BIOSECURITY NEW ZEALAND

Targeted surveillance for non-indigenous marine species in New Zealand

Design report for Opua Marina

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Prepared for MAF Biosecurity New Zealand
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Contents

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 MAFBNZ 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
Local Authority Marina	Opua Marina (09-4027124)	Jane Wubben (Operations Manager)
Local Authority	Far North Holdings Limited enquiries@fnhl.co.nz	
Local Industry	Ashby's Boatbuilders (09-4027483)	
NZ Customs	NZ Customs (Business Hours; 09-6021669) (After Hours; 09-6201669)	Gary Burton (Customs Officer)
Local Authority Biosecurity	MAF Biosecurity Opua (09-4025946) MQSOPUA@maf.govt.nz	Mike Cartwright

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 MAFBNZ'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 Marina	Opua Marina (09-4027124) enquiries@opuamarina.co.nz	Jane Wubben (Operations Manager)
Local Authority	Northland Regional Council (09 4384639)	
Local Authority	Far North District Council (09-4052750)	Kawakawa Office (09-404-1544)
Aquaculture industry	NZ Oyster Industry Association Ltd	Tom Hollings (09-378-7001)
Fisheries regulator	Ministry of Fisheries Biosecurity (09-4025946)	Mike Cartwright (District Compliance Manager)
National government	Department of Conservation	Northland Conservancy Office (09-470-3300)
Research provider	NIWA Bream Bay (09-432 5506)	Graeme MacKay (Field Team Leader)
Research provider	Hollings Resource Management Ltd	Tom Hollings (09-378-7001)
Research provider	Bioresearches (09-379 9417)	
Conservation NGO	Royal Forest and Bird Protection Society	Far North Branch, Kaeo (09-405 1746)

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 the navigational charts of the area (Land Information New Zealand Charts Numbers 5124 and 5125, both published July 1994), 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 charts, 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 Opua Inlet system at high tide is ca 5,213 ha, the shoreline length is ca 232 km and the spring tidal prism (the volume of water entering on the flood tide) is ca 90 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 0.45 (i.e. approximately half of the water present in the harbour at high tide leaves on the subsequent ebb). The index of shoreline complexity is 0.11. This index is 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. The index varies from 1.0 for a simple circular basin to <0.1 for a very complex shoreline with multiple arms). The index for Opua thus indicates the complex and indented form of the harbour's shoreline.

Figure 1a **Map of the sampling area around Opua Marina showing habitats present.**

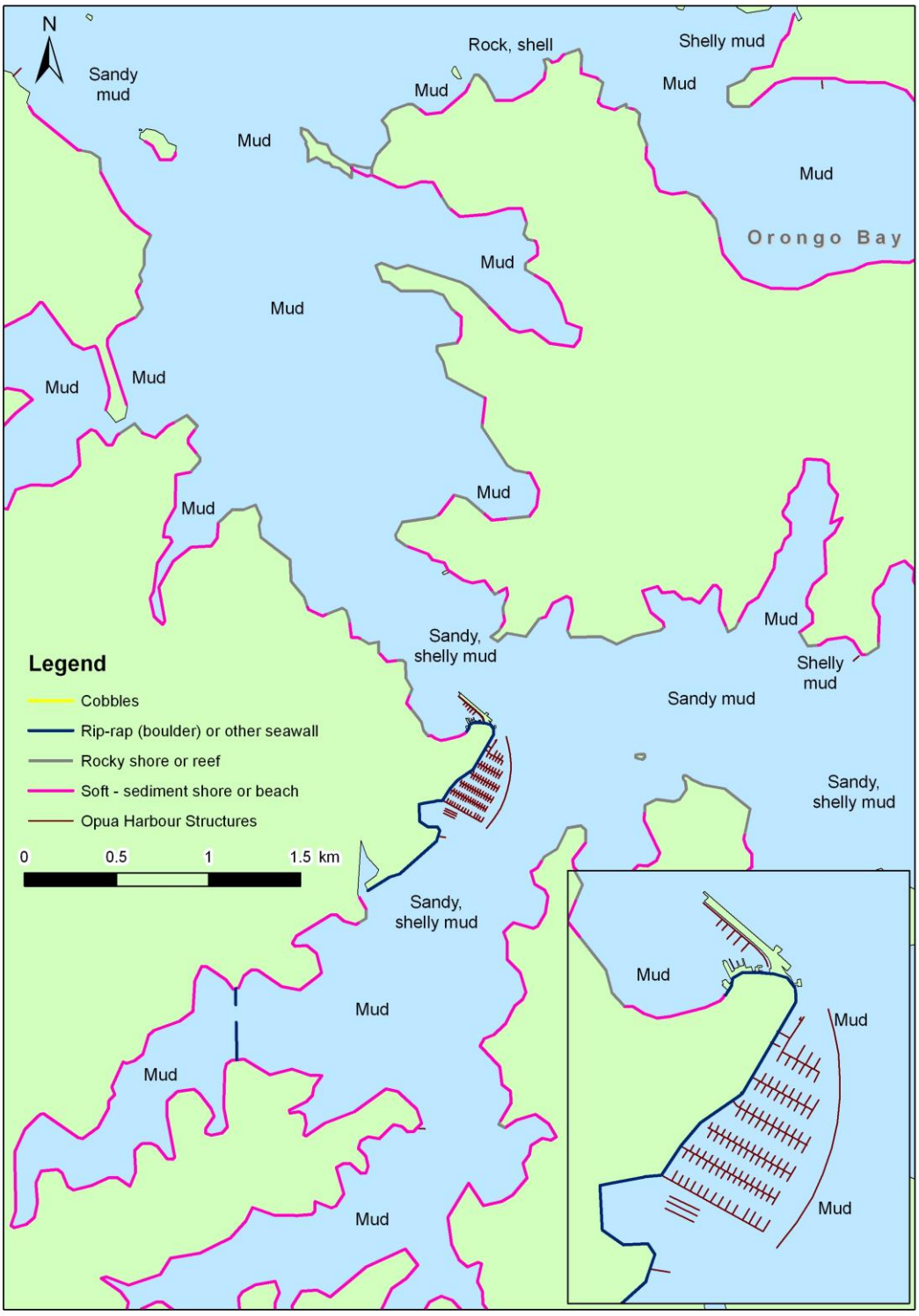
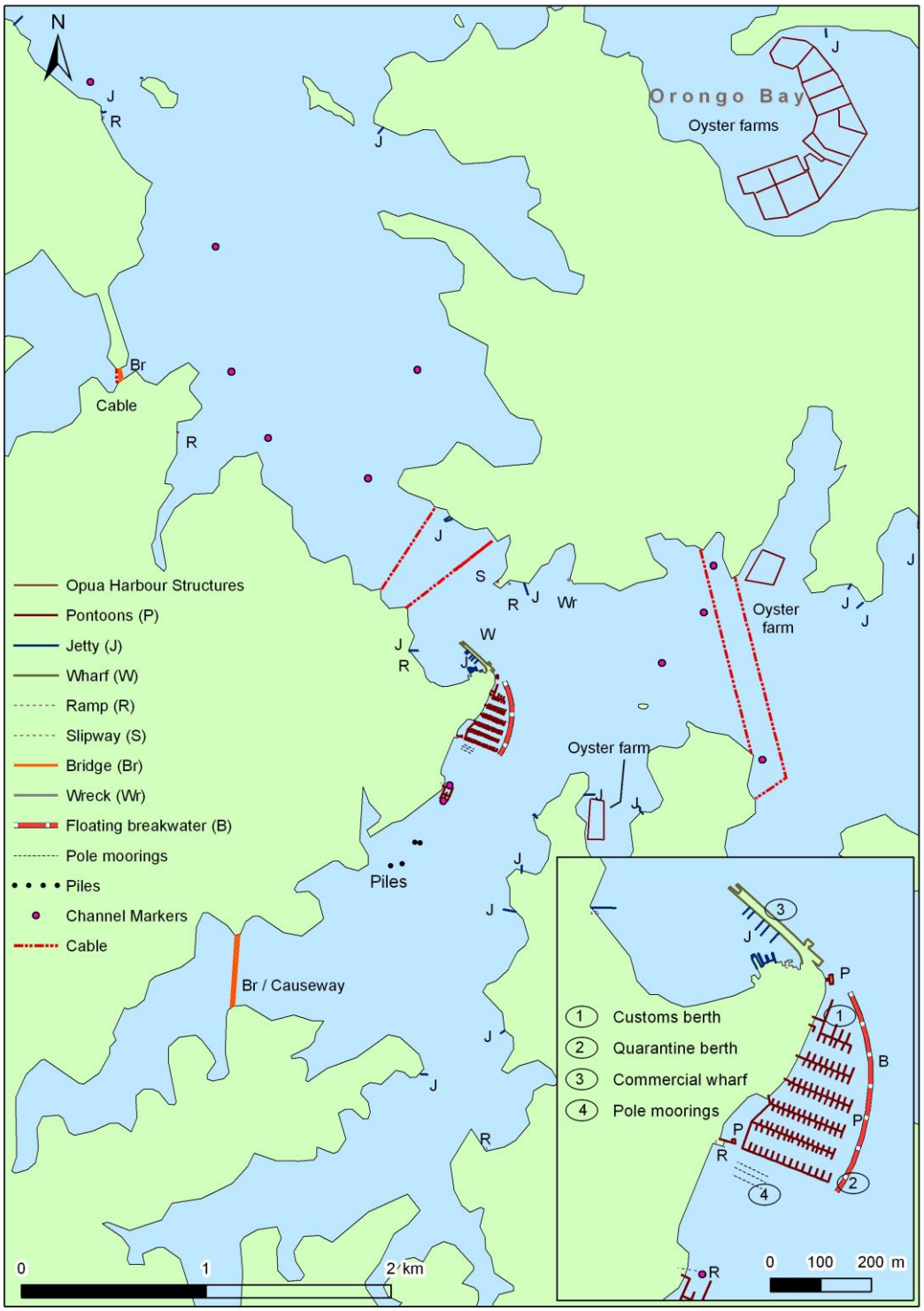


