beds), Aramoana Beach and alongside eastern side of the Harbour
	Sand/mud		Alongside emergent sand flats and main shipping channel on eastern side of Harbour all the way to the head of the upper Harbour. Possesses patchy areas of red macroalgae.
	Sand and crushed shell		Main shipping channel from Harbour entrance to Quarantine Island
Hard-surface	Emergent reef		None in survey area
	Rock (>20cm)		Boulder break-wall mole at Split Beach (Aramoana)
	Artificial structures	Commercial vessel berth	Port Otago and Port Chalmers, Ravensbourne Pier
		Channel marker	Regular channel markers run from Harbour entrance (Harington Point) all the way into Port Otago. Fewer channel markers on shallow eastern side of Harbour.
		Boat ramp	Otago Yacht Club (adjacent to Leith Canal), Back Beach Bay, Careys Bay, and Burns Point
		Marina (pontoons, piles)	Otago Yacht Club (adjacent to Leith Canal)
		Jetty/Breakwater	Jetties at Aromoana Beach groyne wall, Careys Bay, Back Beach Bay (incl. pontoons), Quarantine Island, Portobello Marine Laboratory, Macandrew Bay, and Burns Point. Rubble and rip-rap break-walls in Port Otago and Port Chalmers, rip-rap under Port wharves
		Slipway	Port Otago (Birch Street Wharf) and Careys Bay
		Channel walls	Groyne walls at Aromoana Beach, Omate Beach and Te Rauone Beach, and low tide training wall running from Kilgours Point (Blanket Bay) in to Ravensbourne Pier
		Moorings	Small craft pile and swing moorings at Deborah Bay, Careys Bay, Back Beach Bay, Macandrew Bay, and near Ravensbourne Pier

Table 5 Continued.

Habitat category	Habitat type	Habitat subdivision	Location
		Private boat sheds	Harington Point, Careys Bay, Back Beach Bay, Portobello Marine Laboratory, and in upper Harbour between Burns Point and Andersons Bay
		Bridge (concrete piles)	None in survey area
		Inactive/disused berth	Disused slip at Port Otago (Kitchener Street Wharf) and pile remnants of old wharf at the very head of the upper Harbour
		Ship wrecks	Deborah Bay
		Aquaculture facility	None in survey area
Pelagic	Water column	Top, middle, bottom.	All survey areas

IDENTIFICATION OF VECTOR PARAMETERS

Port Chalmers has three berths, suitable for handling containerised, multipurpose, conventional, and RoRo vessels. Berths are constructed predominantly of concrete decking on steel piles, although the Beach Street Forestry berth is composed of sand-filled sunken concrete casings supporting a concrete deck. The inner and outer berths at George Street are the heart of the Port of Dunedin's container facilities, but are also suitable for geared and conventional vessels. Vessels unable to be berthed immediately in the Port of Dunedin may anchor outside the harbour, west of the harbour entrance towards Blueskin Bay (approximately 45°43'16"S, 170°37'31"E).

The Beach Street forestry berth is suitable for all classes of geared and conventional vessels, and is ideal for the large volumes of logs, lumber and other forestry products that are exported from Port Chalmers. A swinging basin with 700 m diameter and 12.5 m depth enables a wide turning facility for berthing.

The Port of Otago wharf system is suitable for vessels up to 190 m LOA and 31.5 m beam and consists of seven principal berths, suitable for vessels with a shallower draught. Berths construction is a mixture of concrete or wooden decking on Australian hardwood or steel piles. Tankers, fishing vessels and smaller conventional vessels are the principal users. In 2000, the Otago Yacht Club possessed 35 pile moorings in Port Chalmers (Sinner *et al* 2000).

Located between Port Chalmers and Port of Otago is the Ravensbourne fertiliser pier, used by Ravensdown Fertiliser Ltd for discharge of their raw materials to the adjacent manufacturing facility (www.portotago.co.nz).

Regular on-going maintenance dredging is carried out in the shipping channels (trailer suction dredge) and vessel berths (grab dredge) to maintain the required depth. This results in an annual removal of 200-250,000 m^3 of spoil. There are three spoil disposal sites (marked on nautical chart 6612) at, and outside the harbour entrance: Shelley Beach for clean sand disposal (acts as sand replenishment to counter beach erosion), and Spit beach and off Hayward Point for the majority of spoil disposal.

Port Chalmers is the deepest container port in New Zealand with a chart datum draft of 13 m (www.portotago.co.nz). Approximately 50% of the harbour channel is at least 14 m deep. In

order to accommodate the new generation of bigger ships capable of carrying up to 6,000 TEUs[3], Port Otago will need to widen and deepen the harbour channel to between 14 m and 15 m depth. Port Otago is currently investigating the issues and options associated with securing visits by bigger ships. Future development at Port Chalmers will require investment in new infrastructure, the extension of wharves and berths, and channel widening and deepening.

Imports and exports

Port Otago is a major deep-water export port for the South Island. Port Chalmers is New Zealand's deepest container port and the countries third largest port in terms of cargo value, providing an international gateway for some of the countries most important export cargo (www.portotago.co.nz). Since 1997, cargo volumes at the port have increased by more than 300%. The port is capable of handling the largest container ships that visit New Zealand (which have a nominal carrying capacity of 4,100 TEUs).

In 2000, Port Chalmers and Port Otago handled $2 billion of exports in primary products like meat, dairy, fish, wool and timber, and manufactured goods to markets throughout the South Pacific, Europe, North America, Australia, and Asia, and imported $200 million of products (www.cityofdunedin.co.nz). Port Chalmers and Port Otago handled 2.8 million tonnes of cargo in the year 2004, with a total of 537 vessel visits (www.portotago.co.nz). In 2007, the ports handled 2.974 million tonnes of cargo, with a total of 617 vessel arrivals. In 2000, there were 21 registered fishing vessels in Port Otago and 14 in Port Chalmers (Sinner *et al* 2000).

Shipping movements and ballast discharge patterns in the Port of Otago and Port Chalmers

Analyses of shipping arrivals to the Port show that the Ports Chalmers and Otago received 29 international ship visits during 2002/2003 (16 merchant and 13 passenger vessels). During this period, most commercial vessels entering the port arrived from Australia (82.4%), the NW Pacific (11.8%), and the Northwest Atlantic (5.9%) (Campbell 2004).

Since June 2005, vessels have been required to comply with the Import Health Standard for Ships' Ballast Water from All Countries (http://www.biosecurity.govt.nz/imports/non-organic/standards/ballastwater.htm). No ballast water is allowed to be discharged without the express permission of a MAF (Ministry of Agriculture and Forestry) inspector. To allow discharge, vessel masters are responsible for providing the inspector with evidence of: discharging ballast water at sea (200 nm from the nearest land, and at least 200 m depth); or demonstrating that ballast water is fresh (2.5 ppt sodium chloride); or having the ballast water treated by a MAF-approved treatment system. According to Inglis (2001), a total volume of 33,364 m^3 of ballast water was discharged in the Port of Dunedin in 1999, with the largest country-of-origin volumes of 18,697 m^3 from Japan, 7,806 m^3 from Taiwan, 2,028 m^3 from Australia, and 4,834 m^3 unspecified.

Possible vectors for introduction of non-indigenous species

The non-indigenous species located in the port are thought to have arrived in New Zealand via international shipping. Likely vectors of introduction are largely derived from Cranfield *et al* (1998) and indicate that approximately 83% (15 species) probably were introduced to New Zealand waters via hull fouling, and the remaining 17% (three species) could have arrived via either of these mechanisms.

[3] 20' equivalent units - the international standard of measurement for containers.

Assessment of the risk of new introductions to the ports

Many NIS introduced to New Zealand ports, through hull fouling, ships' sea chests, or ballast water, do not survive to establish self-sustaining local populations. Those that do, often come from coastlines that have similar marine environments to New Zealand. For example, approximately 80% of the marine NIS known to be present within New Zealand are native to temperate coastlines of Europe, the North West Pacific, and southern Australia (Cranfield *et al*. 1998).

Compared to other New Zealand commercial ports, the Port of Dunedin receives only a minor amount of commercial shipping. In both 1999 and 2002/2003, Dunedin (Port Otago and Port Chalmers combined) received a total of 36 commercial vessels that came directly from overseas (Inglis 2001; New Zealand Customs Service, unpublished data). Commercial shipping arriving in the port of Dunedin from overseas comes predominantly from temperate regions of Australia (78% of all arrivals in 2002/2003) and the north west Pacific (11%; in particular Japan, Korea and China); environments which are broadly compatible with those in Otago Harbour. Port Chalmers has a high trade volume of bulk cargoes, in particular logs, timber and forestry products, and containers. The Port of Otago deals with a different range of cargoes, in particular fertiliser (Ravensbourne Wharf), bulk petroleum products and liquid petroleum gas (LPG). The Port of Dunedin is mainly an export facility. In both 1999 and 2000, approximately 830,000 tonnes of goods were loaded at Port Chalmers. This is reflected in the relatively high volume of ballast water (given the small number of vessel arrivals) that is discharged in Dunedin Harbour. In 1999, the Port of Dunedin received 33,364 m^3 of reported ballast discharge. On a national scale, this is not a large volume (ranking 8^{th} highest out of 15 ports). Discharged ballast water originates predominantly from Japan (56%), Taiwan (23%) and Australia (6%). Shipping from these regions presents an on-going risk of introduction of new NIS to Otago Harbour.

Assessment of translocation risk for introduced species found in the ports

Dunedin is connected directly to the ports of Bluff, Timaru, Lyttelton and Napier by regular coastal shipping and, indirectly, to most other domestic ports throughout mainland New Zealand and the Chatham Islands (Dodgshun *et al*. 2004). Although many of the non-indigenous species found in the Dunedin survey have been recorded previously in New Zealand, there were two notable exceptions. The annelid *Spirobranchus polytrema* was first described from New Zealand waters during these port surveys, and was found to be present in Dunedin, Wellington and Napier. Little is currently known about this species; however, in the ports where it was encountered it may be competing with native fauna for space, food or other limiting resources. The sponge *Leucosolenia* cf. *discoveryi* was also unknown from New Zealand waters prior to the surveys, but has now been discovered in the ports of Bluff and Dunedin. There is no information on the risks posed by this species to New Zealand's native ecosystems and species. However, it is common in Antarctica and Australian Sub-antarctic Islands, and Bluff and Dunedin represent the northernmost locations where the species has so far been encountered.

The invasive alga, *Undaria pinnatifida*, has been present in Otago Harbour since at least 1990. It has been spread through shipping and other vectors to 11 of the 16 ports and marinas surveyed during the baseline surveys (the exceptions being Opua, Whangarei Port and Marina, Gulf Harbour Marina, Tauranga Port), although a control programme in Bluff Harbour subsequently removed populations established there. Since the baseline surveys it has been recorded on wharf piles in the port of Tauranga. Nevertheless, vessels departing from Dunedin after having spent time at berth within the port may pose a significant risk of spreading this species to ports within New Zealand that remain uninfested. The risk of translocation of *U. pinnatifida* and other fouling species is highest for slow-moving vessels,

such as yachts and barges, and vessels that have long residence times in port. In Port Otago and Port Chalmers, dredge vessels, barges, recreational craft, and seasonal fishing vessels that are laid up for significant periods of time pose a particular risk for the spread of these species. Cruise liners and fishing vessels depart regularly from Dunedin Harbour for Fiordland, the Chatham and Sub-Antarctic Islands. These environments are valued for their unique natural values and marine biodiversity. *U. pinnatifida* and many of the other NIS recorded in this survey are not known to be present in these areas and there is a real threat that they may be spread to them through shipping and other vectors.

LOCAL CONSTRAINING FACTORS ON SURVEILLANCE SUCCESS

Local factors likely to constrain sampling, including those representing hazards to the field team members, are listed in Table 6, together with management options to mitigate them.

Table 6 Hazard analysis for biophysical conditions of surveillance locations

Hazard/Constraining Factor	Effect at Surveillance Location	Present (Y, N or intermittent (I))	Management actions
Water residence time is 4 to 14 days in the upper harbour and 1.2 tidal cycles in the lower harbour (Quin 1978, Reid 1990, Smith and Croot 1993).	Planktonic propagules are likely to remain in the upper harbour for up to 14 days following release and are liable to be dispersed throughout Dunedin Harbour.	Y	Hydrological modelling indicates dispersal will occur throughout most of the harbour when winds are from the N and NE. Dispersal is restricted to the northern shore with SE winds (Inglis *et al*. 2006)
Turbidity (Secchi disk depth ca. 2 m in the upper harbour to ca. 5-6 m in the lower harbour)	Turbidity high in the upper harbour, especially after rain. Lower in the outer harbour.	I	Variability in detection probability of diver searches
Predominant wind directions are N and NE, S and SW. During summer a NE breeze is often generated in the afternoon.	Boat handling can be difficult in exposed areas during high winds. In summer NE in the afternoon may constrain sampling work from boats	I	Port Otago is relatively protected and sheltered areas to work can generally be found. In summer work in more open parts of the harbour need to be completed in the morning before the NE breeze comes up
Wind speed	Seasonally variable but with NE sea breeze in the afternoon in summer	Y	Waves within the harbour are wind generated. Sheltered areas to work in the harbour can generally be found. In summer work in more open parts of the harbour may need to be completed in the morning before the NE breeze comes up
Tidal currents of 0.5 to 0.75 m s^{-1} in the lower harbour and < 0.5 m s^{-1} in the upper harbour (Rainer 1981).	Almost complete exchange of waters in the lower harbour in a tidal cycle with strong currents near the narrow entrance and in the main channel. Tides are moderate with a tidal range of ca. 1.7 m.	I	Diving in locations near the entrance to the port, channels and exposed locations should be done around slack tide. Care also needs to be taken with deployment of traps in these areas to reduce the risk of their being moved by currents or tangled around wharf piles, etc.
Spring-neap tidal cycle	Spring high tides in Dunedin harbour and associated maximal current speeds can produce suboptimal conditions for traps and shore searches	I	Surveys are timed to avoid spring tides because of the associated high current speeds and risk of traps being moved into shipping lanes

Hazard/Constraining Factor	Effect at Surveillance Location	Present (Y, N or intermittent (I))	Management actions
High rainfall can occur throughout the year	High turbidity, risk of sewage spill	I	Sampling (particularly diving) may be postponed after very heavy rain because of poor visibility and (very occasional) sewage contamination
Temperature	Minimum water temperatures in winter are ca 9°C	Y	Diving and other sampling still possible providing divers are adequately equipped (dry suits)
Dangerous animals	Sea lions, fur seals, eagle rays and sting rays are intermittently present	I	Not generally a problem but should be taken into consideration for dive planning
Vessel traffic	Periodic but predictable for main port, frequent and unpredictable at fishing-boat wharves	Y	Sampling of main port can be planned through consultation of Port Otago shipping movement website and communication with the harbour master at the start of each survey and during survey work to monitor (common) changes to schedules. Fishing wharves can be very busy and movements are not posted on the website. In general these wharves are considered too dangerous for diver searches and there is generally no room or unacceptable risk of entanglement for deployment of traps.
Dredging & construction activities	Annual maintenance dredging and periodic construction activity on the port wharves and marina berths	I	May require that parts of the wharves are not sampled at times when construction is in progress, or that channels sites are not sampled with the sled when dredger is on station, but these periods are usually of limited duration. When dredger is working, sampling vessels need to keep clear and not impede its trips out to the spoil ground.
Cables, pipelines and other hazards to navigation	Power cables cross the lower Haven and Marina	Y	Boat handlers to be aware of location of cables and sledding and trapping must avoid these areas.
Pollution (sewer outfall)	No outfalls within the harbour but sewage spills occur occasionally after heavy rain	I	Diving and other work may have to be postponed until Dunedin City Council posts all-clear notice.
Diving related (entanglement)	Areas that are publicly accessible are heavily used by anglers and fishing line presents an entanglement hazard. Wild oysters are a hazard to exposed skin and diving equipment.	Y	Divers carry knives or shears at all times and boat support with standby diver is always present. Divers also wear gloves to avoid oyster cuts.

PORT SECURITY ISSUES

Because entry to the port area is via the water, rather than by land through the port secure area, field teams are not required to obtain formal security clearance before entering the port. Port Otago Security requires the names and identification type and number of all those entering the port security zone during the survey. Team members are to carry photo-

identification when in the port area and will not step onto any area within the port secure area without first obtaining permission from port security. Port Otago Radio (monitored by port security) is to be informed as survey vessels enter the port area. Port security are informed (via Port Otago Radio) prior to deployment of traps in the port area, and when divers are entering the water at each location within the port.

Selection of sampling methods for target species

HABITAT ASSOCIATIONS AND LIFE HISTORIES OF THE TARGET ORGANISMS

Information on the habitat associations and life histories of the primary target species is collated in Appendix 2.

SELECTING LIFE STAGES TO TARGET

It has been agreed with MAF BNZ that sampling for planktonic life stages of target organisms is not currently a feasible option and is not included in the scope of the present contract (*Contract Specification Addendum* page 52). Identification of larval stages of target species is generally considerably more difficult than identification of adults. While molecular probes are available for some non-indigenous species, problems of sampling remain unresolved. These include the volume of water to be sampled, the location of samples and the question of how, if the probe gives a positive result, the location (and size) of the source population can be identified. At present, therefore, although these methods may potentially provide presence/absence information on target species, they are of little practical use for managing any incursions detected. A critical part of operationalising molecular probes for field based sampling is testing their specificity for the target organism. That is, although a gene sequence may have been identified for a pest species, we cannot use it reliably in field surveys until its sensitivity to other, related native species has been tested.