Figure 1b **Map of the sampling area around Opua Marina showing artificial structures present.**



EXISTING INFORMATION ON MARINE PESTS

There appears to be no published biological survey information available for Opua Marina and only limited information available on non-indigenous marine species in the Bay of Islands. The following summary is taken from Inglis *et al*. (in prep.).

Cranfield *et al*. (1998) conducted a desktop review to compile a list of species that are adventive in New Zealand. They reported 151 adventive species and provided an indication of their current ranges within New Zealand, the likely means of introduction, and their probable native ranges. Those listed as having been recorded from Opua, the Bay of Islands or attributed the general range of the north or north east of the North Island were the algae *Colpomenia durvilleae* and *Polysiphonia sertularioides*, the cord grass *Spartina alterniflora*, the sponges *Halichondria panicea* and *Hymeniacidon perleve*, the caryophyllids *Hoplangia durotrix* and *Tethocyathus cylindraceus*, the molluscs *Aeolidiella indica, Crassostrea gigas, Eubranchus agrius, Janolus hyalinus, Limaria orientalis, Lyrodus mediolobatus, Okenia plana, Polycera hedgpethi* and *Theora lubrica,* the decapods *Dromia wilsoni* and *Plagusia chabrus,* and the bryozoans *Bugula flabellata, Bugula neritina, Bugula stolonifera, Conopeum seurati, Cryptosula pallasiana, Electra tenella* and *Schizoporella errata*. Several others were reported to occur throughout New Zealand, including the cord grass *Spartina anglica*, the sponges *Clathrina coriacea, Cliona celata, Dendya poterium, Leucosolenia botryoides, Sycon ciliata* and *Tethya aurantium*, the hydroids *Amphisbetia operculata* and *Plumularia setacea*, and the ascidian *Corella eumyota.*

Occasional reports of the presence of new or rare subtropical species are characteristic of the Bay of Islands, and this region of New Zealand in general (Morley and Hayward 1999; Francis *et al*. 1999). Influxes of tropical and subtropical species occur mostly during warm summers and periods when the East Auckland Current is brought close to the coastline. Many of these species do not persist for longer than a single generation and are often the result of single recruitment events (Francis *et al*. 1999).

Morley and Hayward (1999) used benthic dredging to survey marine molluscs within the Bay of Islands. They reported that the area has the most diverse molluscan fauna of any area of similar size in New Zealand, with 551 species (389 gastropods, 139 bivalves, 20 chitons, two scaphopods and one shelled cephalopod) recorded from intertidal to 60 m depths, estuarine to exposed oceanic, rocky shores to muddy seafloor. The high diversity is a result of the wide range of habitats present and the area's location within the warm waters of the Aupourian Province (east coast of the northern North Island). Among the molluscs recorded were five non-indigenous species: the bivalves *Crassostrea gigas*, *Limaria orientalis*, *Musculista senhousia*, and *Theora lubrica*, and the gastropod *Microtralia occidentalis*.

The Bay of Islands is an important area of commercial oyster aquaculture, with the main species under culture in the bay being the non-indigenous Pacific oyster, *Crassostrea gigas*. The farming method is predominantly rack-culture whereby oysters are seeded onto structures erected at the optimum growing level on the lower intertidal shore. Some growing areas in the Bay of Islands have had increasing problems with water quality as the surrounding human population and use of marine environments in the Bay of Islands have grown. In 1976, one of the major growing areas, Waikare Inlet, was listed as a slightly polluted estuary system (McLay 1976). In 2001 an outbreak of Norwalk-like virus (a virus that causes gastro-enteritis and which is spread through contaminated food or water) shut down production in Waikare Inlet and forced some farmers to abandon their leases.

Sites within the Bay of Islands are regularly surveyed for harmful algae and biotoxins associated with Neurotoxic Shellfish Poisoning (NSP), Paralytic Shellfish Poisoning (PSP), Amnesic Shellfish Poisoning (ASP) and Diarrhetic Shellfish Poisoning (DSP) toxins at levels that present a risk to human health (Hay *et al*. 2000). Following a bloom of the toxic dinoflagellate *Gymnodinium catenatum* in 2000 on the west coast of the North Island, Taylor and MacKenzie (2001) undertook a national delimitation survey to determine the distribution of *G. catenatum* that included a series of sediment samples from Orongo Bay in the Bay of Islands. No evidence was found of resting cysts of this species at the site.

Gust *et al*. (2006a) carried out rapid delimitation surveys on behalf of Biosecurity New Zealand for the invasive tunicate *Styela clava* in 26 ports, marinas and harbours nationwide. The nationwide survey was initiated after the clubbed tunicate was found to be widespread in the Viaduct Basin in Auckland in mid-October 2005 (Gust *et al*. 2005). It is now widespread throughout the Hauraki Gulf, with high density populations (tens to hundreds per m^2) established near Waiheke Island. A southern population is also well established in Lyttelton Harbour near Christchurch (Gust *et al*. 2006a). No *S. clava* were recorded from Opua Marina during the delimitation survey, although this may reflect insufficient sampling power rather than species absence. Opua Marina and nearby structures were searched again specifically for *Styela clava* during further delimitation surveys, following the report of *S. clava* from the hull of the vessel "*R Tucker Thompson*" moored at the old wharf outside Opua Marina in May 2006. Again, no *S. clava* were detected in Opua (Gust *et al*. 2006b).

The following summary is derived from the report on the second baseline biological survey of Opua Marina (Inglis *et al*. in prep.).

An initial baseline survey of Opua Marina was completed in November 2002 (Inglis *et al*. 2005). The report identified a total of 122 species or higher taxa. They consisted of 76 native species, 12 non-indigenous species, 14 cryptogenic taxa (those whose geographic origins are uncertain[2]) and 20 indeterminate taxa (taxa for which there is insufficient taxonomic or systematic information available to allow identification to species level).

Following the first survey, four species were re-classified as a result of new information or re-examination of specimens during identification of material from the second baseline survey. For example, the ascidian *Microcosmus australis* has been re-classified from native to cryptogenic category 1, the bivalve "*Mytilus* sp. OPX" has been re-classified from cryptogenic category 2 to cryptogenic category 1, the crabs *Pilumnopeus serratifrons* and *Plagusia chabrus* have been re-classified from cryptogenic category 1 to native, and the barnacle *Balanus trigonus* has been re-classified from cryptogenic category 1 to native. The revised summary statistics for the first baseline survey of Opua Marina following re-classification were 79 native species, 12 non-indigenous species, 18 cryptogenic taxa and 16 indeterminate taxa. These revisions have been incorporated into the following comparison of data from the two surveys.

Three taxa collected from the first baseline survey of Opua Marina, all of which are considered cryptogenic or indeterminate, had not previously been recorded from New Zealand waters. The three taxa included a mussel whose taxonomic affinities are uncertain (*Mytilus* sp.), an ascidian (*Pyura* sp. OPX) and a sponge (*Haliclona* n. sp. 9).

[2] 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.

The 12 non-indigenous species recorded from the first baseline survey of Opua Marina included representatives of six major taxonomic groups. The non-indigenous species detected were: *Polydora cornuta* (Annelida)*, Apocorophium acutum* (Arthropoda)*, Bugula flabellata*, *Bugula neritina*, *Watersipora subtorquata* (Bryozoa)*, Obelia longissima* (Cnidaria), *Musculista senhousia*, *Crassostrea gigas*, *Limaria orientalis*, *Theora lubrica, Polycera hedgpethi* (Mollusca), and *Polysiphonia sertularioides* (Rhodophyta). Seven out of 12 (58%) non-indigenous species recorded in the Opua Marina first baseline survey were likely to have been introduced in hull fouling assemblages, 8.5% (one species) via ballast water and 33.5% (four species) could have been introduced by either ballast water or hull fouling vectors.

A total of 172 species or higher taxa were identified from the second survey of Opua Marina in November 2005. This collection consisted of 108 native, 15 cryptogenic, and 13 non-indigenous species, with the remaining 36 taxa being indeterminate. In comparison, 125 taxa were recorded from the first survey of the marina in November 2002, comprising 79 native species, 18 cryptogenic taxa, 12 non-indigenous species, and 16 indeterminate taxa. The biota in the second survey included a diverse array of organisms from 13 phyla.

The 108 native species recorded during the second survey of Opua Marina represented 62.8% of all species identified from this location and included diverse assemblages of annelids (31 species), arthropods (27 species), molluscs (15 species), chordates (12 species), rhodophyta (three species), porifera (two species) and a bryozoan. A number of other major taxonomic groups including echinoderms, dinoflagellates and cnidarians were also recorded from the Marina.

Cryptogenic species represented 8.7% (15 species) of all taxa recorded from the Marina. The cryptogenic organisms identified included six (3.5%) cryptogenic category 1 (C1) and nine (5.2%) cryptogenic category 2 (C2) taxa. These organisms included five ascidians, five annelids, four poriferans and one bryozoan. Three of the C1 taxa (the bryozoan *Scruparia ambigua* and the ascidians *Botrylloides leachi* and *Microcosmus squamiger*) and seven of the C2 taxa (the annelids *Perinereis Perinereis*-A, *Eulalia Eulalia-NIWA-2*, *Eusyllin*-unknown *Eusyllin*-unknown-A and *Demonax Demonax*-B and the poriferans *Haliclona* new sp. 2, *Esperiopsis* new sp. 1 and *Dictyociona* cf. *atoxa*) recorded in the second survey were not recorded in the first baseline survey of the marina. Conversely, of the 18 cryptogenic taxa recorded in the first baseline survey of Opua Marina, six of the nine C1 taxa (the ascidian *Microcosmus australis*, the hydroids *Bougainvillia muscus*, *Clytia hemisphaerica* and *Phialella quadrata*, the mollusc *Mytilus* sp. OPX and the dinoflagellate *Gymnodinium catenatum*) and seven of the nine C2 taxa (the annelids *Mystides Mystides*-B, *Phyllodocidae*-unknown *Phyllodocidae*-01, *Branchiomma Branchiomma*-A and *Paraprionospio Paraprionospio*-A [pinnata], the ascidian *Pyura* sp. OPX, and the poriferans *Halichondria* new sp. 4 and *Haliclona* new sp. 9) were not found during the second survey. Several of the C1 taxa (e.g. the ascidians *Astereocarpa humilis*, *Botrylloides leachii* and *Corella eumyota*) have been present in New Zealand for more than 100 years but have distributions outside New Zealand that suggest non-native origins (Cranfield 1998).