SAMPLING METHODS

In comparison to surveys for agricultural pests, survey methods for invasive marine organisms are still relatively undeveloped. Most studies of marine pests have used conventional ecological survey techniques, such as baited traps (Veldhuizen and Stanish 1999, Yamada *et al*. 2001, Thresher *et al*. 2003), diver surveys (Currie *et al*. 2000), and benthic grab (Carlton *et al*. 1990) or sled samples (Parry and Cohen 2001). These methods are relatively non-specific and can be labour-intensive, limiting the number of locations that can be searched effectively. A documented process for the selection of sampling methods and allocation of sampling effort for the target species was developed at the start of the previous phase of the programme (Inglis *et al*. 2006) and included information on the biology and behaviour of the target organisms and sampling methods used for the same or similar species in other parts of their range. Sensitivity (referred to in previous reports as the "efficiency" of the survey method), cost-effectiveness, feasibility and consistency with safe field-working practice were also evaluated in selecting methods, although in most cases the actual sensitivity of the method has not been quantified.

To decide on appropriate sampling methods for each of the target species, we reviewed published information on methods that had been used previously to sample each species and asked experts working on the species in its native or introduced range to comment on the utility of the methods we had proposed for surveillance monitoring (see Appendix 3). The criteria used to select survey methods were:

- effectiveness at capturing the target species when it is present,
- cost and ease of sampling,

- minimal impact on native marine environments and species, and
- safety of field personnel, the general public and property.

Since the purpose of the surveillance programme is detection, not enumeration, techniques in which the presence or absence of the target species could be determined rapidly within a sample were selected, allowing a comparatively large number of locations to be sampled on each survey. Baited box traps were used to sample adult crabs (i.e. *Carcinus maenas* and *Eriocheir sinensis*) and Whayman-Holdsworth starfish traps were used to catch asteroids and other large benthic scavengers. Baited traps do not sample juvenile and subadult *E. sinensis* effectively because these life stages have a largely herbivorous diet. They were therefore sampled with artificial shelters ("crab condos") designed for surveys of *E. sinensis* in San Francisco Bay. An Ocklemann epibenthic sled was used to sample soft sediment habitats for *Potamocorbula amurensis*, *Sabella spallanzanii*, *Asterias amurensis* and *Caulerpa taxifolia*. Divers searched for *S. spallanzanii*, *C. maenas*, *A. amurensis* and S*tyela clava* around piles, floating pontoons and other artificial structures in port and marina environments, and on intertidal and shallow subtidal reefs that were identified as high risk by the dispersal modelling. Timed visual searches for target species were made of intertidal rocky and sandy shorelines.

We considered that the methods selected for the previous phase of this programme (see Table 7) were successful and appropriate, and proposed that they be used in the present study, subject to discussion and approval from MAF BNZ. This was accepted by MAF BNZ, as stated in the *Contract Specification Addendum* (page 51). Note that it has been agreed with MAF BNZ that sampling for planktonic life stages of target organisms is not currently a feasible option and is not included in the scope of the present contract (*Contract Specification Addendum* page 52).

The minimum size of organism retained by the various trapping and sledding methods in governed by the size of mesh used. In the case of the crab (box) traps the netting covering the trap has a 1.3-cm mesh, that on the starfish traps is 2.6 cm and the bag inside the epibenthic sled has a 2-mm mesh.

Table 7 Summary of proposed sampling methods, target organisms and selection factors.

Method	Target species	Habitat	Spatial coverage	Effectiveness	Cost effectiveness	Feasibility	Previous surveillance in NZ	Previous surveillance overseas
Epibenthic sled tows	*Asterias amurensis* *Caulerpa taxifolia* *Didemnum* sp. *Eudistoma elongatum* *Musculista senhousia* *Potamocorbula amurensis* *Sabella spallanzanii*	**Subtidal soft sediments** Particular focus on known shellfish beds (for *Asterias*) and areas next to public access (e.g. wharves, boat ramps, marinas, etc. *Caulerpa*, *Sabella*)	Narrow width but 50 m tow length and high replication (100+ per location) enables a reasonably large area to be sampled (ca 2500m^2 per location)	Reliable sample collection including asteroids, infaunal and epifaunal bivalves and polychaetes and macroalgae	Processing of sled contents can be time consuming	Feasible on all soft-sediment habitats under reasonable weather conditions. Can be limited by the presence of large amounts of benthic macroalgae or soft mud that fill mouth of sled	Yes	Yes
Starfish traps	*Asterias amurensis* and other motile scavengers	Adjacent to wharf pilings and other artificial habitats	Sampled area is dependent on dispersion of bait odour. High replication possible.	Has been used effectively to monitor *A. amurensis* in Australia and benthic predators around marine farms in NZ	Quick to deploy and recover, so high replication possible	Most locations and weather conditions	Yes	Yes (Martin & Proctor 2000)
Box (crab) traps	*Carcinus maenas* *Eriocheir sinensis* *Charybdis japonica*	Intertidal and shallow subtidal rocky shores, breakwalls and saltmarsh Particular focus on habitats with complex physical structure (e.g. mussel beds, seagrass beds)	Sampled area is dependent on dispersion of bait odour. High replication possible.	Effectively sample other species of crabs (*Ovalipes*, *Macrophthalmus*, *Charybdis*)	Quick to deploy and recover, so high replication possible	Most locations and weather conditions	Yes	Yes (Hewitt & Martin 2001, May & Brown, 2001 Thresher *et al*. 2003, Yamada *et al*. 2004)

Table 7 Continued.

Method	Target species	Habitat	Spatial coverage	Effectiveness	Cost effectiveness	Feasibility	Previous surveillance in NZ	Previous surveillance overseas
Crab condos	*Eriocheir sinensis* *Carcinus maenas* *Charybdis japonica*	Intertidal and shallow subtidal banks of rivers. Particular focus on brackish water habitats with complex physical structure (e.g. saltmarsh or fringing vegetation)	High replication possible. Availability of suitable estuarine habitat may limit deployment	Effectively sample other species of crabs (*Helice*, *Macrophthalmus*). Higher rates of detection of crabs than bated traps in muddy river banks (Veldhuizen 2000).	Quick to deploy and recover, so high replication possible	High – access problems at some sites (shallow water, deep mud, private land)	Yes	Yes (Veldhuizen 2000)
Shoreline searches	*Eriocheir sinensis* *Carcinus maenas* *Caulerpa taxifolia* *Charybdis japonica* *Didemnum* sp. *Eudistoma elongatum* *Grateloupia turuturu* *Styela clava*	Sloping sandy shorelines, intertidal rocky reefs and areas where drift material is likely to accumulate. Prevailing winds on preceding days are a useful guide to where material may accumulate	Wide – can cover long stretches of intertidal habitat quickly	Used effectively in delimitation studies of *Styela*	High	High – access to intertidal areas may be limiting	Yes	Yes
Diver searches	*Carcinus maenas* *Asterias amurensis* *Didemnum* sp. *Eudistoma elongatum* *Grateloupia turuturu* *Sabella spallanzanii* *Styela clava*	Wharf piles, marina piles and pontoons and other artificial structures, intertidal and shallow subtidal reefs.	Good – large numbers of piles or lengths of hard substratum can be searched in detail	Dependent on water clarity and level of biofouling	Cost effective in reasonable water clarity, can be time-consuming under poor conditions	Feasibility dependent on water currents, weather, water clarity and safety issues for divers	Yes	Yes

SURVEILLANCE FOR NON-TARGET SPECIES

The secondary objectives of the programme are:

- To detect incursions of non-target non-indigenous or cryptogenic species not previously recorded in New Zealand
- To detect incursions of established non-indigenous or cryptogenic species that are exhibiting invasive characteristics (i.e. range extensions of established organisms).

This objective will be addressed opportunistically. This is inevitable given the taxonomic range of potential new non-indigenous or cryptogenic species and of established non-indigenous or cryptogenic species that might exhibit invasive characteristics. The diversity of specialist taxonomic skills required to identify this range of taxa is unlikely to be present in any one field team, and collection of all potential material for laboratory identification is beyond the scope of this project. In the previous phase of the targeted surveillance programme we identified a suite of non-target, non-indigenous species known to occur in New Zealand (two of which, *Musculista senhousia* and *Didemnum* sp. are now included in the list of secondary target species) that were consistently recorded when encountered during surveys (see Inglis *et al*. 2006 and Morrisey *et al*. 2007). In the present phase, we will retain this suite of species, to be recorded along with the target species whenever encountered. These records will be assessed against the criteria of Chapman & Carlton (1991):

- Sudden appearance in the surveillance location[4]
- Has the species spread subsequently
- Association with, or dependency on, non natural dispersal mechanisms
- Strong association with artificial substrate[5]
- Tendency towards monoculture or high local abundance
- Restricted distribution (e.g. only near a likely point of pest introduction by human activities)
- Rapid increase in abundance
- Disjunctive global distribution
- Are natural dispersal mechanisms inadequate to reach New Zealand
- Genetic or morphological isolation from most similar species distribution elsewhere in the world.

Note that any one of these triggers may immediately indicate an unknown invasive species, however others, such as abundance or distribution, may only become apparent after further surveillance.

TIMING OF SAMPLING ACTIVITY

In the absence of suitable methods of sampling planktonic life stages (see above), sampling is done biannually, in summer (November to March) and winter (May to September) at each location to account for possible changes in abundance of adults of the target species. Adults of all of the primary target species are perennial and likely to be present throughout the year. Timing of sampling is constrained by the need to sample all eight locations (ten during the first round of sampling, when Picton and Opua are also sampled) within each summer and winter period (each survey takes at least a week, with a week in between surveys to allow equipment to be sent on to the next location).

[4] assumes prior knowledge of taxa in surveillance location.
[5] assumes comparable sampling of artificial and natural substrata has occurred.

DETERMINATION OF SAMPLING EFFORT

MAF BNZ have specified, in consultation with NIWA (as set out in the *Contract Specification Addendum*), that the total sampling effort in each harbour and survey (i.e. total number of sites surveyed and samples taken) will be governed by a fixed cost, since at the time the tender was let, criteria were not specified for the size of infestation to be detected or the desired confidence of detection, both of which are necessary to estimate a statistically robust sample size (Carter 1989, Binns *et al*. 2001). The budg*et al*lowed a field team of six people (operating from two vessels) to work in each harbour for up to six days using the six different survey methods. During the first surveillance programme (2002-2004) we established the average time taken to obtain samples with each method and the number of sites that could be surveyed in the allotted time. This varied somewhat among harbours according to the size of the harbour and the availability of suitable habitat for the target species. The initial estimates of sample time were then used to set targets for the numbers of sites sampled with each technique in subsequent surveys. The allocation of effort among the different survey techniques (Table 8) reflected the relative abundance of each type of habitat in the harbours. For example, most sample effort was allocated to sledding (soft-sediment habitats) and crab trapping (structurally complex habitats including wharf structures and subtidal rocky habitats) because these habitats typically covered the largest part of the survey area.

Table 8 Allocation of sampling effort among the survey techniques proposed.

Sampling method	Target number
Crab condo lines[1]	8
Crab (box) trap lines[2]	60
Starfish trap lines[3]	20
Epibenthic sled tows	100
Diver searches	30
Shore searches	25

[1] 3 traps per line
[2] 3 traps per line
[3] 2 traps per line

The numbers of samples taken in each harbour during the field surveys in the 2002-2004 sampling programme were similar to those used in the present programme. They generally provided low probabilities of detection of manageable-sized incursions (i.e. <1.5 ha.) for most of the target species (see Inglis *et al*. 2006b for a description of the methods for estimating probabilities of detection). The chance of a sizeable incursion being missed because of statistically low sample numbers, sparse distribution of an incursion and the chance placement of survey locations is amply illustrated by Waitemata Harbour where less than 0.6% of the total linear distance of the artificial structures could be sampled on each survey. As a result, even a relatively large infestation in Waitemata Harbour over a combined linear distance of 1 km could be expected to be found in only one out of every 10 surveys (i.e. probability of detection = 0.11). Such infestations are not usually distributed contiguously, but can be comprised of many small clusters of abundance distributed over a large area. In these circumstances (i.e. statistically low sample number and sparsely distributed incursion) a sizeable incursion can be missed by the chance placement of survey locations.

As stated in the *Contract Specification Addendum*, the elements of the survey design required to set realistic targets for the desired level of confidence and the minimum detectable incursion size may be explored and determined between MAF BNZ and NIWA when available research provides sufficient information on which to base these determinations. The surveillance survey design may then be varied to take account of this new information. It is expected that opportunities for continued improvement will be explored and implemented

where appropriate and agreed to during the course of this contract. Determining an appropriate level of sampling requires explicit consideration of the following:

(1) the minimum size of incursion that is required to be detected by the survey (the "design prevalence");
(2) how confident the manager wishes to be that an incursion of that size or greater will be detected (the. "confidence of detection"), since absolute confidence is not possible (Cannon 2002; Cameron 2002; Venette *et al*. 2002; Inglis *et al*. 2006; Hayes *et al*. 2005);
(3) intuitively, it seems obvious that smaller incursions might be contained more easily than larger ones, but there is little guidance in the literature about how big (or small) such a target should be;
(4) resources available.

Estimate of sampling effort and results of discussions with BNZ prior to contact

SPATIAL ALLOCATION OF SAMPLING EFFORT

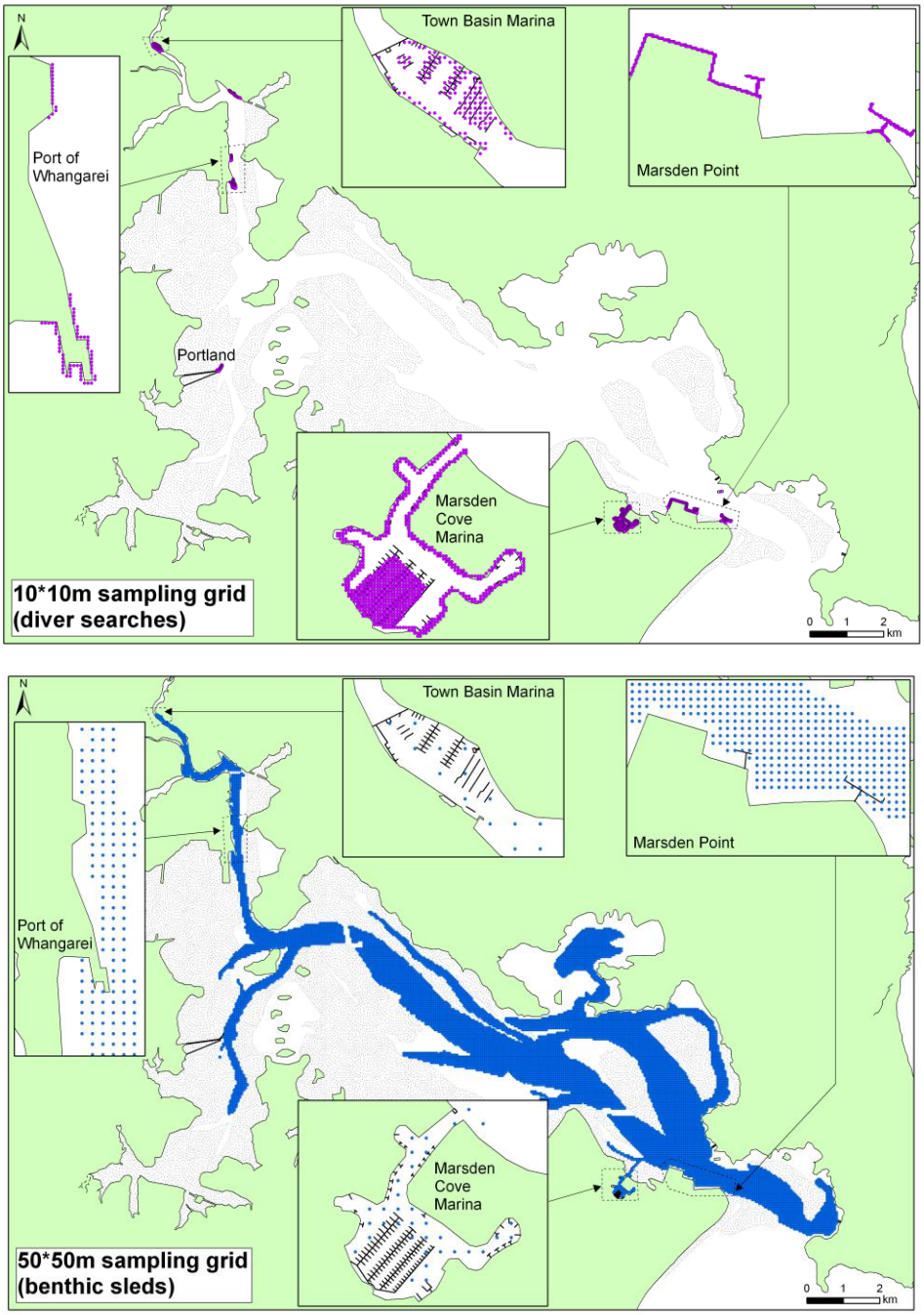
Allocation of sampling effort in the present programme follows the strategy used in previous programmes (Inglis *et al*. 2006, Morrisey *et al*. 2007). Survey plans were developed for each sampling method and harbour based on the known distribution of habitat for the target species and outputs from the hydrodynamic modelling. We originally anticipated combining the predictive habitat models with the outputs from the plume dispersal simulations in each harbour to identify risk zones in each harbour (the habitat and hydrodynamic modelling are described by Inglis *et al*. 2006). The area of each habitat in each risk zone (the "search area") could then be determined and detection limits estimated quantitatively for each zone. However, the major constraint to achieving this was the limited availability of spatially explicit data on the key environmental variables needed to project the predicted habitat distributions in each harbour. Instead, we allocated sampling effort based predominantly on the results of the hydrodynamic modelling (Appendix 4) and our existing knowledge of the distribution of habitats in each harbour, with highest priority given to suitable habitat for the target species within the predicted dispersion plume. Each harbour was subdivided into large strata (3-4 per harbour) that reflected broad environmental gradients (e.g. head/entrance of the harbour) and the concentrations of particles simulated in the hydrodynamic modelling. Generally, ~60% of locations surveyed were allocated to the stratum where moderate to high weighted mean concentrations of simulated particles were predicted, with the remainder distributed among the remaining strata.