The 13 non-indigenous species recorded in the second survey of Opua Marina included seven bryozoans, two molluscs, one annelid, one red alga, one amphipod and one ascidian. None of these NIS are new records for New Zealand. Seven of the NIS found in the second survey were not recorded during the first baseline survey in November 2002. These were the polychaete *Hydroides ezoensis*, the bryozoans *Bugula stolonifera*, *Conopeum seurati*, *Schizoporella errata* and *Bowerbankia gracilis*, the ascidian *Ciona intestinalis* and the red alga *Neosiphonia subtilissima*. Six NIS recorded in the first survey were not recorded in the

second survey. These were the polychaete *Polydora cornuta*, the hydroid *Obelia longissima*, the molluscs *Musculista senhousia*, *Limaria orientalis* and *Polycera hedgpethi* and the red alga *Polysiphonia sertularioides*.

Thirty-six organisms from Opua Marina were classified as indeterminate taxa. If each of these organisms is considered a species of unresolved identity, then together they represent 21% of all species collected during the survey. Indeterminate taxa from Opua Marina included eight arthropods, seven annelids, four cnidarians, four molluscs, three myzozoans, two chlorophytes, three echinoderms, two chordates, two rhodophytes and one poriferan.

Table 3 Non-indigenous marine species recorded from Opua Marina during the first (T1) and second (T2) baseline surveys. Likely vectors of introduction are derived mainly from Cranfield *et al*. (1998), where "H' indicates hull fouling and 'B' indicates ballast water transport. Novel NIS not listed in Cranfield *et al*. (1998) or previously encountered in New Zealand are marked as new records (NR). For these, and other species for which information is scarce, we provide dates of first detection rather than probable dates of introduction.

Phylum, Class	Order	Family	Genus and species	Date of introduction or detection	Probable means of introduction	T1*	T2*	Location in Opua Marina
Annelida								
Polychaeta	Sabellida	Serpulidae	*Hydroides ezoensis*	April 2003	H	0	1	Jetty B, Quarantine Berth, Jetty C Pontoon
Polychaeta	Spionida	Spionidae	*Polydora cornuta*	Pre-1972	H or B	1	0	D44
Arthropoda								
Malacostraca	Amphipoda	Corophiidae	*Apocorophium acutum*	Pre-1921	H	1	1	Jetty B, Jetty C, Jetty D, Jetty E, D44
Bryozoa								
Gymnolaemata	Cheilostomata	Bugulidae	*Bugula flabellata*	Pre-1949	H	1	1	Jetty D, Jetty F, Quarantine Berth, Quarantine
Gymnolaemata	Cheilostomata	Bugulidae	*Bugula neritina*	Probably 1949	H	1	1	Jetty B Pontoon, Jetty C Pontoon, Quarantine
Gymnolaemata	Cheilostomata	Bugulidae	*Bugula stolonifera*	1962	H	0	1	Jetty B, Jetty C, Quarantine Berth
Gymnolaemata	Cheilostomata	Electridae	*Conopeum seurati*	Pre-1963	H	0	1	Quarantine Berth
Gymnolaemata	Cheilostomata	Schizoporellidae	*Schizoporella errata*	Pre-1960	H	0	1	Jetty B Pontoon, Quarantine Berth
Gymnolaemata	Cheilostomata	Watersiporidae	*Watersipora subtorquata*	Pre-1982	H or B	1	1	Quarantine Berth, C3, D4
Gymnolaemata	Ctenostomata	Vesiculariidae	*Bowerbankia gracilis*	Pre-1965	H or B	0	1	Quarantine Berth, Jetty C Pontoon
Chordata								
Ascidiacea	Enterogona	Cionidae	*Ciona intestinalis*	Pre-1950	H	0	1	Jetty D
Cnidaria								
Hydrozoa	Hydroida	Campanulariidae	*Obelia longissima*	Pre-1928	H	1	0	Quarantine

Table 3 Continued.

Phylum, Class	Order	Family	Genus and species	Date of introduction or detection	Probable means of introduction	T1*	T2*	Location in Opua Marina
Mollusca								
Bivalvia	Mytiloida	Mytilidae	*Musculista senhousia*	1978	H or B	1	0	C3, D44
Bivalvia	Ostreoida	Ostreidae	*Crassostrea gigas*	1961	H	1	1	Quarantine, C3, D44, end of B – C, Jetty B, Jetty D, Jetty F
Bivalvia	Pterioida	Limidae	*Limaria orientalis*	Pre-1972	H or B	1	0	D44
Bivalvia	Veneroida	Semelidae	*Theora lubrica*	1971	B	1	1	Quarantine Berth, Outer break wall, , Old wharf outer, Jetty B Pontoon, Jetty A, Jetty B, Jetty C, Jetty D, Jetty E, Jetty F, D44, C3, between B - C, between C - D, between D - E, between E - F, end of D - F, between Customs - B, F, outside of F
Gastropoda	Nudibranchia	Polyceridae	*Polycera hedgpethi*	1970s	H	1	0	Quarantine
Rhodophyta								
Florideophyceae	Ceramiales	Rhodomelaceae	*Neosiphonia subtilissima*	Pre-1974	H	0	1	Jetty B Pontoon
Florideophyceae	Ceramiales	Rhodomelaceae	*Polysiphonia sertularioides*	Pre-1938	H	1	0	C3

* 1 = Present, 0 = Absent

Table 4 Cryptogenic marine species recorded from Opua Marina in the first (T1) and second (T2) baseline surveys. "C1"- category 1 cryptogenic species, "C2" - category 2 cryptogenic species (refer to text for definitions).

Phylum, Class	Order	Family	Taxon name	Status	T1*	T2*
Annelida						
Polychaeta	Phyllodocida	Nereididae	*Perinereis Perinereis*-A	C2	0	1
Polychaeta	Phyllodocida	Phyllodocidae	*Mystides Mystides*-B	C2	1	0
Polychaeta	Phyllodocida	Phyllodocidae	*Phyllodocidae*-unknown *Phyllodocidae*-01	C2	1	0
Polychaeta	Phyllodocida	Phyllodocidae	*Eulalia Eulalia*-NIWA-2	C2		1
Polychaeta	Phyllodocida	Syllidae	*Eusyllin*-unknown *Eusyllin*-unknown-A	C2	0	1
Polychaeta	Sabellida	Sabellidae	*Branchiomma Branchiomma*-A	C2	1	0
			Demonax Demonax-B	C2		1
Polychaeta	Scolecida	Maldanidae	*Asychis Asychis*-B	C2	1	1
Polychaeta	Spionida	Spionidae	*Paraprionospio Paraprionospio*-A [*pinnata*]	C2	1	0
Bryozoa						
Gymnolaemata	Cheilostomata	Scruparіidae	*Scruparia ambigua*	C1	0	1
Chordata						
Ascidiacea	Enterogona	Rhodosomatidae	*Corella eumyota*	C1	1	1
Ascidiacea	Pleurogona	Botryllinae	*Botrylloides leachi*	C1	0	1
Ascidiacea	Pleurogona	Pyuridae	*Microcosmus australis*	C1	1	0
Ascidiacea	Pleurogona	Pyuridae	*Microcosmus squamiger*	C1	0	1
Ascidiacea	Pleurogona	Pyuridae	*Pyura* sp.	C2	1	
Ascidiacea	Pleurogona	Styelidae	*Asterocarpa humilis*	C1	1	1
Ascidiacea	Pleurogona	Styelidae	*Styela plicata*	C1	1	1
Cnidaria						
Hydrozoa	Hydroida	Bougainvilliidae	*Bougainvillia muscus*	C1	1	0
Hydrozoa	Hydroida	Campanulariidae	*Clytia hemisphaerica*	C1	1	0
Hydrozoa	Hydroida	Campanulinidae	*Phialella quadrata*	C1	1	0
Mollusca						
Bivalvia	Mytiloida	Mytilidae	*Mytilus* sp. OPX	C1	1	0
Myzozoa						
Dinophyceae	Gymnodiniales	Gymnodiniaceae	*Gymnodinium catenatum*	C1	1	0

Table 4 Continued.

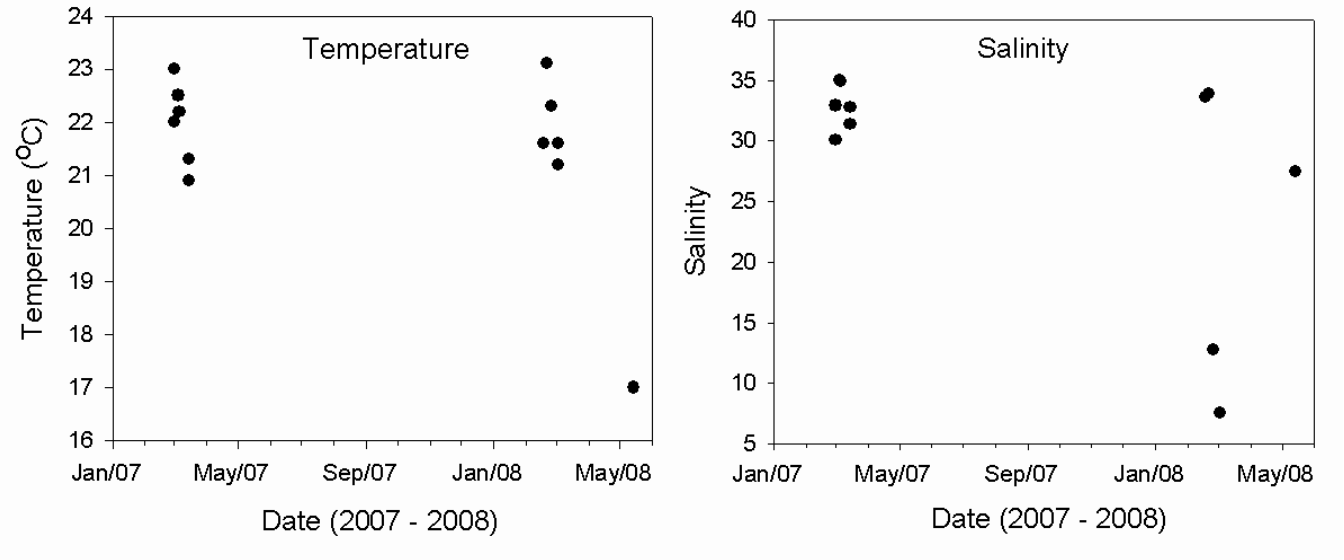
Porifera						
Demospongiae	Halichondrida	Halichondriidae	*Halichondria* new sp. 4	C2	1	0
Demospongiae	Haplosclerida	Chalinidae	*Haliclona* new sp. 2	C2	0	1
Demospongiae	Haplosclerida	Chalinidae	*Haliclona* new sp. 3	C2	1	1
Demospongiae	Haplosclerida	Chalinidae	*Haliclona* new sp. 9	C2	1	0
Demospongiae	Poecilosclerida	Esperiopsidae	*Esperiopsis* new sp. 1	C2	0	1
Demospongiae	Poecilosclerida	Microcionidae	*Dictyociona* cf. *atoxa*	C2	0	1

* 1 = Present, 0 = Absent

BIOPHYSICAL CONDITIONS

Data collected by Northland Regional Council 200 m southwest of Opua Marina (site number 105715) in March 2007, and February, March and June 2008 show that water temperatures varied between ca. 21 and 23°C in March of both years to ca 17°C in May 2008 (Figure 2). Salinity varied between 30 psu and 35 psu in March 2007. In February/March 2008 salinity decreased rapidly from ca. 34 to 7.

Figure 2 Water temperature (°C) and salinity 200 m southwest of Opua Marina in 2007 and 2008. Data courtesy of Northland Regional Council.



HABITAT TYPES WITHIN THE SURVEY AREA

Opua Marina (35° 18' S, 174° 07 'E) on the east coast of the North Island is located on Waimangaroa Point in the southwest of the Bay of Islands (Figure 1). The marina is linked to the main Bay of Islands via the Veronica Channel to the north-west. The Kawakawa River is to the immediate south and Waikare Inlet to the immediate east of the marina. The marina is 27 km from Cape Brett, the entrance to the Bay of Islands, and within close proximity to the towns of Russell and Paihia. Opua Marina is a major hub for recreational vessels in the North Island (Inglis 2001). It is often the first port of entry for recreational vessels entering New Zealand. A convex breakwater protecting the marina bounds the main Veronica Channel eastwards, which is only 350 m wide at its narrowest between Waimangaroa Point and Tapu Point. The smaller northern entrance of the marina is approximately 60 m wide, whilst the larger southwestern entrance is approximately 160 m wide. Minimum depth in the marina is 2 m at LWS. The marina seabed is composed mostly of sand. Tidal range varies between 1.6 and 2.0 m.

Types of habitat present in Opua Marina and the surrounding area are listed in Table 5. Given that much of the available habitat for incoming non-indigenous species is represented by artificial substrata within the port, 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 the whole port and the available habitat area is structurally extremely complex. As part of the delimitation survey for *Styela clava* Gust *et al*. (2006a) estimated the length of artificial habitat (piling, pontoons, breakwalls) present in Opua Marina to be 5196 m. This value was derived from GIS maps of the areas and represents the horizontal length of structures present. Clearly it does 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 11300 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*. 2006a).