Because marine organisms are typically aggregated in their spatial distribution, they tend to be absent from, or in comparatively low abundance at most locations and in large densities in relatively few places (Gray 2002). This pattern is even more extreme for the small founder populations of introduced species, which, at least initially, are likely to be absent from most areas and to occur in aggregations at relatively few locations (Gaston 1994). For example, during the initial stages of its invasion of Port Phillip Bay, Australia, the seastar *Asterias amurensis* was found at only two out of more 70 locations surveyed in the bay (Garnham 1998). This pattern of distribution – locally abundant, but geographically restricted founder populations – suggests that, in most instances, the probability of detection within locations where the species is present is likely to be greater than its expected rarity among locations. Since eradication and control efforts are likely to be most successful when infestations are relatively localised, surveys that optimise the number of locations surveyed will stand the best chance of detecting founding populations with aggregated distributions (Green & Young 1993). Thus, given limited resources, surveying a relatively large number of discrete locations using rapid sampling techniques is likely to be more effective than intensive searches of a few

key locations (although there will be a point at which the survey sensitivity is compromised by under-sampling at each location). This basic assumption - the need to sample a large number of survey locations in each harbour - formed the foundation for our choice of survey methods.

Within each harbour, a grid was overlain on the areas to be sampled in GIS (10-m grid-cell size for highest risk areas, such as wharves and marinas, and 50-m grid-cell size in other areas: Fig. 3). The individual locations surveyed within each habitat type and stratum were then dispersed uniformly across the grids. Sampling locations were offset by one grid cell for each subsequent survey, so that no location will be sampled more than once over the course of the surveillance programme. These predetermined locations were exported from GIS as map coordinates and loaded into GPS units to allow the field teams to locate positions in the field. Where a preassigned location could not be sampled because of constraints such as the depth of water, presence of a vessel on a berth, or source of danger to the field team (such as areas of high vessel movement), a new location was chosen (referring to maps of past sampling locations to ensure that locations were not inadvertently resampled) and its location recorded and later mapped in GIS.

Figure 3 **Example of (upper) the 10x10-m grid used to allocate sampling locations in highest-risk parts of a survey area (Whangarei is used here as an example) and (lower) the 50x50-m grid used in other parts of the survey area. Each dot in the figures represents a grid cell.**



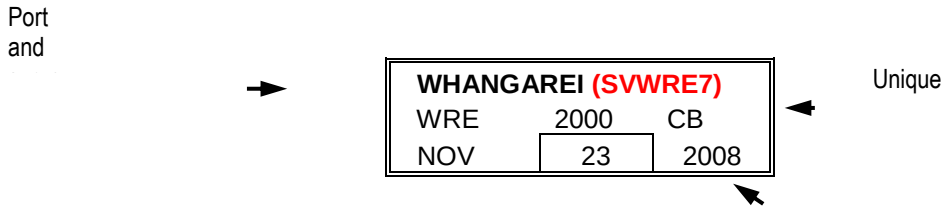
SAMPLE LABELLING AND PROCESSING

A documented labelling and audit system for biological samples collected during each survey was developed at the start of the first phase of this programme. It proved to be very effective and provided traceability of samples/specimens from collection to identification. It included the use of standardized recording sheets for each sampling method used and log sheets for material retained for subsequent identification (for both the biological material, material subsampled for DNA analysis, and any photographs taken of the material at the time of

collection). Recording and log sheets were formatted in Microsoft Excel, and data were transcribed to Excel spreadsheets at the end of each survey. Data recorded for each sample included date, time, precise location (including GPS coordinates), method of sampling, numbers of target and selected non-target species collected, individual identifying numbers for any material retained, and environmental data. This system will be retained for the proposed study and will be formally documented in the Design Report for MAF BNZ's approval. Collection and recording of environmental data will include the items listed in Table 10 of the contract. Electronic data recording devices (Hewlett Packard iPAQ hand-held computers) were used during the related port baseline surveys (ZBS2000-04) and their use will be trialled the present study and will be used if they prove reliable and reduce time needed for recording. Copies of the data sheets to be used in the present phase of the programme are included in Appendix 5.

Sample labelling

All samples are sorted on site and any specimen to be retained (all primary target species, representative samples of *Didemnum* sp. and *Eudistoma elongatum*, and any suspicious individuals whose identity is uncertain) is allocated a label (see below) with a unique identifying number (the "sample lot code" including the identity of the port) and placed, with the label, in an individual container for return to the laboratory. The sample lot code is recorded on the sample data sheet against the sample in which it was found, linking the specimen(s) to its exact location and date of collection (which are included on the data sheet – see Appendix 5). Sample lot codes are pre-allocated for each survey so that their format is consistent among surveys and there is no possibility of duplication of codes among or within surveys. The sample lot code, date of collection, method of sampling, sample number, number of specimens retained and a description of the specimens (minimally the relevant taxon) are also recorded on a field sample lot register sheet (Appendix 5.1), providing a list of all specimens retained during the survey in question, by date and type of sample (crab trap, sled, etc.).



Sample processing

At the end of each day, all specimens retained are returned to the field laboratory and their labels and sample lot codes checked against the sample register. Where the sample container contains more than one taxon, specimens are separated into taxa and placed in separate containers (suitable for intermediate-term storage – i.e. until they are processed by MITS) with a label bearing the sample lot code and a 2-letter taxon code (which will thereafter form part of the unique identifier for that specimen). Specimens are preserved in the chemical appropriate to that taxon (the team member responsible for sample processing is provided with a list of the appropriate fixative and preservative to use with each taxon), and all samples are entered into a sample record sheet (Appendix 5.5), showing the number of individuals of each taxon present in that sample (as identified by the sample lot code).

Taxon-specific methods have been developed for fixing/preserving specimens of target and non-target species (Table 9). Note that specimens will be transferred to the appropriate long-term preserving agent by MITS.

Table 9 Methods for fixing/preserving specimens of target and non-target species collected during surveillance surveys.

Fixing/preserving agent	Taxon	Notes
5% formalin	Algae except bladed red forms	
10% formalin	Ascidians (colonial)	Relax first in menthol and photograph
	Brachiopods	
	Ctenophores	Photograph
	Ectoprocts	
	Fish	Photograph
	Hydroids	
	Jellyfish	Relax first in menthol and photograph
	Nudibranchs	
	Sea anemones	Relax first in menthol and photograph
	Worms	
80% ethanol	Ascidians (solitary)	Photograph
	Bryozoans	
	Crustaceans	
	Echinoderms	Photograph holothurians
	Hard corals	
	Molluscs (no shell)	Relax first in menthol and photograph
	Molluscs (with shell)	
	Soft corals	Relax
	Sponges	Photograph
Other	Red bladed algae	Press. Keep piece for DNA analysis
		(clean off epiphytes, wrap in tissue
		and place in bag with silica gel).

Sample reporting and despatch to MITS

Any suspected Unwanted Species (primary target species, excluding *Styela clava*) or suspected non-indigenous or cryptogenic species not previously recorded in New Zealand will be reported as soon as possible (and within 48 hours) by the field team leader to one of the project leaders (Graeme Inglis or Don Morrisey) who will, in turn, inform the MAFBNZ Exotic Diseases hotline (0800 80 99 66) and the MAF BNZ Biosecurity Surveillance Group Manager (again, within 48 hours of discovery). In the event that the field team leader is unable to contact either of the project leaders within 48 hours, they will contact the hotline and MAF BNZ Group Manager directly. MAF BNZ will issue a submission number to be attached to the specimen (in addition to its existing unique NIWA identifier) and will alert MITS that it is to be dealt with as a priority.

Samples reported via the MAFBNZ hotline will be despatched to MITS as soon as possible. MITS will classify these samples as *urgent* and, where possible, will log them, send them to the relevant taxonomist, and receive an identification back within 48 hours[6]. The person despatching the samples will inform MITS when they have been sent and provide the name of a field-team contact person, and will include a copy of the sample register with the specimens. An electronic version of the sample register will be sent as soon as possible.

[6] Email correspondence between Brendan Gould (MAFFBNZ) and Shane Ahyong (MITS) 24 July 2008.

All other specimens are then submitted to MITS as soon as possible, and within a week of completion of fieldwork (this allows for travel back to base from remote ports, completion of sample logging, packaging and despatch). If despatch is likely to be delayed beyond a week, MITS and the project leader(s) are to be informed of the delay and advised of the likely date of despatch. This allows for samples to be held until all sampling is completed when this is not possible within the main block of field work, so that all samples from the survey can be despatched together. The person despatching the samples will inform MITS when they have been sent and provide the name of a field-team contact person, and will include a copy of the sample register with the specimens. An electronic version of the sample register will be sent as soon as possible. MITS will treat these samples as *priority* and aim for a 1-week turnaround (from receipt of sample to receipt of identification).

All shipments will need to be accompanied by dangerous-goods documentation appropriate to the preserving chemicals used.

Contact and delivery details for MITS

Delivery details:
Serena Cox
NIWA Marine Invasives Taxonomic Service
NIWA
301 Evans Bay Parade
Greta Point
Wellington
NEW ZEALAND

Contact details:
s.cox@niwa.co.nz
Phone: 04-386-0300 (ext 7364)

Special Requirements:
Please provide MITS with as much advance notice of the dates of fieldwork as possible, to allow preparation.

Data entry and archiving

Data recorded on the field sheets are entered into an Excel spreadsheet (designed in the same format as the datasheets) and checked (not by the same person who entered them). Coordinates of all sampling locations are then mapped in GIS (ArcView) and all data are imported to a Microsoft Access database for final storage. All files are stored on the project server at NIWA's Greta Point, Wellington campus and are backed up daily. GIS data are currently georeferenced to WGS84 but will be converted to NZGD2000 before being provided to MAFBNZ, along with the Access database.

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Appendices

APPENDIX 1: LETTER SENT TO STAKEHOLDERS

Fields highlighted in yellow are replaced with appropriate text for each survey at each location.

Targetted surveillance for non-indigenous marine species in New Zealand,

PORT NAME MONTH YEAR

We propose to carry out this survey during the period INSERT DATES. The work will cover the whole of the harbour, including INSERT NAMES OF PORT/WHARF AREAS TO BE SAMPLED.

Background to the survey

The survey is being done by NIWA with funding from Ministry of Agriculture and Forestry Biosecurity New Zealand (MAF BNZ), and repeats the surveillance work done in 2002-2004 at ports around the country. This project provides surveillance for a group of potentially invasive marine animals and plants that MAF BNZ believes present a significant threat to New Zealand. One of them – the sea squirt *Styela clava* – is already present in New Zealand, and in this case the project will monitor its spread). These surveys will be repeated at six-monthly intervals.

Sampling methods

We will be sampling by setting traps for crabs and starfish, dredging for animals on the seabed using a small (1-m wide mouth) scallop dredge, and diving to inspect wharf piles, walls and rocky shores. **All access to port areas will be from the water**, using vessels of 4-6 m length, equipped with VHF radio. We will inform PORT NAME Harbour Radio whenever we enter and leave port areas. INSERT NAME OF ANY MARINAS TO BE SAMPLED will be accessed by boat or from the shore (pontoons). NIWA staff will not board any boat berthed in the marina at any time. ADD INFORMATION RELEVANT TO ANY OTHER STAKEHOLDERS THIS WILL BE SENT TO

- Crab and starfish traps will be deployed on lines with anchors and a marker buoy for periods of 24 hours. Buoys bear NIWA's name and contact telephone number.
- All traps will be deployed away from shipping lanes and will only be deployed on berths when the notice of shipping movements on the INSERT NAME OF PORT AUTHORITY website indicates that the berth will be empty during the period of deployment. We will contact PORT NAME Harbour Radio just prior to deployment to confirm that there have been no changes to advertised shipping movements. Traps in marinas will be placed so that they do not interfere with the movements of vessels. If there is any doubt about deployment we will contact the Marina Manager.
- Dredging and diving around port areas will also avoid shipping lanes, and diving on wharf piles and walls will be timed to avoid shipping movements or the presence of ships on berths. A support boat showing a dive flag will accompany the divers. Again, we will confirm with PORT NAME Harbour Radio prior to

starting to sample. In the marina a surface observer with a dive flag (either in a boat or on the pontoons) will monitor the diver and warn vessels that there is a diver in the water.

We are very grateful to PORT NAME, the marinas and their staff for their cooperation with this project. If you have any questions regarding any aspects of the work, please do not hesitate to contact:

The field-team leader, ADD YOUR NAME, DDI AND EMAIL,

NIWA programme leaders
Don Morrisey, telephone 03-545-7744, email d.morrisey@niwa.co.nz
Graeme Inglis telephone 03-348-8987, email g.inglis@niwa.co.nz

or

MAF BNZ contact
Brendan Gould telephone 04 819 0548, email Brendan.Gould@maf.govt.nz

APPENDIX 2: SUMMARIES OF THE HABITAT ASSOCIATIONS AND LIFE HISTORIES OF THE TARGET SPECIES

Northern Pacific seastar (*Asterias amurensis*)

General information

The northern Pacific seastar, *Asterias amurensis*, naturally inhabits the northern coast of China, the coasts of Korea and Japan, and along the Russian coast to the Bering Strait. It is also found occasionally in Alaska and northern Canada (Morrice, 1995). Its distribution has since increased to several other countries, including Australia. (http://www.fish.wa.gov.au/hab/broc/marineinvader/marine01.html).

Fully-grown seastars reach sizes of 40-50 cm in diameter, with reproduction possible at 10cm, when the seastar is around one year old (CRIMP, 2000). The seastar can increase its diameter by 8cm each year. (http://www.fish.wa.gov.au/hab/broc/marineinvader/marine01.html). Increasing size is also a response to food. When food is short the seastars shrink: their sexual organs also shrink which reduces fertilisation success (http://www.marine.csiro.au/PressReleasesfolder/99releases/seastar4jun99/backgrnd.html#gaps).

Timing of reproduction and recruitment

In the southern hemisphere, spawning occurs during winter (July-October) when temperatures are around 10 to 12 ^{o}C. Fertilisation takes place externally (http://www.parliament.vic.gov.au/enrc/ballast/Ballast-30.htm). Small eggs of approximately 150µm in diameter hatch, and develop into free-swimming larvae through a series of stages - coeloblast, gastrula, bipinnaria and brachiolaria (Bruce, 1998). A single adult female seastar can produce 10-20 million eggs each year for about 5 years. Both the eggs and larvae are planktonic, drifting in the ocean for up to two months before they settle and metamorphose into juvenile seastars. (http://www.parliament.vic.gov.au/enrc/ballast/Ballast-30.htm). Based on this 60-day larval period, settlement in Australian waters has been shown to occur during mid-September (Parry *et al*. 2001). The northern Pacific seastar lives for up to five years. It is known to reach outbreak proportions that occur in three to ten year cycles, and which last two to three years (http://www.fish.wa.gov.au/hab/broc/marineinvader/marine01.html).

Habitat and biology

Morrice (1995) suggests that in Tasmanian studies, it is unclear whether the northern Pacific seastar is present in areas due to specific habitat requirements or whether their location is dependant on their rate of spread.

Substratum type

The preferred substrata for *A. amurensis* are mud, sand or pebbles (http://www.fish.wa.gov.au/hab/broc/marineinvader/marine01.html), extending to a mixture of rock, algae and seagrass (Morrice, 1995). It is rarely found on reefs or places subject to high wave action.

However, a benthic habitat is not essential - in Tasmania, both adults and juveniles have been recorded attached to scallop longlines, mussel and oyster lines, salmon cages and spat bags (http://www.fish.wa.gov.au/hab/broc/marineinvader/marine01.html). Research has shown that substratum seems important for the induction of settlement and metamorphosis - brachiolaria have shown high rates of settlement on non-geniculate coralline algae, followed by rock and mud. Sand and mussel shell did not induce settlement well. Bacterial cover on mussel lines, accompanied by the fine algae that grows on the ropes, may also provide a very attractive settlement surface (Morris & Johnson, 1998).

Food preferences

The seastar is a predator of many organisms but has a particular preference for shellfish (http://www.fish.wa.gov.au/hab/broc/marineinvader/marine01.html). Other prey include sponges, crustaceans, polychaetes and fish (http://www.parliament.vic.gov.au/enrc/ballast/Ballast-30.htm), as well as tunicates, bryozoans and echinoderms (Morrice, 1995).

Physiological tolerances (range and preferences)

Temperature

The seastars prefer water temperatures of between 7 and 10 ^{o}C in their natural range (http://www.fish.wa.gov.au/hab/broc/marineinvader/marine01.html), but can tolerate a range of 5 – 20^{o}C. In Japan, water temperatures above 20^{o}C limit the seastars' range, with adults losing weight and larvae dying above this temperature (Morrice, 1995, Bruce, 1998). The survival of larvae is temperature dependant, with the optimal range being between 8 to 16 ^{o}C (Bruce, 1998). However, adult seastars have been shown to adapt to warmer temperatures of up to 22 ^{o}C in countries outside their natural range, such as Australia (http://www.fish.wa.gov.au/hab/broc/marineinvader/marine01.html).

Depth

The seastar is mainly found in sublittoral to subtidal areas, but can also be present at depths of up to 200m

(http://www.fish.wa.gov.au/hab/broc/marineinvader/marine01.html). In Australia, it occurs in the intertidal zone down to a depth of 25m (CRIMP, 2000). Parry & Cohen (2001) have observed that in some parts of Port Philip Bay, the density of the seastar decreases at depths of <15 metres. Morrice (1995) states that in the northern Pacific, the seastar inhabits deeper water in the summer and moves into shallower water in the winter. This may be to survive summer temperatures and to move between areas.

Salinity

Little research appears to have been conducted on salinity tolerances of the northern Pacific seastar, but adults seem to be restricted to salinities above 28 psu (Morrice, 1995). In general, the seastar is sensitive to any changes in salinity and as a result is unlikely to tolerate fluctuating salinities (http://www.fish.wa.gov.au/hab/broc/marineinvader/marine01.html).

Optimal salinity for larval survival is 32 psu. The larvae become adversely affected by 10 minute exposures to salinities <17.5 psu and do not survive exposures to salinities <8.55 psu, when extensive cellular damage has been found to occur (Bruce, 1998).

Route of introduction

The most likely route is as seastar larvae contained in the ballast water of international vessels, although research suggests that 'sea chests' are another potential method of transport (Dodgshun & Coutts, 2002). Juvenile seastars found on mussel lines in Port Philip Bay, Australia, indicate a further risk of spread (Garnham, 1998).

Methods of sampling

- Parry & Cohen (2001) used a 2.7 m wide peninsula scallop dredge, covered by 25mm mesh to sample *Asterias*. Estimates of field densities were based on the number of seastars collected in a 60 second tow at a speed of 5.7 +_ 0.3 knots. The average tow length was around 170 m (Parry & Cohen, 2001).

- Whayman/Holdsworth seastar traps have been designed to catch *Asterias*. Traps with a mesh size of 26mm catch more seastars than larger mesh (65mm) traps. Most seastars are caught within the first 24-48 hours. Pilchards are the more attractive bait but only for short soak times (24-48 hours). The traps effectively fish an area of approximately $30m^2$ (Martin, 1998).

- Vertical distribution of larval asteroids can be measured using vertical tows of a 100µm free-fall plankton net with a 500mm diameter mouth and 5m in length. A choking bridle closes the net when hauled. Vertical tows are undertaken to depths of 5m, the depth at which the net completely submerges, or 15m. A small float can be tied to the end of the plankton net by 10m of fine line – when this submerges, the net has reached the appropriate depth of 15m (Parry *et al*, 2001).

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Asian clam (*Potamocorbula amurensis*)

General information

The Asian clam, *Potamocorbula amurensis*, is a native of estuaries from southern China (22° N latitude) to southern Siberia (53° N) and Japan (Cohen & Carlton, 1995). However, it has extended its range to establish abundant populations in California, USA, particularly San Francisco Bay. Asian clams are euryhaline at all stages of development, and reach settlement 17-19 days after fertilisation (Nicolini & Penry, 2000).

Timing of reproduction and recruitment

Field studies in San Francisco Bay suggest that the clam spawns throughout the year, although site-specific seasonal reproduction appears to be related to food supply (Parchaso & Thompson 2002). The eggs are negatively buoyant, so fertilisation and initial development occur in more saline bottom waters. It takes 48 hours for development to the straight hinged larval stage through several life phases – fertilised egg, two-cell stage, four-cell stage, blastula, trochophore). At 17 – 19 days after fertilisation, the bivalve settles at a shell length of approximately 135 μm. Newly settled clams can reproduce within a few months (Nicolini & Penry, 2000). Juvenile clams studied in San Francisco Bay had a mean shell length of 1.7 mm. By the time they were under a year old, shell length was approximately 11 mm (Cohen & Carlton, 1995). Adults generally reach a length of 20 – 30 mm (NZ Ministry of Fisheries, 2001).

Studies in San Francisco Bay have shown that the clam displays a complex picture of patchy recruitment in space and time, which is expected for an invasive eurytopic species (Carlton *et al*. 1990). The zone of greatest recruitment shifts dramatically with changes in flow - high riverine outflow conditions may reduce clam densities, but the clams are quick to repopulate brackish water habitats when high flows abate (Peterson, 1998).

Habitat and biology

Substratum type

The Asian clam is pervasive with regard to habitat. It can invade environments which are nearly freshwater, creeks and sloughs, intertidal sand-mud flats, and on a wide range of subtidal soft bottomed substrata - flocculant mud, coarse sand, peat and hard clay (Carlton *et al*, 1990). It typically sits with one-third to one-half of its length exposed above the sediment surface. (Cohen & Carlton, 1995). It has been found in very high densities in the benthic layer in the majority of San Francisco Bay estuary, at up to 48,000 individuals.m^{-2} (Peterson, 1998). Research in laboratory aquaria has shown that its behaviour can lead to the formation of depressions in the underlying substrate, which can significantly disturb sediment layers to a depth of about 1cm. The highly altered, complex surface left behind may cause difficulties for other mobile and sedentary infauna, thus allowing the clam to dominate (Carlton *et al*, 1990).

Feeding

Potamocorbula amurensis is an efficient suspension feeder (Thompson *et al*, 1991). Examination of faeces from specimens collected in San Francisco Bay show that the clam ingests both planktonic and benthic diatoms. It also filters bacterioplankton as well as phytoplankton, though at lower efficiency, and assimilates both with high efficiency. Laboratory experiments have shown that the bivalve can also readily consume certain copepod nauplii (Kimmerer *et al* 1994). Other research suggests it may feed on the larvae of other benthic organisms (Cohen & Carlton, 1995).

Physiological tolerances (range and preferences)

The Asian clam is one of the few species of bivalves able to tolerate virtually any salinity, withstand tropical or cold temperate waters and survive in polluted environments. Research in San Francisco Bay suggests that the Asian clam has spread rapidly, irrespective of sediment type, water depth and salinity (Thompson *et al*. 1991). The following information highlights the wide range of physiological tolerances that this species displays.

Temperature

Their latitudinal range in Asia suggests that Asian clams can survive a temperature range of 0 – 28ºC (Cohen & Carlton, 1995). There is very little information for *P. amurensis*, but data for the similar Chinese corbulid *P. laevis* (found at approximately the same latitude as San Francisco Bay) suggest that gametogenesis requires water temperatures ranging from 12 – 23ºC. Reproductively active *P. amurensis* have been seen in San Francisco Bay in water temperatures ranging from 6 – 23ºC (Parchaso and Thompson 2002). Fertilised eggs of *P. laevis* are shed at temperatures of between 16 and 20ºC. Growth rates are greatest when water temperatures are between 22 and 28ºC. Growth rates decline below 17ºC, and growth ceases below 11.8ºC (Carlton *et al*, 1990).

Depth

The clams live both subtidally and intertidally (Cohen & Carlton, 1995), but primarily subtidally (Carlton *et al*, 1990).

Salinity

The Asian clam can survive in a range of salinities from almost freshwater (< 1 psu) to full-strength seawater (32 – 33psu) (Cohen & Carlton, 1995, Carlton *et al*, 1990, http://www.fish.wa.gov.au/hab/broc/marineinvader/marine08.html) but long-term survival of adults is highest at salinities from 5 to 25 psu (Nicolini & Penry, 2000). Spawning and fertilisation can occur at salinities from 5 – 25 psu, with a maximum at about 10 – 15 psu. Eggs and sperm can tolerate at least a 10-psu step increase or decrease in salinity. Studies have shown that fertilisation and initial development tend to occur in the more saline bottom waters of San Francisco Bay. Embryos of two hours old have been shown to tolerate salinities from 10 – 30 psu, and at 24 hours old they can tolerate the same wide range of salinities that adult clams can. However, any *rapid* changes in salinity may adversely affect larval growth (Nicolini & Penry, 2000).

Route of introduction

The initial introduction of the Asian clam to San Francisco Bay seems to have been as veliger larvae transported in ballast water by trans-Pacific cargo ships. The clams' ability to tolerate wide changes in salinity suggests it can survive incomplete oceanic exchanges of ballast water (Nicolini & Penry, 2000). The infaunal habitat of the clam suggests that it did not arrive as a fouling organism (Carlton *et al*, 1990).

Methods of sampling

- Carlton *et al*. (1990) described a combination of sampling devices that were used to sample *Potamocorbula*, including a modified Van Veen grab, a Ponar grab and a Van Veen grab, that sampled between 0.05 and 0.1 m^2 of sediment. Samples were sieved through screens of 0.5 mm to 1mm mesh size. Between 3 to 5 replicate grabs were taken at each sampling station.

- Peterson (1998) describes an extensive survey for *Potamocorbula* in San Francisco Bay using a Ponar grab.

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Chinese mitten crab (*Eriocheir sinensis)*

General Information:

The Chinese mitten crab *Eriocheir sinensis* is a burrowing crab native to mainland China and coastal rivers and estuaries of the Yellow Sea. It is a palm-sized greyish- brown grapsid crab with small white pincers protruding from hairy brown claws. The native range of the mitten crab extends from the southern border of North Korea (40^{o}N latitude) to Hong Kong (22 ^{o}N). It has established introduced populations in Vietnam, northern Europe and the west coast of America. The first specimens to be found in Europe were reported from near Hamburg in Germany 1912 (Panning 1939). Since then, mitten crabs have spread from Finland to the Atlantic coast of southern France and to the UK, Russia, Holland, Belgium, the Czech republic, Denmark, Sweden, France, Poland and Portugal and Spain. The first reported occurrence of the mitten crab in North America was in the Detroit River in 1965 by the city of Windsor, Canada. Later, in 1973, commercial fishermen netted several crabs in Lake Erie near Erieau and Port Stanley, Ontario, Canada (Nepszy & Leach, 1973). In June 2006 a specimen was caught in Chesapeake Bay (SERC 2004). On the west coast, it was first reported from San Francisco Bay in 1992 where it has since become well-established (Halat & Resh 1996). Ballast water introductions have been blamed, but speculation also exists about possible deliberate release into the U.S.A.

Eriocheir sinensis is a catadromous species that lives most of its life in freshwater environments. Mature males and females migrate during late summer to tidal estuaries where they mate and spawn. Adults (Maximum body size 10-cm carapace width, but more commonly between 5 and 8 cm) are capable of very long distance migrations e.g. over 1000km in the Yangtze River (Cohen & Carlton 1995). After mating the females are thought to continue seaward, over-wintering in the deeper water and returning to brackish water in the spring to hatch their eggs (Panning 1939). The movement of crabs to deeper water and the timing of egg hatching/larval release is temperature dependent. Winter temperatures are much colder in Europe than San Francisco, which is probably why crabs there move to deeper water and why hatching is delayed until spring. In the San Francisco Estuary, preliminary data indicate that the adult crabs remain in the spawning areas (~ 20psu) and hatching occurs in November/December and again in March. The timing of hatching varies yearly depending upon winter water temperatures. Settled juvenile crabs gradually move upstream into brackish (1-5 psu) and fresh water to complete the life cycle.

Mitten crab ‘plagues’ of extreme numbers have been reported from Germany in the mid 1930’s (Panning 1939) and in the Netherlands in 1981 (Ingle, 1986). Adults are capable of emerging from water and crossing dry land when migrating.

Timing of reproduction and recruitment

Crabs mature at different ages according to locality. Maturity has been reported at ages of 3 to 5 years in Europe (Panning 1939), 1 to 2 years in China (Cohen & Carlton 1995) and 2 to 3 years in California (Veldhuizen and Stanish 1999. Each female produces from 250,000 to 1 million eggs, which hatch in late spring or early summer. In laboratory culture, the larval period lasts for 1 –2 months and the larvae develop through five zoeae and a megalopa stage (Kim & Hwang 1995). After the final larval moult the juvenile crab settles to the bottom in late spring and begins its migration upstream (Panning, 1939; Ingle, 1986; Anger, 1991). Experiments indicate that complete development of larvae is not possible in rivers or in brackish estuarine conditions (Anger 1991).

Habitat and Biology:

Substratum type

The normal habitat of the juveniles is the bottoms and banks of brackish and freshwater rivers and estuaries, individuals prefer hard bottoms and areas covered with submerged plants (Nepzy & Leach 1973). Older juveniles are found in a diversity of habitats including silt, gravel, and open unvegetated stream channels. In freshwater habitats of San Francisco Bay, *E. sinensis* is most common in areas with steep, vegetated banks that are high in clay content. Burrows are concentrated underneath the root profile of the aquatic macrophytes lining the banks, which mainly consists of *Scirpus* (Halat & Resh 1996). Submerged aquatic vegetation is an important component to the habitat. It provides cover and high concentrations of invertebrates (Veldhuizen 2000).

In Asia and Europe mitten crabs live in burrows dug in river banks or in rice paddies in coastal areas (Cohen & Carlton 1995). Young mitten crabs are found in tidal freshwater areas and usually burrow in banks and levees between high and low-tide marks. Optimal rearing habitat for juveniles is areas with still or slow velocity water, a stable water depth, low turbidity, and warm temperatures (ranging from 20ºC to 30ºC, with optimal growth at 24ºC to 28ºC) (Veldhuizen 2000). Mitten crabs apparently do not burrow as extensively in non-tidal areas. Older juveniles are found further upstream than young ones and both adults and juveniles can move hundreds of km.

In China, recently settled juvenile mitten crabs are harvested during spring tides in late May and June when they congregate over sandy bottom areas in water of 1 to 3 $^{0}/_{00}$ (Hymanson *et al*. 1999)

Food preferences

The mitten crab is known to be predominantly an omnivorous, opportunistic feeder, although feeding habits change as they mature. Juvenile crabs mainly eat vegetation (Halat & Resh 1996) primarily filamentous algae (Veldhuizen & Stanish 1999). As they mature they also prey on small invertebrates, especially worms and clams so that adults and juveniles are considered omnivorous (http://www.wsg.washington.edu). Gut content analysis of crabs in the San Francisco Bay area revealed a high proportion of vegetative matter, with low amounts of invertebrates, regardless of the size of the crab or the habitat from which it was captured (Rudnick *et al*. 2000).

Vegetation type

Juveniles were observed taking cover in floating vegetation, especially water hyacinth in the USA (Hieb & Veldhuizen 1998). An ongoing study by Veldhuizen is currently assessing habitat

associations for this crab in the San Joaquin Delta, but results are presently unavailable. In Asia, the juveniles can be associated with rice paddies (Panning 1939). An attempt to characterise habitat associations of mitten crabs in the San Joaquin River in 2000 failed to capture any individuals (May & Brown 2000).

Physiological Tolerances (range and preferences):

Temperature

Adult mitten crabs exhibit a wide range of temperature tolerances. Growth ceases only at temperatures below 7°C and above 30°C (Rudnick *et al*. 2000). All larval stages of the Chinese mitten crab show a clear preference for warm water, however (15° to 18°C), and temperatures below 12°C do not allow any development beyond the first zoeal stage in the laboratory (Anger 1991). Adults can tolerate temperatures as low as 0 °C for a week and temperatures up to 31 °C are suitable for juveniles (Veldhuizen & Stanish 1999).

Depth

Juvenile mitten crabs appear to occur mostly in shallower waters (i.e. < 10m) (Veldhuizen & Stanish 1999, preliminary results), with largest densities found in areas with an average depth of 2 m, which corresponds to the depth of submerged aquatic vegetation (Veldhuizen 1999). However, through the winter sexually mature females are thought to move to "deep" water to develop their fertilised eggs. Adult mitten crabs are highly tolerant of desiccation and are able to remain on land for several hours without mortality. Veldhuizen (pers. comm.) compared the relative abundance of juvenile mitten crabs among six different habitat types - shallow (0-2.4 m) vegetated natural substrate, shallow unvegetated natural substrate, shallow vegetated rock substrate, shallow non-vegetated rock, mid-depth channels (2.5 – 4.9 m), and deep channels (5 - 10 m) - that occurred in a tidal freshwater marsh. Crabs occurred in all habitat types, but were overall more abundant in shallow (0 to 2.4 m) vegetated areas with natural substrate. Most of the crabs ranged in size from 20 to 38 mm, average size was 28 mm.