Table 5 Types of habitat present in the survey area.

Habitat category	Habitat type	Habitat subdivision	Location
Soft-surface	Mud		Opua Marina, Orongo Bay, Pipiroa Bay, Te Wahapu Inlet, Veronica Channel, Kawakawa River, Haumi River
	Sandy shelly mud		Veronica Channel, Waikare Inlet, Kawakawa River
	Sandy Mud		Waikare Inlet, Paihia
	Mangroves		Kawakawa River, Whangae River
Hard-surface	Emergent reef		Motutokape Island, Motuarahi Island, Lewin Rocks
	Rocky shore		Okaito Point, Tapu Point, Toretore Island, Mukimuki, Te Wahapu Point
	Artificial structures	Commercial vessel berth	Opua Wharf
		Channel markers	Veronica Channel, Waikare Inlet
		Boat ramp	Opua Wharf
		Marina (pontoons, piles)	Opua Marina
		Jetty/Breakwater/sea walls	Rubble and rip-rap breakwalls at Opua Marina and Wharf. Jetties at Opua Wharf, Tapu Point, Okaito Point. Boulder wall at causeway over Whangae River and Haumi River
		Slipway	Opua Wharf, Tapu Point, Okaito Point
		Moorings	Opua Marina
		Bridge (concrete piles) and causeway	Whangae River and Haumi River
		Inactive/disused berth	Not in survey area
		Aquaculture facility (Oyster farms)	Orongo Bay, Waikare Inlet
Pelagic	Water column	Top, middle, bottom.	Throughout survey area

IDENTIFICATION OF VECTOR PARAMETERS

Opua Marina has been operational since 2000 and is owned by Far North Holdings Ltd. The Marina has 235 berths, ranging in length from 10.5 to 27 m, with floating concrete berths on H6 marine-grade pine pilings inside the marina and steel pilings on the breakwater (www.opuamarina.co.nz). An additional 30 swing moorings plus several pile moorings are

also available outside the marina breakwater. There are numerous private swing moorings in the nearby bays, particularly in the Waikare Inlet, which can provide additional mooring for vessels waiting to enter the marina. A commercial wharf with berthage of up to 40 m is also available by prior arrangement.

Located adjacent to the marina southwards is Ashby's Boatyard with slipway and floating berths, which services and builds recreational vessels (www.ashbyboats.co.nz). The quarantine and customs area within Opua Marina is located on the northern half of the marina breakwater.

During its construction, the depth inside Opua Marina was dredged down to a baseline of 2 m. No dredging inside the marina has been required since then, and the main channel adjacent to the marina is not dredged.

In terms of future development, it is proposed to extend the marina southwards towards the Kawakawa River within the next five years, with extra berthing for 60-80 vessels up to 30 m in length (www.opuamarina.co.nz). Far North Holdings Ltd opened 162 m of marina-style facilities for smaller super yachts (10 berths up to 50 m) alongside the old Opua Wharf in 2004 and propose to further develop the old wharf, which is currently used by charter and fishing vessels. This development is intended as a step towards the overall development of Opua as a servicing and support centre for visiting recreational boats (www.fnhl.co.nz). Indicators point to burgeoning recreational vessel traffic within the Bay of Islands.

The original wharf at Opua (immediately to the northwest of the marina) was constructed in the early 1880's to load coal from Kawakawa, and continued this role until the late 1890's. In 1921 when the freezing works at Moerewa became operational, Opua developed into a significant meat export port although water depth restricted loading to the use of lighters. Dredging and wharf extension occurred in 1922, with further development in the 1950's. The last cargo vessel to use the wharf was in 1983, with passenger cruise ships using the wharf between 1983 and 1992. Customs and MAF were originally located at the wharf, but relocated to the new marina in 2000. A ferry operates between Russell and Opua more or less continuously from morning to late evening, carrying cars and passengers.

Vessel movements and ballast discharge patterns at Opua

Since June 2005, vessels arriving in New Zealand have been required to comply with the Import Health Standard for Ships' Ballast Water from All Countries (www.fish.govt.nz/sustainability/biosecurity). No ballast water is allowed to be discharged without the express permission of an MAF (Ministry of Agriculture and Forestry) inspector. To allow discharge, vessels Masters are responsible for providing the inspector with evidence of either: discharging ballast water at sea (200 nautical miles from the nearest land, and at least 200m depth); demonstrating ballast water is fresh (2.5 ppt sodium chloride) or having the ballast water treated by a MAF approved treatment system. The Bay of Islands, in which Opua Marina is situated, is a no-discharge area for vessels.

The number of overseas yachts travelling to New Zealand has increased dramatically over the last three decades. Data from the New Zealand Customs Service show that around 900 international yachts visited New Zealand in 2000, almost three times as many as in 1993 (Inglis and Floerl 2002). Overseas recreational vessels visiting New Zealand from international waters commonly arrive from Fiji, Tonga, New Caledonia, Australia (Coffs Harbour, Lord Howe Island, Brisbane, Sydney, Norfolk Island, Bundaberg, Gladstone, Southport, Townsville, Launceston), Cook Islands, Vanuatu, Western Samoa, American Samoa, Niue, French Polynesia and the US Pacific Dependency (Inglis and Floerl 2002).

Interviews with marina operators suggest that the majority of overseas vessels entering New Zealand waters spend most of their time in Northland (which encompasses Opua) and Auckland and do not travel further south than Tauranga. The peak period for arrivals of international yachts is between October and December as the vessels move south to avoid the austral tropical cyclone season, with most vessels departing in April and May when the cyclone season has ended (Inglis and Floerl 2002).

Opua Marina accounts for almost 70% of all international recreational craft visits to New Zealand (Campbell, 2004). In 2002/2003, there were 552 international pleasure vessel arrivals to Opua Marina, and an additional four international arrivals by larger commercial vessels (one merchant and three passenger vessels; Campbell, 2004). The New Zealand Customs Service estimates that at least 80% of the boats that clear customs in Opua spend at least one night at Opua Marina. Telephone surveys of marina operators in 2005 were used to gather estimates on annual domestic and international vessel movements from marinas nationwide (O. Floerl, NIWA, unpublished data). Estimates indicate that around 950 vessels arrive at Opua Marina annually, with around 47% of these being international arrivals and 53% domestic arrivals. An estimated 5,000 trips are undertaken by local boats per year from Opua Marina. Most of these occur during the summer season (70%), followed by 15% of trips undertaken in autumn, 10% in spring and 5% in winter (O. Floerl, NIWA, unpublished data).