Salinity

Juvenile and adult Chinese mitten crabs are extremely euryhaline (i.e. high range of tolerated salinities) and its osmoregulatory abilities appear well developed (Onken 1996). By hyper-regulating the ionic content of their body fluids, the crabs can quickly adapt from high to low salinity environments (Welcomme & Devos 1991 cited in Rudnick *et al*. 2000). Different larval stages are known to vary in their salinity tolerances. The first zoeal stage, which occurs in seawater, is strongly euryhaline, but successive zoeal stages become increasingly stenohaline (low range of tolerated salinities) and prefer more typical marine salinities (e.g. >30 psu). The megalopa, which migrates to freshwater, is euryhaline, with an optimal growth response in brackish waters (5-25 psu) (Anger 1991). Salinities in the areas where *E. sinensis* has been found range from 0-5 psu in San Francisco Bay (Halat & Resh 1996). It cannot spawn in fresh water and larval growth cannot go to completion in rivers or brackish waters (Anger 1991). Mating and fertilisation in the San Francisco estuary occur in late autumn and winter, generally at salinities of 15- 20 psu. In China, most mating occurs in brackish water (10 – 16 psu) (Hymanson *et al*. 1999). A large increase in the abundance of this species in England coincided with a drought and a large change in the salinity of the estuaries they occupied (Atrill & Thomas 1996).

Methods of Sampling:

Methods of sampling mitten crabs need to differ between adults and juveniles to reflect their different diets and habitats. Adults migrate downstream in late summer to spawn. These crabs are sexually mature. Only juveniles migrate upstream. Juveniles are found in creeks, rivers, and tidal freshwater and brackish marshes and sloughs. Juveniles burrow and occupy burrows but also remain in the subtidal zone.

- Panning (1938) found that because juveniles are mostly vegetarian, capturing them with baited traps didn't work and they had to be excavated from their burrows during low tides. Capturing juveniles in the USA has involved intertidal searches at low tide where all cavities such as burrows and root tunnels were excavated and all debris, driftwood and small puddles were examined. Juveniles were also successfully captured in 'crab condos', submerged artificial structures of PVC tube used for shelter.

- A comparison of trapping techniques by Veldhuizen *et al* (1999) suggested that traditional crab sampling techniques are not very effective for this species due to the change in diet between juveniles and adults, the diversity of habitats occupied, and their escape tendencies. For juvenile crabs she recommends using artificial shelter substrates ("crab condos") made of 12 vertical PVC tubes (6 in long, 2 in diameter) and burrow searches for juveniles in the banks of silty, tidally influenced streams. Crab condos are typically submerged for 48 hours to allow the crabs to enter (Veldhuizen 2000), but significant increases in catch are achieved with longer soak times (3, 5and 9 days).

- Beach seining for adults was possible in shallow intertidal areas and subtidal areas. Baited traps were not recommended for juvenile mitten crab, or for monitoring and detection programs where adult densities may be very low.

- Various other baited traps, snares and ring nets have also been trailed, with variable success. Ring nets are most successful when densities of crabs are high. The crabs appear to be most active in the two weeks surrounding the full moon.

Impacts

The crab has caused numerous problems in Europe when found in extremely high densities. The burrows that it excavates can destabilise river banks and lead to accelerated bank erosion. The sharp claws of *E. sinensis* cut up commercial fish nets, increasing operating costs of fishing operations. The most widely reported economic impact of mitten crabs in Europe has been damage to commercial fishing nets and the catch when the crabs are caught in high numbers. Because of the severe problems the crab has caused in European waters, *E. sinensis* recently has been listed as a federally injurious species in the United States.

The ban on importing live Chinese mitten crabs to the USA was enacted due to concern over potential damage from its burrows to levees or rice fields in the Central Valley, and because the crab is a second intermediate host of a human parasite, the oriental lung fluke *Paragonimus westermanii* (Cohen &

Carlton 1995). The Chinese mitten crab has been widely reported to be an intermediate host for the Oriental lung fluke, a parasite that uses a snail as its primary host, freshwater crayfish and crabs as intermediate hosts, and a variety of mammals, including humans, as final hosts in its life cycle (Chandler & Read 1961; Lapage 1963). Humans can become infected with the parasite through ingestion. The fluke settles in the lungs and other parts of the body, and can cause significant bronchial or, in cases where it migrates into the brain and/or muscles, neurological illnesses. It is believed that no species of snail that is in the family of the primary host currently occurs in Europe, and no appropriate snail host has been found in the San Francisco Bay-Delta system (Clark *et al*. 1988; Veldhuizen & Stanish 1999). Armand Kuris and Mark Torchin of U. C. Santa Barbara found no parasites of any kind in 25 mitten crabs from San Francisco Bay (A. Kuris, pers. comm., 1995).

The potential ecosystem impacts of large numbers of crabs invading new areas are unknown but authors have often speculated on possible effects to benthic invertebrate communities. There is concern that the crab will consume benthic invertebrates, salmon and trout eggs and may affect other species through direct predation or competition for food resources. In England there is some concern that it may compete with the native crayfish in fresh water (Clarke *et al* 1998). In China and Korea Juvenile mitten crabs have been reported to damage rice crops by consuming the young rice shoots and burrowing in the rice field levees. Since *E. sinensis* often inhabit areas that may contain high levels of contaminants, bioaccumulation of contaminants could also be transferred to predators or humans.

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European green crab (*Carcinus maenas*)

General information

The European green crab, *Carcinus maenas*, is native to the Atlantic, Baltic and North Sea coasts of Europe, but has established populations outside this range on the Atlantic and Pacific coasts of North America, in South Africa, and Australia. Green crabs produce planktonic larvae that pass through six developmental stages – a prezoea, 4 zoeal stages, and a megalopa – before metamorphosis to the benthic, juvenile crab phase. The crabs themselves grow through 18 to 20 moult cycles before reaching maximum size and terminal anecdysis (Parry *et al*. 1996). In its native range, the green crab can live up to 5 years and males reach a size of 86 mm carapace width. In western North America, adult males can be up to 92 mm carapace width within 2 years (Grosholz & Ruiz 1996).

Timing of reproduction and recruitment

Green crabs mate after the females moult, usually between spring and autumn. In warmer waters, females carry eggs for around four months. Egg-bearing females tend to migrate into deeper water during winter and prezoeae hatch from the eggs predominantly in spring (http://www.wa.gov/wdfw/fish/ans/greencrab.htm). The prezoeae pass through four zoeal stages in the plankton before moulting into the megalopal stage. Megalopae appear in early-mid summer and metamorphose and settle into the juvenile crab phase in late summer (Parry *et al*. 1996). The average development time for *C. maenas* larvae varies with temperature. At 10ºC development takes around 75 days, and at 25ºC it can take as little as 13 days (Parry *et al*. 1996). The timing of settlement is related to the number of months in which water temperatures are below 10ºC. In cooler waters, settlement occurs in late summer. In warmer waters, megalopae can begin to settle in late autumn (Yamada *et al*. 2001). Settlement occurs predominantly at night around the time of high tide (Zeng *et al*. 1997).

Habitat and biology

Substratum type

In its native range, the Green Crab, *Carcinus maenas*, occurs on both hard (rocky) and soft intertidal and shallow subtidal habitats in semi-exposed soft-sediment bays (Moksnes 2002). In Europe, eastern North America, Australia and South Africa, green crabs occur in protected embayments and on moderately exposed rocky shores. In western North America green crabs occur only in sheltered embayments and only in soft-sediment environments (Grosholz & Ruiz 1996). A recent survey of the distribution of *C. maenas* in southern Australia found crabs in a range of soft-sediment habitats in low energy embayments. Substratum type, depth and water quality were all poor predictors of its presence and abundance in traps set in these habitats (Thresher *et al*. 2003).

Post larvae (megalopae) settle and metamorphose predominantly in shallow (< 1 m) sheltered or semi-exposed areas that have some form of structured habitat that provides shelter from predators (e.g. seagrass, macroalgae, mussels, shell debris, etc). Small crabs are often found in close proximity to vegetation such as beach grass, reeds, and eelgrass, although they also occur in exposed areas such as bare mud. Larger crabs do not need vegetative cover. In Sweden, young crabs are concentrated in greatest densities within structurally complex habitats, such as mussel beds, shell debris, seagrasses

and filamentous algae. Much smaller densities occur in adjacent sand or mud. Densities of juvenile crabs (2^{nd} – 9^{th} instar) are significantly greater in mussel beds and shell habitats (mean = 206 crabs.m^{-2}) than in eelgrass (45 crabs.m^{-2}), filamentous green algae (24 crabs.m^{-2}) or sand (13 crabs.m^{-2}). Settlement of megalopae occurs predominantly to structurally complex habitats such as filamentous algae (231 settlers.m^{-2}), eelgrass (159 settlers.m^{-2}) and mussel beds (114 settlers.m^{-2}), rather than to open sand (4 settlers.m^{-2}), but larger animals redistribute themselves among these habitats. Indeed, adult crabs are highly mobile and are capable of foraging over large areas (km to 10's km).

Food preferences

Green crabs are omnivorous. Adult crabs feed predominantly on bivalves (rank = 1), small crustaceans (rank = 2) and smaller numbers of polychaetes and green algae (rank = 3 to 4) (Grosholz & Ruiz 1996).

Physiological tolerances (range & preferences)

Temperature

Carcinus maenas can tolerate a wide range of temperatures. In its native and introduced ranges, animals can tolerate average summer water temperatures of 22°C and average winter temperatures of 0 °C, although adult mortality has been recorded at sustained winter temperatures of 0 °C or below (Cohen *et al*. 1995). Crabs stop moulting and drastically reduce their activity below 10°C, and stop feeding when temperatures are below 7°C (Yamada *et al*. 2001). Successful embryonic development occurs at temperatures between 11 and 25 °C.

Depth

Green crabs are found predominantly in the mid-intertidal zone, between about 1.3 m to 1.7 m above datum, and shallow subtidal, although adults have been recorded as deep as 60 m (Cohen *et al*. 1995). Juveniles (0-1+ age, 1-20 mm carapace width) are found mainly < 1 m water depth (Moksnes 2002). In Bodega Harbour, California, green crabs were caught between +0.7m and 1.4 m above mean lower low-water, with crabs being most abundant at +1.2 m (Grosholz & Ruiz 1995: see Figure 3). Parry *et al*. (1996) and Thresher *et al*. (2003) report greatest catches of adult *C. maenas* in water depths < 10 m. However, in Sweden, subadults and adults are found commonly between 0.1 to 20 m depth (occasionally to 60 m).

Salinity

Green crabs tolerate a wide range of salinity, but appear to prefer more saline areas (Proctor 1997). Adults reside in water from 4 psu to 34 psu. Populations breed successfully at salinities down to at least 13 psu, although larvae may only settle at salinities above 17 psu (Cohen *et al*. 1995). Survival of eggs to larval stages occurs at salinities between 26 and 39 psu and larval development may be prevented at < 13 psu (http://www.wa.gov/wdfw/fish/ans/greencrab.htm*)*. In the laboratory, adult *Carcinus* prefer salinities of 22-41 psu, but can tolerate maximum salinities of up to 54 psu (Cohen *et al*. 1995).

Methods of sampling

- Standard baited minnow traps (cylindrical with inverted cone entrances of ~ 57 mm) are set near the edge of vegetation or along mud/peat banks, generally far from the low tide drainage channels. Set 5-10 traps with openings perpendicular to the incoming tide with a rock in the trap to hold it in place, and possibly a rock "cradle" made in the substrate to keep the traps from being moved by wave action (http://www.pac.dfo-mpo.gc.ca/ops/fm/shellfish/Green_Crab/FIND.HTML).

- Shore searches along the high tide wrack line where storm driven vegetation accumulates for exuviae of molting crabs. This is most profitable in areas with some vegetation intertidally or subtidally, as molting crabs prefer to have cover available during this vulnerable process (http://www.pac.dfo-mpo.gc.ca/ops/fm/shellfish/Green_Crab/FIND.HTML).

- Yamada *et al*. (2001) compared 4 types of traps for catching *Carcinus*: unbaited pitfall traps, minnow traps, fish traps and box traps deployed in intertidal and shallow subtidal environments. In high intertidal areas, pitfall traps were successful for sampling crabs < 45 mm carapace width. Folding traps and box traps successfully caught crabs > 40 mm. The box traps typically yielded larger catches than other types and caught crabs in their second or third summer.

- Thresher *et al*. (2003) used collapsible box traps (62 cm x 42 cm x 20 cm) to survey populations of *C. maenas* in southern Australia. Traps were typically baited with oily fish and deployed over night for 15-24 hours. Average catch rates from a single overnight set were occasionally as high as 44 crabs.trap^{-1}.

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Mediterranean fanworm (*Sabella spallanzanii*)

General information

Sabella spallanzanii is a large (up to 70 cm length) tube-building polychaete that is native to the Mediterranean and Atlantic coasts of Europe. Introduced populations of *S. spallanzanii* have been recorded in Brazil, and in the southern states of Australia (Western Australia, South Australia and Victoria) where it occurs in large densities attached to a variety of substrata. The worm's tubes are constructed of a tough but flexible material with the outer layer often incorporating deposits of silt and mud. The base of the tube is usually secured to hard substrata such as rocks, jetty pilings or shell fragments (Clapin & Evans 1995), but they may inhabit soft sediments where there are some solid particles (e.g. shell fragments, pebbles) on which the tubes can attach.

Timing of reproduction and recruitment'

Sabella spallanzanii is a gonochoric broadcast spawner that releases strings of mucus containing eggs or sperm into the water column (Giangrande *et al*. 2000). Worms attain sexual maturity at around 50 mm length after 6 months of growth. Spawning is thought to occur in autumn and winter in Victoria (Currie *et al*. 2000), although there is some evidence for summer spawning in Western Australia (Clapin & Evans 1995). Females are highly fecund and can produce >50 000 eggs which appear to be fertilised either internally or in situ (Giangrande *et al*. 2000). The fertilised egg masses are negatively buoyant and sink rapidly to the bottom (Giangrande *et al*. 2000). As the egg membrane disappears, free-swimming trochophore larvae emerge. These larval stages have a planktonic life of up to 21 days before they settle to the adult habitat. Settling larvae are gregarious and new recruits often occur in dense clusters. In Victoria, small worms (10-14 cm length) have been recorded in late November (Parry *et al*. 1996). Larvae spend about 2 weeks in the plankton before they settle and metamorphose (CRIMP 2001), but appear to travel only short distances (<20 km) from their parent stock prior to settlement (Parry *et al*. 1996).

Habitat and biology

Substratum type

Sabella spallanzanii grows preferentially in sheltered, nutrient enriched waters that are not subject to waves (Currie *et al*. 2000). In its native range it occurs predominantly on hard substrata and, in Port Phillip Bay, Australia, it is particularly abundant on man-made hard surfaces such as wharf pilings, channel markers, marina piles, etc. It is not common on the hulls of ships (Giangrande *et al*. 2000). Largest densities occur on hard surfaces between 2 m and 7 m depth (Currie *et al*. 2000). In unconsolidated sediments, *Sabella* occurs in areas where suitable attachment substrata (rocks, concrete, wood, steel, bivalves, ascidians, etc) are present and tends to be aggregated in smaller densities. Although it has become established in most subtidal habitats in Port Phillip Bay, Currie *et al*. (2000) suggest that the larger densities on pilings and artificial hard surfaces reflect a preference for settlement on vertical surfaces.

Feeding

Sabella spallanzanii is a filter feeder that traps suspended food particles using its fan-shaped crown of tentacles. It has apparently been reared in the laboratory on a variety of food, but few details of actual diets are available (Parry *et al*. 1996).

Physiological tolerances (range and preferences)

Temperature

Spawning of *S. spallanzanii* occurs when seawater temperatures range between 11°C and 14°C **(**Giangrande *et al*. 2000). Optimum conditions for growth are at temperatures of between 10-19°C.

Depth

Sabella spallanzanii has been recorded in water depths of 1 m to 30m (Parry *et al*. 1996). In soft sediments, densities tend to be larger at depths of < 7 m, but decline significantly at greater depth (17 to 22 m) (Currie *et al*. 2000). Densities on hard surfaces in Port Phillip Bay generally increased with depth, but were largest between 2 m and 9 m depth (Currie *et al*. 2000).

Salinity

There are few data on the salinity preferences of *S. spallanzanii*. In its native and introduced ranges, it is abundant in sheltered harbours and ports that are subject to fluctuations in salinity, but most studies have been of populations in relatively saline (> 32 psu) waters.

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Aquarium weed (*Caulerpa taxifolia*)

General information

Caulerpa taxifolia is a green single-celled alga (Chlorophyta: order Caulerpales, family Caulerpaceae) native throughout many areas of the tropical Pacific and Caribbean (GISP 2002). It is a popular aquarium plant, and prolonged breeding in aquaria and associated exposure to chemicals and UV light are thought to have produced a hardier strain that differs from native plants genetically and has a higher tolerance to cold water temperatures (Jousson *et al*. 1998). *C. taxifolia* has been introduced to at least three geographical regions outside its native range: the Mediterranean Sea on the coasts of Croatia, France, Italy, Monaco, and Spain, (2) the southern Californian coast near San Diego, and (3) parts of the coasts of New South Wales and South Australia (Meinesz 1999; Campbell & Tebo 2001). However, the "aquarium hypothesis" has been challenged by recent work on the temperature tolerance of native populations in eastern Australia (Chisholm *et al*. 2000; see below).