Data on the domestic movements of recreational yachts to and from Opua Marina were derived from a questionnaire survey of approximately 1,300 yacht owners, conducted in 2002 - 2003 (O. Floerl, NIWA, unpublished data). National survey information was used to create an epidemiological model simulating yacht movements between main marinas around NZ. Annual movements of yachts between marinas were calculated from a 10-year simulation. The calculated average annual number of recreational vessels departing Opua Marina and heading to one of 36 domestic destination ports was 1,282. The five most common destination ports for vessels travelling from Opua were: Auckland's Westhaven Marina (290 vessels departing Opua for this marina annually), Gulf Harbour Marina (192), Whangarei Marina (139), Tutukaka Marina (120) and Auckland's Bayswater Marina (113). A similar trend was seen in recreational vessels arriving at Opua Marina (1,266 arrivals annually). The five most common origin ports were Auckland's Westhaven Marina (288), Gulf Harbour Marina (187), Whangarei Marina (135), Tutukaka Marina (120) and Auckland Bayswater Marina (111; O. Floerl, NIWA, unpublished data).

Possible vectors for the introduction of non-indigenous species

The non-indigenous species located in Opua Marina are thought to have arrived in New Zealand via international movement of recreational vessels. They may have reached Opua Marina directly from overseas or through domestic spread (natural and/or anthropogenic) from other New Zealand ports. Likely vectors of introduction are largely derived from Cranfield *et al*. (1998) and expert opinion. They suggest that only one of the 19 NIS (5%) probably arrived via ballast water, 13 species (68%) were most likely to be associated with hull fouling, and five species (26%) could have arrived via either of these mechanisms.

Assessment of the risk of new introductions to the marina

Many non-indigenous species introduced to New Zealand ports by movement of recreational and commercial vessels 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 northwest Pacific, and southern Australia (Cranfield 1998).

Between 2002 and 2003, there were 556 vessel arrivals from overseas to Opua Marina, with all but four being pleasure vessels (Campbell 2004). The majority of international recreational vessel arrivals to New Zealand come from the South Pacific (around 80%) or Australia (16%; O. Floerl, NIWA, pers. comm., Feb 2007). These vessels commonly arrive from Fiji, Tonga, New Caledonia, Australia (Coffs Harbour, Lord Howe Island, Brisbane, Sydney, Norfolk Island, Bundaberg, Gladstone, Southport, Townsville, Launceston), Cook Islands, Vanuatu, Western Samoa, American Samoa, Niue, French Polynesia and the US Pacific Dependency (Inglis and Floerl 2002). Almost all of these are tropical locations with coastal environments dissimilar to those of New Zealand. However, southern Australian locations, such as Sydney, are in temperate regions that have coastal environments similar to New Zealand's. Due to the environmental similarities and relatively short transit times, vessels arriving from Sydney present perhaps the greatest risk of introducing new non-indigenous species to the Opua Marina.

Assessment of translocation risk for introduced species found in the marina

The Westhaven Marina in Auckland, Gulf Harbour Marina, Whangarei Marina, Tutukaka Marina, and the Bayswater Marina in Auckland were the next ports of call for the most domestic vessel movements from Opua. Although many of the non-indigenous species found in the second survey of Opua Marina have been recorded in other locations throughout New Zealand, they were not detected in all of the other ports surveyed. Therefore, there is a risk that species established in Opua Marina could be spread to other New Zealand locations.

Several other species recorded during the second baseline survey have relatively restricted distributions nationwide and could, therefore, be spread from Opua to other locations. These include the annelid *Hydroides ezoensis* and the bryozoans *Bugula stolonifera*, *Schizoporella errata* and *Bowerbankia gracilis*.

LOCAL CONSTRAINING FACTORS ON SURVEILLANCE SUCCESS

Local factors likely to constrain sampling, including those representing hazards to field team members, are listed in Table 6, together with management actions 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 for Opua is unknown	Planktonic propagules are only likely to remain in the marina and wharf area for a limited time following release and are liable to be dispersed to other parts of the harbour.	Y	Dispersal of larvae is likely to occur throughout the inner harbour and these areas are therefore included in the survey area.
Turbidity (Secchi disk depth < 5 m)	Turbidity high in the marina and wharf area, especially on ebbing tide and after rain, and average in the lower reaches of the harbour.	I	Variability in detection probability of diver searches
Predominant wind direction is variable.	No modelling of dispersal from Opua Marina to predict larvae accumulation in the area. Boat handling can be difficult in exposed areas during high winds and may constrain sampling work from boats.	I	Dispersal of sampling effort incorporates the areas that larvae are predicted to reach under predominant wind conditions, based on local knowledge of the area. Opua Marina is relatively protected and sheltered areas to work can generally be found.
Wind speed	Seasonally variable.	Y	Opua Marina is relatively protected and sheltered areas to work can generally be found.
Tidal currents may be strong around the wharfs during flood and ebb tides, especially during spring tides	Tidal range is ca. 2 m and current velocities on the ebb and flood tides can be strong.	I	Diving around the wharf and Marina berths needs to be done at slack tide or as drift dives. 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 and mooring piles, etc.
Spring-neap tidal cycle	Spring high tides associated maximal current speeds and suboptimal conditions for shore searches	I	Surveys are timed to avoid spring tides because of the associated high current speeds and risk of traps being moved into harbour channels.
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 9oC	Y	Diving and other sampling still possible providing divers are adequately equipped (dry suits)
Dangerous animals	Jellyfish	I	Full-face AGA masks may be used to protect against jellyfish.

Table 6 Continued.

Hazard/Constraining Factor	Effect at Surveillance Location	Present (Y, N or intermittent (I))	Management actions
Vessel traffic	Frequent and predictable car ferry movement at Opua Wharf. Frequent and unpredictable recreational boat movement at Opua Marina.	Y	Sampling around Opua Wharf can be planned through communication with Opua Wharf at the start of each survey and during survey work to monitor changes to schedules. Marina traffic can be busy and unpredictable and will be monitored through communication with the operations manager during the survey.
Dredging & construction activities	No regular dredging in the Marina or entrance channels.	I	N/A
Cables, pipelines and other hazards to navigation	No power cables in the Marina area.	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 Marina but sewage spills may occur occasionally after heavy rain	I	Diving and other work may have to be postponed until the all-clear is posted.
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.

MARINA SECURITY ISSUES

Because the marina area is publicly accessable and entry to the marina is via the water, rather than by land, field teams are not required to obtain formal security clearance before entering the marina (Operations Manager, Opua Marina, pers. comm.). However, permission from MAF Quarantine or New Zealand Customs Service is required to access the Quarantine Berth. TheMarina Operations Manager and MAF Quarantine will be informed prior to deployment of traps and dredging. A dive flag will be displayed at all time divers are in the water and the operations manager will be informed when divers enter and exit the water.

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 MAFBNZ 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 & 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 MAFBNZ. This was accepted by MAFBNZ, as stated in the *Contract Specification Addendum* (page 51). Note that it has been agreed with

MAFBNZ 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[3]
- Has the species spread subsequently
- Association with, or dependency on, non natural dispersal mechanisms
- Strong association with artificial substrate[4]
- 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[5]
- 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, as each survey takes at least a week and a week in between surveys is required to allow equipment to be sent on to the next location.