The basic morphology consists of a thallus with horizontal stolons that give off rhizoids and erect feather-like branches, with pinnately arranged pinnules (GISP 2002). In its native range, *C. taxifolia* occurs mostly in small isolated clumps that reach an average height of 25 cm. In the Mediterranean Sea, however, introduced *C. taxifolia* forms dense "astroturf-like" mats with a height of up to three feet, and up to 213 m of stolon growth and 5,000 emerging fronds per square metre (Meinesz 1999; Anderson & Keppner 2001; Yip 2001). *C. taxifolia* produces several types of secondary metabolites (caulerpenyne) that are toxic to potential competitors or grazers belonging to a range of taxa.

Timing of reproduction and recruitment

Little information exists on the reproduction of *C. taxifolia*. Reproduction in native tropical populations can occur sexually during a short period of the year by synchronised (light intensity) release of anisogamous gametes and formation of zygotes (Zuljevic & Antovic 2000). However, Mediterranean and other introduced populations appear to be able to produce only male gametes, and are thus not capable of sexual reproduction. Therefore, reproduction and dispersal of *C. taxifolia* in the introduced range appear to be solely vegetative (asexual) or by fragmentation (Smith & Walters 1999; Anderson & Keppner 2001; Ramey 2001). *C. taxifolia* is pseudoperennial, with highest rates of stolon growth (up to 8 cm day^{-1}) in summer and autumn, followed by a short resting period from January to April (GISP 2002; Neill 2002). Successful recruitment of dispersed fragments of *C. taxifolia* (as small as 10 mm) can occur throughout the year, but establishment probabilities are highest during summer (Ceccerelli & Cinelli 1999).

Habitat and biology

Substratum type

Caulerpa taxifolia occurs on all types of substrata in both native and introduced range. The alga flourishes equally well on rocky, sandy, mud or clay substrata, both in sheltered and exposed conditions, and in polluted and pristine waters (Meinesz *et al*. 1993; Williams & Grosholz 2002). Dense mats of *C. taxifolia* in the Mediterranean smother other benthic biota, including corals, sponges, and other seaweeds (Meinesz 1999; Neill 2002). *C. taxifolia* can adjust its growth strategy to suit the

type of substratum available. For example, in the San Diego population, upright fronds developed adventitious rhizoids and stolons when lying on sediments, and stolons when entwined within existing algal canopy (Williams & Grosholz 2002).

Food preferences

Caulerpa taxifolia occurs in both polluted and nutrient-poor (e.g. the Mediterranean) habitats (Meinesz 1993). The rhizoid system is used to take up major nutrients from the substratum (Anderson & Keppner 2001), and the extensive biomass of *C. taxifolia* mats acts as a vast nutrient trap (P and N) (Yip 2001). Non-native populations of *C. taxifolia* lack severe nutrient (P and N) limitation (Delgado *et al.* 1996), which may be an important factor enabling it to out-compete native macrophytes.

Physiological tolerances (range & preferences)

Temperature

Mediterranean (introduced) populations of *C. taxifolia* have a temperature range of 9 – 32.5 ^{0}C. Some reports claim observation of live plants at 5 ^{0}C (Makowka 2000). Survival without growth occurs at temperatures of 10 – 12.5 ^{0}C; frond and stolon development commence at 15 and 17.5 ^{0}C, respectively, with optimum growth occurring at 25 ^{0}C (Gillespie *et al*. 1997; Komatsu *et al.* 1997). The lower temperature tolerance limit is thought to occur only in introduced strains, and to have developed during decades of aquarium-breeding. It is common opinion that *C. taxifolia* within the native range do not grow in water colder that 20 ^{0}C (Meinesz & Boudouresque 1996). However, recent research from eastern Australia showed that native populations are able to survive temperatures of 11 ^{0}C for a period of four weeks, and that a temperature of 13 ^{0}C is sufficient to maintain existing tissue biomass (Chisholm *et al.* 2000). Maximum growth occurs at > 20 ^{0}C (Komatsu *et al.* 1997).

Depth

Dense mats of *C. taxifolia* commonly occur at depths of 1 – 30 m, but the alga is known to occur down to a depth of ~ 100 m (Meinesz 1999; Anderson & Keppner 2001; Yip 2001). See "*Light*" (below) for more information.

Salinity

No specific information on *C. taxifolia*'s salinity tolerance range exists in the literature. Populations in the San Diego area were sampled at 34 psu (Williams & Grosholz 2002). Congeners of *C. taxifolia* are able to grow at salinities of 10 – 40 psu (*C. racemosa*; Carruthers *et al.* 1993) and 15 – 50 psu (*C. lentillifera*; Liao & Cheng 1989).

Light

Stolon and frond growth occur at very low light levels (27 μmol m^{-2} s^{-1}); the optimal light intensity ranges from 88 to 338 μmol m^{-2} s^{-1} (Mediterranean population; no upper irradiation limit established; Komatsu *et al.* 1997). Other studies report highest growth rates at an irradiance of 75 μmol m^{-2} s^{-1} (Gillespie *et al*. 1997). *C. taxifolia*'s annual productivity pattern is less affected by fluctuations in light and temperature than what has been reported from endemic seaweeds (Gacia *et al.* 1996). Photosynthetic assays suggest depth limits for colonisation at 80 m (clear water) and 50 m (turbid water) (Gacia *et al.* 1996). Mediterranean *C. taxifolia*'s maximum photoautotrophic growth limit was determined as 24 m during winter. Although this correlates reasonably with the distribution of dense

populations on the Monaco coastline, the limit is greatly inferior to the maximum reported depth of ~ 100 m, and implies significant heterotrophic carbon acquisition at depths much greater than 24 m (Chisholm & Jaubert 1997).

Methods of sampling

There appears to be no single "best" sampling method for *C. taxifolia* due to its occurrence on a range of substrata. Sampling methods that have been used to detect *Caulerpa* and estimate its abundance include visual transects, video transects, quadrat surveys (hard and soft substrata), grab samples (soft bottom) or sled samples (soft bottom).

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Clubbed tunicate (*Styela clava*)

General information

The clubbed tunicate, *Styela clava*, is a solitary ascidian native to the northwest Pacific from the Sea of Okhotsk, southern Siberia, Japan, Korea and the coast of China south to Shanghai (Millar 1970, Cohen 2005). It has a club-shaped body, up to 160 mm long, with a distinct stalk and basal disc with which it attaches to the substratum. Small individuals (<30 mm) may lack a stalk (Lützen 1999). The body wall (test) is leathery and variable in colour (commonly brown-white, yellow-brown or red-brown), with conspicuous tubercles on the upper part and longitudinal ridges on the stalk.

Like all ascidians, *Styela clava* is hermaphroditic (but not self-fertile) and gametes are shed into the water column. The "tadpole" larvae peculiar to ascidians are planktonic and hatch from the eggs after ca 12 hr, although the duration of this period varies with egg size and water temperature (Svane & Young 1989, cited in Bourque *et al*. 2005). The larvae are active for a similar period before settling to the substratum (Holmes 1969, cited in Holmes 1976, Minchin *et al*. 2006). The larvae do not feed and at first tend to swim upwards, though this behaviour later reverses (Millar 1970).

In those species of ascidians that have been studied, life-spans are generally 12-20 months, although some may live for several years (Millar 1970). Minchin *et al*. (2006) stated that the size of individual *Styela clava* (75-180 mm) collected in Ireland "suggests that they were between one and two years old", although they did not give any reason for this conclusion. Individuals that settled in the Limfjord, Denmark in mid-August grew to 17-48mm by the end of October (Lützen 1999), after which growth ceased during the colder months. Considerable mortality of smaller individuals also occurred during winter. Survivors reached lengths of 50-75 mm by June and became fully mature, spawning in July and August at 75-95 mm. Many small (12-40-mm-long) individuals were also present in early and mid-summer, representing late settlers from the previous year. These, and some of the larger individuals, probably survive a second winter to reach a length of 110-120 mm and reproduce for a second time aged 1.75-2 years old. The lifespan of individuals in southern England was found to be shorter, only rarely exceeding 15 months (Holmes 1969, cited in Lützen 1999). Death may results from senescence, predation or adverse environmental conditions. Reported predators of juvenile *Styela clava* include gastropods (*Mitrella lunata* in eastern North America) and fish (NIMPIS 2002).

The first recorded occurrences of *Styela clava* outside its native range were at Newport Bay (1932) and Elkhorn Slough (1935, a single specimen and no longer present at this site), both in California (Cohen 2005). It subsequently spread along the Pacific coast of North America, north as far as Puget Sound (collected in 1998) and Vancouver Island (collected in 1994) and south as far as Baja California (collected at Ensenada in 2000). On the east coast of North America, it was collected in Massachusetts in 1970, New York in 1972, Connecticut, Maine, New Hampshire and Rhode Island in the 1980s and, more recently, in New Brunswick and Prince Edward Island (1998) (Cohen 2005).

Styela clava was recorded in southwest England in 1953 (Carlisle 1954, Houghton & Millar 1960, both cited in Eno *et al*. 1997) and has since spread to northwest England, southwest Scotland and southern Ireland (collected 1972: Minchin & Duggan 1988). It has also been found in France (1968),

the Netherlands (1974), Denmark (1978-1979), Germany (1997), Portugal (2003) and Spain (2004) (Lützen 1999, Cohen 2005, Davis & Davis 2005).

The first record of *Styela clava* in Australia was in 1972 in Port Phillip Bay, Victoria (Holmes 1976) and in 1977 it was reported from Sydney Harbour, New South Wales (Cohen 2005). It was first recorded in New Zealand in the Viaduct Harbour, Auckland in August 2005 and there appear to be well-established populations in the Waitemata Harbour, Hauraki Gulf and Firth of Thames (Gust *et al*. 2006a). More localised populations have also been found in Lyttelton Port, Lyttelton Marina, Tutukaka and Opua Marinas (Northland) (Gust *et al*. 2006a and b) and Nelson Port (Morrisey *et al*. 2006).

Timing of reproduction and recruitment

Reproduction is usually restricted to warmer seasons in ascidians living in temperate and cold seas (Millar 1970). Holmes (1969, cited in Holmes 1976) reported that *Styela clava* bred throughout all but the coldest 2-3 months in southern England, with a marked peak of settlement in mid-late summer (late July-early September). A similar pattern of settlement was observed in the Limfjord, Denmark (Lützen & Sørensen 1993, cited in Lützen 1999). Monthly sampling of *S. clava* in southern Ireland (Parker *et al*. 1999) showed gametogenesis (presence of ripe gametes in the gonads) from February-November, with a peak in August-October, and spawning in September-October (when average water temperatures were 15.2ºC (±0.4 SD) – 14.1ºC (±1.3 SD)).

Spawning in ascidians generally occurs in response to a period of light following a period of darkness (Svane & Young 1989, cited in Bourque *et al*. 2005). The rapidity of response to this period of light varies among species and, therefore, not all species spawn at the same time of day. Time of spawning may also vary among populations of the same species from different locations (Bourque *et al*. 2005). In *Styela plicata*, the duration of the light period required to stimulate spawning decreases with increase in the preceding period of darkness (West & Lambert 1976, cited in Bourque *et al*. 2005). Light intensity may also affect the duration of the light period prior to spawning (Forward *et al*. 2000, cited in Bourque *et al*. 2005). Bourque *et al*. (2005) found that concentrations of larvae of *Styela clava* in the upper 1-m of the water column at a field location in Prince Edward Island, eastern Canada, peaked around noon. They pointed out, however, that timing of peak concentrations of larvae may vary among locations and over time at the same location, in response to factors such as day-length, water temperature and light intensity. Cohen 2005 and ISSG Global Invasive Species Database 2006 indicate that *Styela clava* is only able to spawn at water temperatures above 15ºC and salinities above 25-26 psu (no sources are given for this information).

Larvae of *Styela clava* do not usually travel more than a few centimetres by active swimming (Minchin *et al*. 2006). Consequently they tend to congregate close to the parent population, although they can be passively dispersed over distances covered by 1-2 tidal excursions (equivalent to the duration of the larval period). Larvae are negatively buoyant but negatively geotactic and positively phototactic, particular at higher hydrostatic pressures, and consequently tend to settle near the water surface (Davis 1997, cited in Minchin *et al*. 2006). Suitable conditions for establishment occur in sheltered localities with salinities of >22 psu and temperatures ≥16ºC for several weeks (Minchin *et al*. 2006). Individuals apparently reach maturity at 3-10 months (Cohen 2005).

Habitat and biology

Styela clava occurs in low wave-energy environments and sheltered embayments from the upper sublittoral zone to at least 25 m depth (ISSG Global Invasive Species Database 2006). It is especially abundant 10-200 cm below the sea surface (Lützen 1999), and the fact that it has been recorded up to 30 cm above the level of extreme low water of spring tides in southern England (Holmes & Coughlan 1975, cited by Lützen 1999) suggests that it is able to withstand a degree of regular exposure to air. It can apparently survive for up to 3 days out of water under cool, damp conditions (Lützen & Sørensen 1993, cited in Minchin *et al*. 2006). Based on a survey of the distribution of *S. clava* in harbours of the Southern Californian Bight, Lambert & Lambert (2003) noted that the species was consistently more abundant closer to the entrances to bays, where water currents were stronger and that it differed from *S. plicata* in this respect.

Substratum type

Natural substrata for attachment of *Styela clava* include rocks, the blades of macroalgae and the shells of live and dead bivalves (Lützen 1999, NIMPIS 2002, Bourque *et al*. 2005). *S. clava* is also found on a range of artificial structures, including floating pontoons, tyre fenders, vessels, buoys and anchors, and diverse materials, including concrete, cement, wood, ropes and the steel or fibreglass hulls of vessel (Bourque *et al*. 2005, Gust *et al*. 2005, 2006a, ISSG Global Invasive Species Database 2006, Minchin *et al*. 2006). In a survey of harbours in southern California, Fay & Johnston (1971, cited in Lambert & Lambert 2003) recorded *Styela clava* only on floats and pilings and not on any natural substrata.

According to Holmes (1976), *Styela clava* colonises only those surfaces bearing a well-developed epibiota. It can attach to larger individuals of its own species and individual *S. clava* may be extensively fouled with smaller tunicates of their own or other species, algae, sponges, hydroids and bryozoans (Lützen 1999, Cohen 2005, Minchin *et al*. 2006).

On natural substrata, such as rocks or bivalve shells, *Styela clava* is reported to reach population densities of 50-100 m^{-2} (Lützen 1999). On artificial substrata, however, much higher densities have been reported (500-1500 m^{-2}: Holmes 1976, NIMPIS 2002).

In New Zealand *Styela clava* has been found attached to floating pontoons, wooden pier piles, suspended mooring lines and vessel hulls (Gust *et al*. 2006a). It has also been reported attached to dead bivalve shells on a muddy shore in the Tamaki Estuary, Auckland (Chris Hickey, NIWA, pers. comm.).

Food preferences

Styela clava is a suspension feeder, feeding on suspended, particulate matter, such as phytoplankton, zooplankton and organic detritus, filtered from water pumped through its branchial sac.

Physiological tolerances (range and preferences)

Temperature

Styela clava is reportedly able to tolerate temperatures ranging from –2 to 23ºC (Minchin *et al*. 2006). Holmes (1969, cited in Holmes 1976) described a population living in southern England, where water temperature raged from 2-23ºC, and breeding in all but the coldest 2-3 months of the year. On the Pacific coast of North America it has been found at water temperatures ranging from 11-27ºC (Cohen 2005). Larvae are able to survive temperatures from 10 - 30ºC (Boothroyd *et al*. 2003).

Parker *et al*. (1999) reported no evidence of gametogenesis in individuals sampled in early February in southern Ireland, when the water temperature was 3-4ºC, but small numbers of ripe gametes in individuals sampled in the middle of the same month, when the temperature had risen to 8ºC. There was evidence that gonad maturation occurred at temperatures below 8ºC. Gametogenesis and spawning peaked in August-October, when water temperatures ranged from 14-18ºC.

Depth

The reported depth range for *Styela clava* ranges from just above the level of extreme low water of spring tides (in southern England: Holmes & Coughlan 1975, cited by Lützen 1999) to at least 25 m (NIMPIS 2002). Lützen (1999) described *S. clava* as a "predominantly littoral species, which is especially abundant 10-200 cm below the sea surface in areas without tides or when attached to floating objects….The species may extend to depths of 15-25 m…but a record of 40 m depth…is probably exceptional".

Salinity

Styela clava appears to avoid areas with estuarine conditions (Lützen 1999). Sims (1984, cited in Lützen 1999) found that Californian specimens showed poor vital functions after 3-d immersion in 26.5 psu seawater. This corresponds with Lambert & Lambert's (2003) observation of die-offs of *S. clava* on floating structures in southern California after heavy rain (followed by rapid recolonisation). They also cited an earlier study (MacGinitie 1939) in the same area that found complete mortality of *S. clava* below a sharp halocline that formed at a depth of ca 2.2 m following heavy rain. Below this depth there was no evidence of any mortality. Individuals can, however, survive shorter periods of salinity as low as 8 psu, presumably by closing their siphons (Sims 1984, cited in Lützen 1999).