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

DETERMINATION OF SAMPLING EFFORT

MAFBNZ 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*. 2000). 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 of replicates
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*. 2006 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 MAFBNZ 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 (Cameron 2002, Cannon 2002, Venette *et al*. 2002, Hayes *et al*. 2005, Inglis *et al*. 2006);
(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.

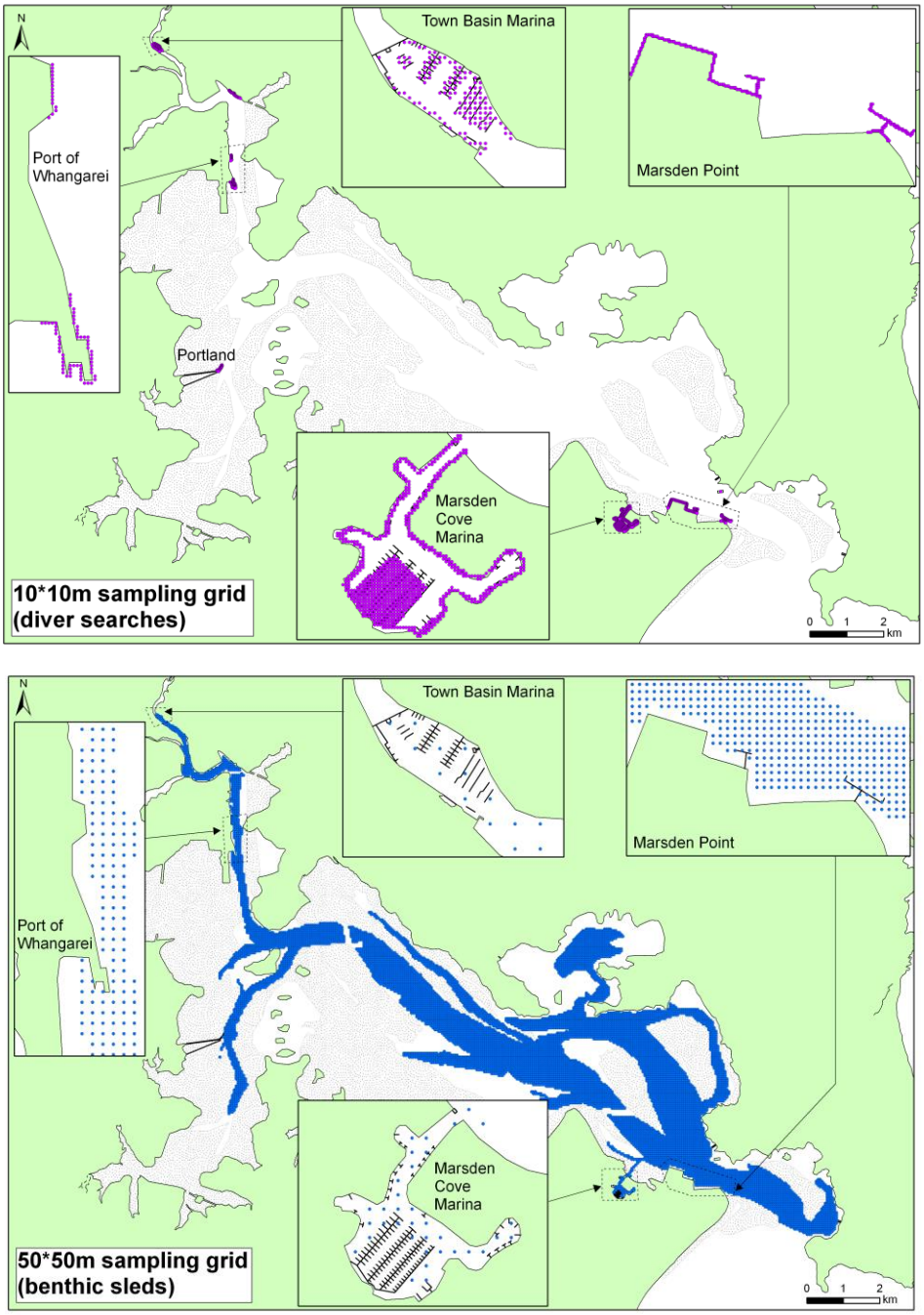
SPATIAL ALLOCATION OF SAMPLING EFFORT

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. Furthermore, because Opua was not included in the first phase of the surveillance programme (2002-2004), no hydrodynamic modelling was done for this location. Consequently, we allocated sampling effort based on our existing knowledge of the distribution of habitats in the marina and surrounding area, with highest priority given to suitable habitat for the target species and samples allocated in proportion to the relative areas of each habitat.

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.**



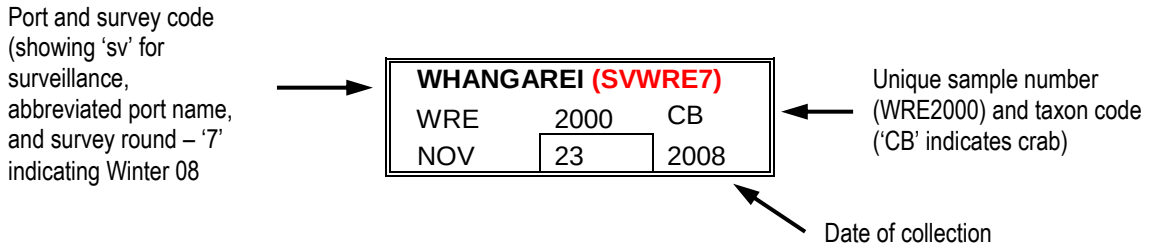
SAMPLE LABELLING AND PROCESSING

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 (2002-2004). 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 MAFBNZ'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 4.

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 4). 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 4.1), 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 4.5), 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 MAFBNZ 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 MAFBNZ Group Manager directly. MAFBNZ 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[5]. 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.

[5] Email correspondence between Brendan Gould (MAFBNZ) 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.

Acknowledgements

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Appendices

APPENDIX 1: TEMPLATE OF 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 (MAFBNZ), 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 MAFBNZ 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

MAFBNZ 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: SAMPLING DATA SHEETS

A4.1 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				

A4.2 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

A4.3 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					

A4.4 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					

A4.5 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					

A4.6 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					

A4.7 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														
												WORMS		ALGAE			ASCIDIANS			SS	OTHERS					
Site ID (e.g SVLYT6001)	**GPS CO-ORDINATES** including all symbols & decimal points (e.g. for latitude: 36º 42.887'S) **START POINT**	**END POINT**	**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)	**Sabella spallanzanni** (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)					**SAMPLE LOT NO.** (separate transect = separate sample lot no.)	**NOTES** if you can't get to pre-allocated site include reason here too
				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:																		