Other populations of *Styela clava* may be more tolerant of lower salinities than those studied in California. In the eastern Limfjord (Denmark), populations exist in salinities averaging 26-28 psu, with decreases to <20 psu for periods of several days (Lützen 1999). Individuals experimentally exposed to stepwise decreases in salinity from 31-18 psu showed >50% survival for 40 d (at 12ºC) and 50% survival when the salinity was further reduced to 16 psu (Lützen & Sørensen 1993, cited in Lützen 1999). Lützen (1999) cited a report that larvae of *S. clava* from the Sea of Japan were able to complete metamorphosis at salinities of 20-32 psu, but that <18 psu was "deleterious" (no definition given). Cohen (2005) stated that adult *S. clava* die in salinities <10 psu, but did not give a source for this information.

In summary, salinity tolerance of adults and larvae appears to extend as low as 18 psu for extended periods (and much lower for short periods), but may be dependent on the salinity regime to which the population has previously been exposed.

Route of introduction

Styela clava may have reached the Pacific coast of North America as fouling on ships' hulls, but it may also have been introduced as fouling on imported live oysters (Cohen 2005). It is known to occur on oysters (*Crassostrea gigas*) in Japanese oyster farms, and oysters from Japanese farms were transplanted to Elkhorn Slough (California) in 1929-1934, roughly coincident with its date of first detection in California (1932). From Elkhorn Slough it could have been transported to other parts of California as fouling on coastal shipping or via further transfer of oyster stock (including its recent appearance in Humboldt Bay: Cohen 2005).

The introduction of *Styela clava* to southern England is commonly ascribed to fouling on naval vessels returning from the Korean War in 1952 (Minchin & Duggan 1988, cited in Minchin *et al*. 2006), having acquired fouling in the Yellow Sea. It is likely to have spread from the original site of introduction to other parts of the United Kingdom and continental Europe on coastal shipping or, locally, by dispersal of eggs and larvae (Lützen 1999). It has also been suggested that *S. clava* reached the Danish coast, where it was first recorded on an oyster bed in the Limfjord, attached to oysters imported from the English Channel and re-laid in the Limfjord (Lützen 1999). Oyster spat imported from Japan in the 1970s, or transplanted within the English Channel region, may have contributed to the establishment of Dutch and French populations (Lützen 1999).

Given the distances involved, the introduction of *Styela clava* to Australia and New Zealand is likely to have occurred via fouling on ships' hulls, either from its native range or from introduced populations in Europe or North America. In view of the disjunct distribution of *S. clava* in New Zealand's North and South Islands, several inoculation events may have occurred (Gust *et al*. 2006a). Research is currently underway to determine the genetic relationships among populations of *S. clava* in New Zealand.

Minchin *et al*. (2006) noted that *S. clava* tend to be stripped from ships' hulls at speeds above ca 5 kt, unless they occur in more protected habitats such as sea-chests, thruster tubes, or in the lee of stabilisers and other structures on the hull. Lützen (1999) also described *S. clava* as rheophobic (i.e. avoiding strong currents), reducing the likelihood of individuals surviving as fouling on exposed parts of the hulls of rapid vessels in continuous service. Attachment to drifting macroalgae provides another potential means of dispersal. Lützen (1999) stated that fronds of *Sargassum muticum* (a macroalga introduced to Europe from Asia in the early 1970s) with *Styela clava* attached are often washed up on shores in the Limfjord. Fronds become detached from their holdfasts towards the end of the growth cycle and can float for "considerable distances".

Davis & Davis (2004) suggested that a combination of transport mechanisms, including translocation on oyster shell, dispersal on flotsam such as drift macroalgae, fouling on vessel hulls, transport of eggs and larvae in ballast water, and fouling of sea-chests are probably required to explain the present distribution of *S. clava*. Davis (2005) suggested that sea-chests were potentially of greatest importance

because they offer a means of transport for established colonies of individuals, and translocated colonies are more likely to establish new populations than a single inoculum of larvae.

Slow-moving and towed vessels are particularly likely mechanisms of introduction, because of the reduced likelihood of individuals being removed from the hull by water currents during transit. Such vessels may also spend longer periods moored in ports of origin and destination than vessels in continuous service. Specimens of *S. clava* found on vessels in New Zealand have been on a tug (Lyttelton), recreational launches and yachts (Auckland, including one that subsequently travelled to Waikawa Marina, Picton, where it was found to harbour a single individual) and fishing vessels (Nelson) that had been berthed for long periods of time (possibly months in one case, years in another). Of these, recreational vessels are perhaps the most likely to have been the vector of inoculation in the ports where they were found, as the other types of vessel tend to spend most of their time in their home port.

Methods of sampling

- Lambert & Lambert (2003) sampled harbours by examining the sides and bottom edges of pontoons and vessels in marinas, manually removing clumps of fouling organisms to arms' depth, and recovering 5-m long ropes deployed 4 years previously.

- Minchin *et al*. (2006) sampled floating pontoons, supporting piles and quay walls by feeling for specimens by hand, or by scraping adhered biota from the surfaces.

- Gust *et al*. (2005, 2006a,b) employed above-water searches from shore or boat to detect *S. clava* on pontoons, pilings, breakwalls, buoys, heavily-fouled vessels and mooring lines. Submerged ropes were pulled up and examined. Selection of vessels to search was based on a risk-profiling approach based on empirical relationships between level of fouling and probability that the fouling assemblage includes solitary ascidians.

- Gust *et al*. (2005, 2006a,b) also used in-water diver searches of the undersides of pontoons, wharf piles and breakwalls. For safety reasons, and because previous studies had shown that 70% of all *S. clava* detected were found within this depth, searches were confined to the upper 5 m of the water column.

- The probability of *S. clava* being detected by searchers when it is present can be estimated for each type of substratum in a given harbour. These estimates require information on the proportion of the total area of the substrate searched and the sensitivity of the search method under prevailing environmental conditions, particularly water clarity (Gust *et al*. 2006a,b). Sensitivity, the ability of the searchers to detect *S. clava* when present, can be determined by searches for experimentally-deployed mimics of the organism. Details of the methods are given in Gust *et al*. (2006a,b).

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APPENDIX 3. EXPERTS CONTRACTED TO REVIEW THE HABITAT SUMMARIES AND SAMPLING METHODS FOR THE TARGET SPECIES

Species	Expert	Affiliation
Asterias amurensis	Greg Parry	Marine & Freshwater Resources Institute, PO Box 114, Queenscliff 3225, Australia
	Craig Johnson	School of Zoology, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, GPO Box 252-05, Hobart TAS 7001, Australia
Sabella spallanzanii	Greg Parry	Marine & Freshwater Resources Institute, PO Box 114, Queenscliff 3225, Australia
	Adriana Giangrande	Departimento de Biologia, Stazione de Biologia Marina, Laboratorio de Zoologia, Universita de Lecce, I-73100 Lecce, Italy
Potamocorbula amurensis	Jan Thompson	US Geological Survey, Reston, VA, USA
	Heather Peterson	California Department of Water Resources, 3251 "S" Street, Sacramento, CA 95816, USA
Carcinus maenas	Ed Grosholz	Environmental Science & Policy, University of California , One Shields Way , Davis, CA 95616 -8576, USA
	Per-Olav Moksnes	Kristineberg Marine Research Station, SE-450 34 Fiskebäckskil, Sweden
	Sylvia Behrens-Yamada	Department of Zoology, Oregon State University, Corvallis, Oregon 97331-2914, USA
Eriocheir sinensis	Tanya Veldhuisen	California Dept Water Resources, Sacramento, USA
	Leif-Matthias Herborg	University of Newcastle, Dept. Marine Sciences and Coastal Management, Ridley Bldg Newcastle upon Tyne NEI 7RU, UK
	Debra Rudnick	Dept Environmental Science, Policy & Management, University of California, Berkley, USA
Caulerpa taxifolia	Alexandre Meinesz	Laboratoire Environnement Marin Littoral, Equipe d'Accueil "Gestion de la Biodiversité" (EA 3156), Université de Nice-Sophia Antipolis (UNSA), Faculté des Sciences Parc Valrose 06108 Nice Cedex 2, France
	Susan Williams	Director, Bodega Marine Laboratory , P.O. Box 247 , Bodega Bay, CA 94923-0247, USA
Undaria pinnatifida	Wendy Nelson	NIWA, Greta Point, Wellington
	Bob Fletcher	Earth & Environmental Sciences Research Centre, University of Portsmouth, Burnaby Building , King Henry I Street , Portsmouth , PO1 3QL, UK

APPENDIX 4. RESULTS OF HYDRODYNAMIC MODELLING

Dark blue areas represent lowest concentrations of propagules (larvae, etc.), red areas represent highest concentrations four days after release.

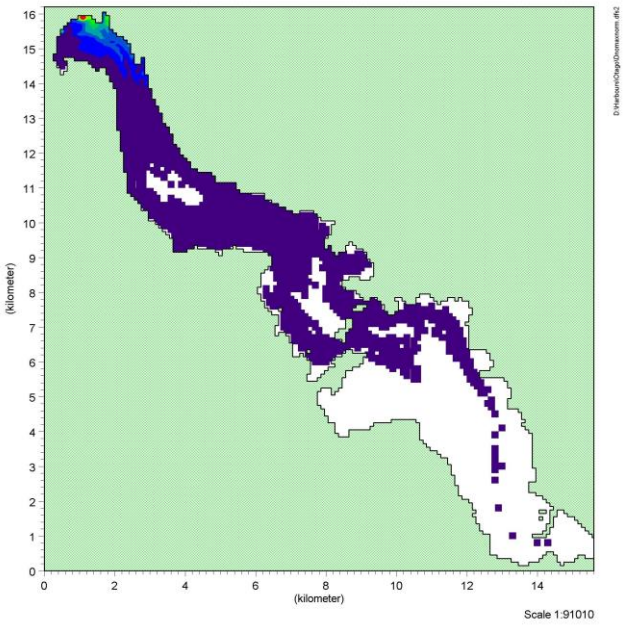

Figure A4.1 Simulation under northerly winds from release point in the Port of Otago.

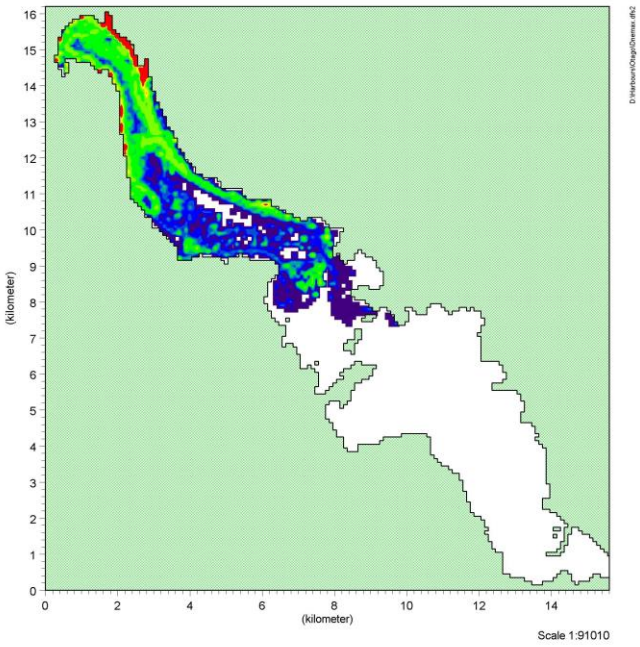

Figure A4.2 Simulation under north easterly winds from release point in the Port of Otago.

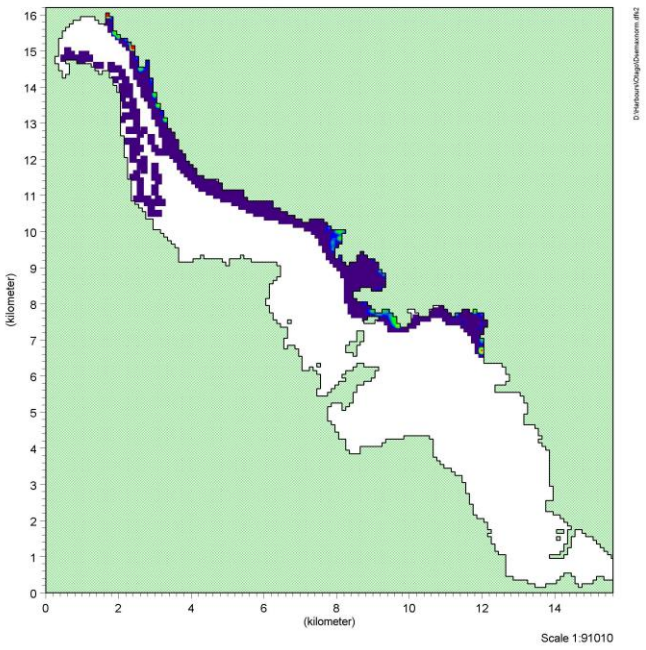

Figure A4.3 Simulation under south easterly winds from release point in the Port of Otago.

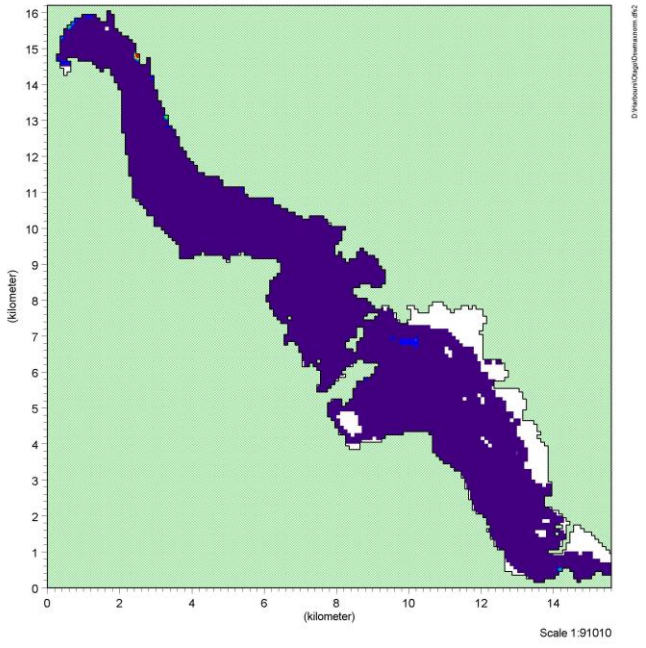

Figure A4.4 Simulation under south westerly winds from release point in the Port of Otago.

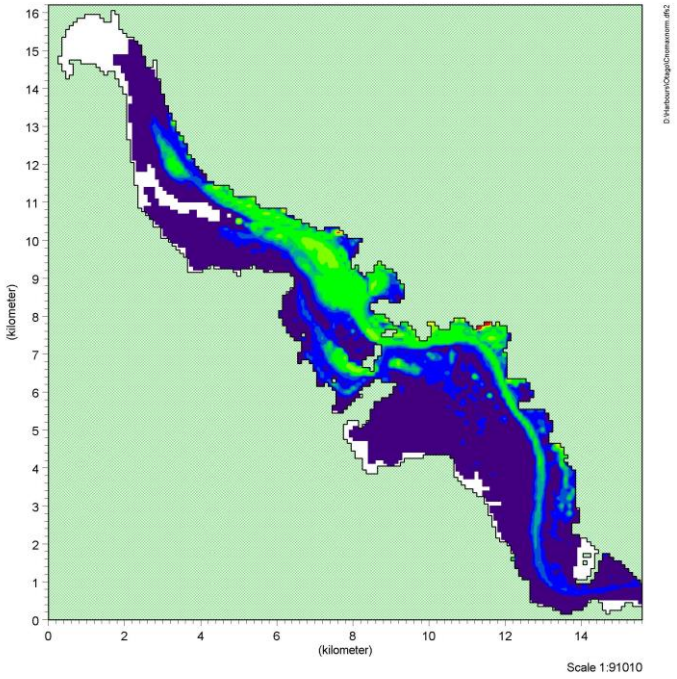

Figure A4.5 Simulation under northerly winds from release point in Port Chalmers.

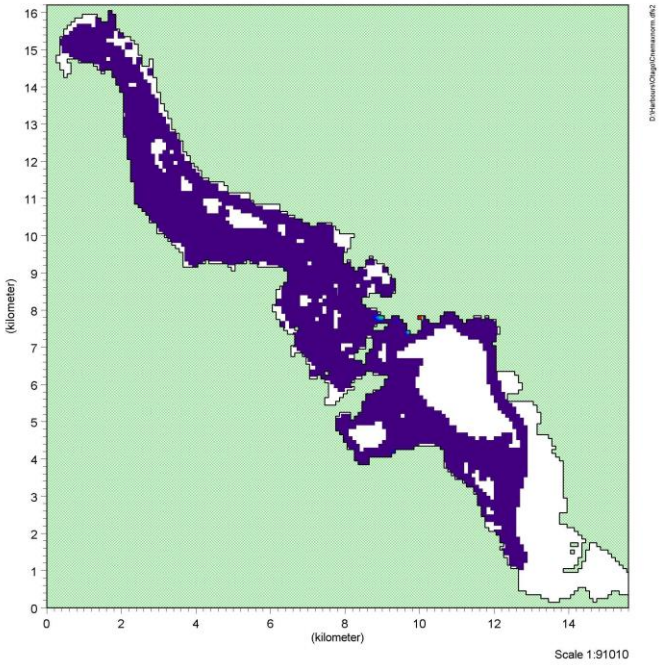

Figure A4.6 Simulation under north easterly winds from release point in Port Chalmers.

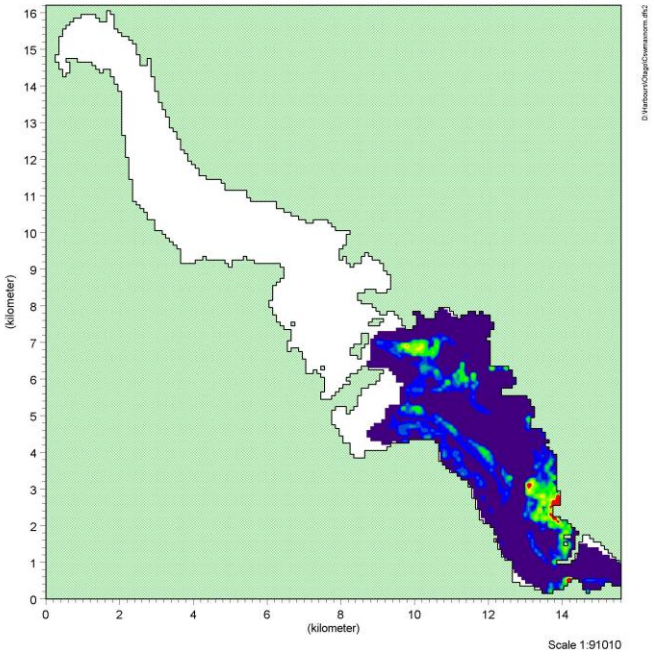

Figure A4.7 Simulation under south westerly winds from release point in Port Chalmers.

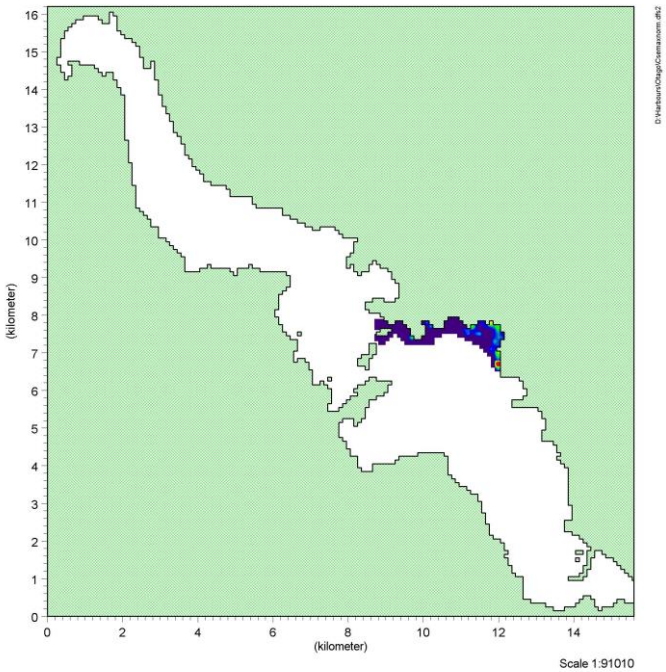

Figure A4.8 Simulation under south easterly winds from release point in Port Chalmers.

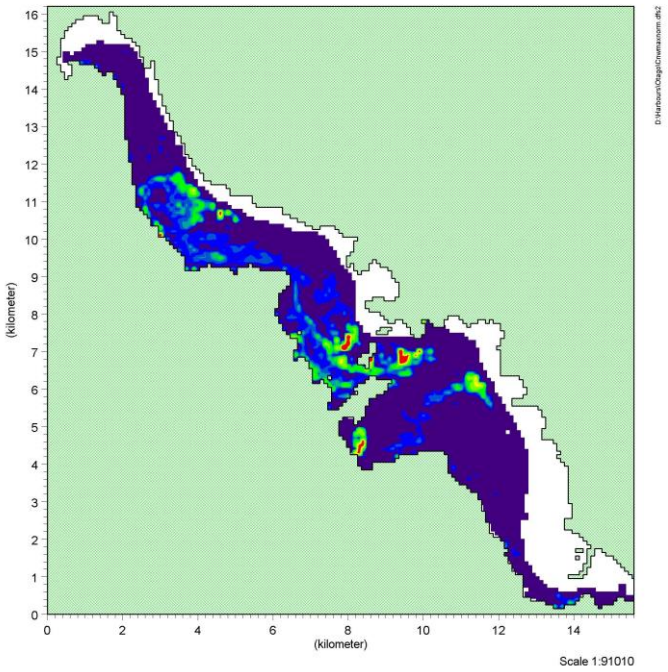

Figure A4.9 Simulation under north westerly winds from release point in Port Chalmers.

APPENDIX 5: SAMPLING DATA SHEETS

A5.1 Sample lot register (record of sample lot code allocated to a sample from which a specimen has been collected for submission to MITS).

TARGET SURVEILLANCE **SAMPLE LOT REGISTER**

Surveillance round: _____ (e.g WINTER_08)

Survey code: _____ (e.g. SVBLU7)

PORT: _____

Survey code	SAMPLE LOT CODE	DATE	SAMPLE METHOD	Site ID	TRAP TYPE
(e.g. enter SVBLU7 for Bluff winter08)	enter port code (e.g. BLU)	eg. 1/01/2001	(BSLD / STFTP / CRBTP / CONDO / VISD / SHORE)	(e.g. SVLYT7001)	(STFTP, CRBTP OR CONDO) & TRAP NO.
	7000				
	7001				
	7002				
	7003				
	7004				
	7005				
	7006				
	7007				
	7008				
	7009				
	7010				
	7011				
	7012				
	7013				
	7014				
	7015				
	7016				
	7017				
	7018				
	7019				
	7020				

A5.2 Image register (record of identity of specimen that has been photographed to aid identification).

TARGET SURVEILLANCE **IMAGE REGISTER**

Surveillance round & survey code: ____________ (e.g WINTER08 & SVBLU7)

PORT: ____________

DATE	IMAGE NO.	SAMPLE LOT CODE	TAXA	NOTES

A5.3 Sledding data sheet.

Target surveillance	**SLEDDING : 100+ sled tows per port**				**Port:**
	(Sled tows = 2 mins @ 2 knots (80-100m) - Please note if shorter/longe				**SVL round:**
Sediment type: 1- Sandy mud**, 2-** Muddy sand**, 3-** Sand, **4-** Sandy gravel**, 5-** Shelly gravel**, 6-** Sand foul					**Survey code:**
7- Sand reef, **8-** Reef, **9-** Other (Please state), **10** - Mud.					**Boat:**
Habitat type: 1- Seagrass bed, **2-** Oyster bed **(2.1** = Pacific**, 2.2** = Flat oysters**), 3-** Horse mussels**, 4-** Scallops,					**Recorder:**
5- Large bivalves (**5.1** = Cockles, **5.2** = Pipis, **5.3** = Others), **7-** Algae, **8-** Sponge bed, **9-** Nothing					

Site ID (e.g. SVLYT6001)					
Start point of tow (GPS co-ords) include all symbols and decimal points					
End point of tow (GPS co-ords) include all symbols and decimal points					
DATE (day/month/year)					
Sounder depth (m)					
Secchi depth (m)					
Salinity					
Water temp					
Wind speed					
Wind direction					
SEDIMENT TYPE (1-10)					
HABITAT TYPE (1-9)					
	No. of individuals & enter (K) if sample is kept				
SEASTARS					
Asterias amurensis (nthn pacific)					
Coscinasterias (11 arm)					
Pateriella (cushion)					
BIVALVES					
Potamocorbula amurensis (asian clam)					
Musculista senhousia (asian date msl)					
Theora lubrica					
WORMS					
Sabella spallanzanii (meditern fan)					
Chaetopterus (parchmnt.)					
ALGAE					
Caulerpa taxifolia (aquarium wd)					
Undaria pinnatifida (japan. kelp)					
Codium fragile (brocco wd)					
CRABS					
Carcinus maenas (grn. euro. shore)					
Eriocheir sinesis (chinese mitten)					
Charybdis japonica (asian paddle)					
Pyromaia tuberculata (fire crab)					
Metcarcinus sp. (cancer crab)					
Nectocarcinus integrifrons (red swimmer)					
Macrophthalmus hirtipes (stlk eyed mud)					
Hemigrapsus crenulatus (hairy hand)					
Hemigrapsus sexdentatus (cmn rock)					
Halicarcinus (spider crab)					
Pagarus novizealandaea (hermit)					
Plagusia capensis (red rock)					
Petrolisthes elongatus (porcelain)					
Helice crassa (tunnel mud)					
Notomithrax sp. (deco / cammo)					
Ovalipes catharus (paddle)					
ASCIDIANS					
Styela clava (clubbed sea-squirt)					
Eudistoma elongatum (colonial ascidian)					
Didemnum sp.(colonial ascidian)					
OTHERS (pls note):					
SAMPLE LOT NO. (e.g LYT546) include taxa code on pot lable					
NOTES					

A5.4 Shore search data sheet.

Target surveillance	**SHORE SEARCH :**	**Port:**			
	Target = 25+ sites per port	**SVL round:**	(e.g. Winter08)		
	(10 minute seaches)	**Survey code:**	(e.g. SVBLU7)		
Shore type: 1 - SAND, **2** - SAND & SHELL GRAVEL, **3** - SHELL GRAVEL, **4** - SAND & ROCKS, 5 - ROCKY, 6 - MUD, **7** - MANGROVES, **8** - OTHER (PLEASE STATE)		**Recorder:**			

Site ID (e.g. SVLYT6001)					
Start point of search (GPS co-ords) include all symbols and decimal points					
End point of search (GPS co-ords) include all symbols and decimal points					
Date & time					
SHORE TYPE (1-8)					
Observers names					
Wind speed					
Wind direction					
Secchi depth (if viewing from boat)					
Sounder depth (if viewing from boat)					
Water temp (if viewing from boat)					
Salinity (if viewing from boat)					
	No. of individuals & (K) if sample is kept				
BIVALVES					
Potamocorbula amurensis (asian clam)					
Musculista senhousia (asian date msl)					
WORMS					
Chaetopterus (parchmnt.)					
ALGAE					
Caulerpa taxifolia (aquarium wd)					
Undaria pinnatifida (japan. kelp)					
Codium fragile (brocco wd)					
CRABS					
Carcinus maenas (grn. euro. shore)					
Eriocheir sinesis (chinese mitten)					
Charybdis japonica (asian paddle)					
Pyromaia tuberculata (fire crab)					
Nectocarcinus integrifrons (red swimmer)					
Metacarcinus sp. (cancer crab)					
Macrophthalmus hirtipes (stlk eyed mud)					
Hemigrapsus crenulatus (hairy hand)					
Hemigrapsus edwardsi (cmn rock)					
Halicarcinus (spider crab)					
Pagarus novizealandaea (hermit)					
Plagusia capensis (red rock)					
Petrolisthes elongatus (porcelain)					
Helice crassa (tunnel mud)					
Notomithrax sp. (deco / cammo)					
Ovalipes catharus (paddle)					
ASCIDIANS					
Styela clava (clubbed sea-squirt)					
Eudistoma elongatum (colonial ascidian)					
Didemnum sp. (colonial ascidian)					
OTHERS (pls note):					
SAMPLE LOT NO. (e.g LYT546) include taxa code on pot label					
NOTES					

A5.5 Sample record (record of taxa present in each sample collected for submission to MITS).

TARGET SURVEILLANCE **SAMPLE RECORD**

Surveillance round: ____________ (e.g WINTER08)

Survey code: ____________ (e.g. SVBLU7)

PORT: ____________

		AG	AM	AN	BN	BV	BR	CB	DP	EC	FH	FW	GP	HT	HY	IS	JF	MY	OS	CT	MU	PY	SN	SS	ST	SP	TN	UK	WH	WM					
SAMPLE LOT CODE	DATE	ALGAE	AMPHIPODS	ASCIDIANS	BARNACLES	BIVALVES	BRYOZOA	CRABS	DECAPODS	ECHINOIDS	FISH	FLATWORMS	GASTROPODS	HOLOTHURIANS	HYDROIDS	ISOPODS	JELLYFISH	MYSIDS	OSTRACODS	OTHER CRUSTACEANS	OTHER MOLLUSCS	PYCNOGONIDS	SEA ANENOMES	SEA STARS	SEDIMENT	SPONGES	TANAIDS	UNKNOWNS/MISC.	WASH	WORMS					

A5.6 Crab and starfish trapping data sheet (also used for crab condos).

TARGET SURVEILLANCE:	**CRAB & STARFISH TRAPPING**	**PORT:**
SVL round: (e.g WINTER08)	**24 HR SOAKS =** Crab trap (CRBTP) lines = 3 traps to each line (minimum 60 CRBTP lines per port) Starfish trap (STFTP) lines = 2 traps to each line (minimum 20 STFTP lines per port)	**BOAT:**
Survey code: (e.g. SVBLU7)	**72 + HR SOAKS =** Crab CONDO lines = 3 traps to each line (as many as possible, min 8 CONDO lines per port)	**RECORDER:**

Site ID (e.g SVLYT6001)	**GPS co-ordinates** include all symbols & decimal points (e.g. for latitude: 36º 42.887'S or 36º 42' 34.778"S)	**SOUNDER & SECCHI DEPTH (m)** (when traps deployed)	**DATE & TIME IN** (day / month)	**DATE & TIME OUT** (day / month)	**Environmental data** (include speed and direction for wind)	**TRAP TYPE** (CRBTP, STFTP or CONDO)	**TRAP NO.** (1,2,3 or X if no trap)	**CONTENTS OF TRAP** * ENTER (K) NEXT TO ORGANISM IF KEPT *	**SAMPLE LOT NO.** Assign only ONE Sample Lot No. per trap, Include taxa code on pot label (e.g. LYT546	**OTHER NOTES** If you can't get to pre-allocated site, include reason here too
		Sounder depth	/	/	Salinity					
					Water temp					
		Secchi depth	:	:	Wind					
		Sounder depth	/	/	Salinity					
					Water temp					
		Secchi depth	:	:	Wind					
		Sounder depth	/	/	Salinity					
					Water temp					
		Secchi depth	:	:	Wind					
		Sounder depth	/	/	Salinity					
					Water temp					
		Secchi depth	:	:	Wind					
		Sounder depth	/	/	Salinity					
					Water temp					
		Secchi depth	:	:	Wind					

A5.7 Diver search data sheet.

TARGET SURVEILLANCE
SVL round: ______________ (e.g. WINTER08)
Survey code: ______________ (e.g. SVBLU7)
PORT: ______________
BOAT: ______________
RECORDER: ______________

DIVING
30+ LOCATIONS
10 piles (or equivalent area of pontoon, breakwall or other substrate, please state)

ENTER NO. PILES SEEN ON (or approx # per m if breakwall) AND (K) IF SAMPLE KEPT

Site ID (e.g SVLYT6001)	GPS CO-ORDINATES including all symbols & decimal points (e.g. for latitude: 36º 42.887'S)		DATE (day/month)	Environmental data			Diver names	Transect depth (TD) (2m or 4m or specify) / Max depth (MD)	Time & Air IN	Time & Air OUT	TOTAL # OF PILES SURVEYED (or distance of pontoon, breakwall etc)	WORMS		ALGAE			ASCIDIANS			SS	OTHERS				SAMPLE LOT NO. (separate transect = separate sample lot no.)	NOTES if you can't get to pre-allocated site include reason here too
	START POINT	END POINT										**Sabella spallanzanii** (mediterranean fan)	**Chaetopterus** (parchmnt.)	**Caulerpa taxifolia** (aquarium wd)	**Undaria pinnatifida** (japanese kelp)	**Codium fragile** (brocco wd)	**Styela clava** (clubbed sea-squirt)	**Eudistoma elongatum** (colonial ascidian)	**Didemnum sp.** (colonial ascidian)	**Asterias amurensis** (nthn pacific seastar)						
				Sounder depth:	Wind speed:	Salinity:	Diver 1:	TD:																		
								MD:																		
			/	Secchi depth:	Wind Direction:	Water Temp:	Diver 2:	TD:																		
								MD:																		
				Sounder depth:	Wind speed:	Salinity:	Diver 1:	TD:																		
								MD:																		
			/	Secchi depth:	Wind Direction:	Water Temp:	Diver 2:	TD:																		
								MD:																		
				Sounder depth:	Wind speed:	Salinity:	Diver 1:	TD:																		
								MD:																		
			/	Secchi depth:	Wind Direction:	Water Temp:	Diver 2:	TD:																		
								MD:																		
				Sounder depth:	Wind speed:	Salinity:	Diver 1:	TD:																		
								MD:																		
			/	Secchi depth:	Wind Direction:	Water Temp:	Diver 2:	TD:																		
								MD:																		
				Sounder depth:	Wind speed:	Salinity:	Diver 1:	TD:																		
								MD:																		
			/	Secchi depth:	Wind Direction:	Water Temp:	Diver 2:	TD:																		
								MD:																		