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REVISED PROTOCOLS FOR BASELINE PORT SURVEYS FOR INTRODUCED MARINE SPECIES: SURVEY DESIGN, SAMPLING PROTOCOLS AND SPECIMEN HANDLING

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SUMMARY

A prerequisite for any attempt to control the introduction and spread by shipping of non-indigenous marine pest species in Australian waters is knowledge of the current distribution and abundance of introduced species in Australian ports. This information base is lacking for a majority of Australian ports. The Australian Ballast Water Management Advisory Council (ABWMAC), the Standing Committee on Agriculture and Resource Management (SCARM), and the Australia and New Zealand Environment and Conservation Council (ANZECC) State of the Environment (SoE) Reporting Task Force, have all recognised the need for baseline studies to determine the extent to which introduced species have established in Australian waters. In response to these needs, the CSIRO Centre for Research on Introduced Marine Species (CRIMP) and various state agencies have commenced a national port survey program designed to define the occurrence of non-indigenous species in Australian ports.

Given the number of agencies and research organisations that will potentially participate in a national port survey program, a high priority was given to developing a standardised set of survey methods that would provide a consistent basis on which to assess the introduced species status of individual ports. Surveys designed to identify all non-indigenous species in a port will inevitably be subject to scientific, logistic and cost constraints that will limit both their taxonomic and spatial scope. Recognition of these constraints led CRIMP to adopt a targeted approach that concentrates on a known group of species and provides a cost-effective collection of baseline data for all ports. While these surveys specifically target designated pest species, they are also designed to determine the distribution and abundance of other introduced species in a port. The surveys will also identify species of uncertain status (cryptogenic, that is not known if they are endemic or introduced) that are abundant in a port and/or are likely to become major pest species.

This report reviews the general protocols developed by CRIMP for introduced species port surveys in 1996, and updates and provides evidence to support the recommended methodologies. The survey design and sampling protocols are outlined to encourage the adoption of a broad and consistent approach to the problem. Triggers for post survey monitoring regimes and factors influencing the frequency of resurveying are also discussed.

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1 INTRODUCTION

The intentional and accidental transport and introduction of marine species to new regions is currently perceived to be one of the primary threats to biological diversity (Hatcher et al. 1989; Lubchenco et al. 1991; Norse 1993; Suchanek 1994). Australian State of the Environment (SoE) reporting guidelines have identified the distribution and abundance of introduced species as one of the key biodiversity threat indicators (ANZECC 2000). Surveys have detected marine (and estuarine) biological introductions in all oceans of the world (Table 1). However, these studies differ in the scale of evaluation and the survey methodologies used and, as a consequence, it is not possible to determine if the observed patterns of biological invasions in different regions are the result of real differences between systems or merely artefacts of sampling effort or identification capacity. An understanding of invasion patterns is critical if we are to develop strategies to manage marine bio-invasions and to effectively target the limited resources that are currently available for the management of non-indigenous species.

Two primary methods can be used to identify patterns of invasions: (i) evaluations of literature records and/or specimen collections, and (ii) field surveys, targeting those habitats and areas most closely linked with known introduction vectors. Evaluations of literature and museum collection provide the broadest coverage for a region, however these evaluations are usually inconsistent in scope and typically variable in effort. Patterns derived from these sources alone can result in misleading indications of invasion rates and vector strengths (Coles et al. 1999a; Hewitt et al. 1999). Field surveys, even when targeted, are costly and will of necessity be limited in their spatial scope. If based on a consistent set of sampling methods, surveys will however, eliminate differences in effort and provide a consistent basis on which regional comparisons can be made.

Australia has ~72 trading ports that receive international and coastal shipping (Figure 1). In total these ports receive over 10,000 ship visits and around 160 million tonnes of ballast water each year (Kerr 1994). Local and overseas experience suggests that, in the absence of effective controls on ballast water uptake and discharge, and with the level of fouling on the hulls of vessels determined in part by the economics of vessel operations, the risk of the introduction of non-indigenous species to Australian waters and their subsequent translocation between ports by shipping, must be considered to be high. The Australian Ballast Water Management Strategy (AQIS 1995) recognises that "there is no known total solution to the problem at this point in time, but there are measures that can be taken to minimise the risk." A prerequisite for the adoption of a risk management approach to controlling the spread of introduced marine pest species by shipping, is a knowledge of the distribution and abundance of non-indigenous species in Australian ports (Hayes and Hewitt 1998). This information is still lacking for the majority of Australian trading ports (Figure 1).

The Australian Ballast Water Management Advisory Council (ABWMAC)[1], the Standing Committee on Agriculture and Resource Management (SCARM), and the Australia and New Zealand Environment and Conservation Council (ANZECC) SoE Task Force, have all recognised the need for baseline studies to establish the extent to which introduced species have established in Australian waters. The Joint SCC/SCFA National Task Force on the Prevention and Management of Marine Pest Incursions (SCC/SCFA 1999) recommended that baseline evaluations be undertaken for all Australian first ports of call and that a targeted approach be adopted to surveying other ports and marinas.

As no single agency or organisation is likely to undertake all surveys, key requirements for a national survey program are (i) uniformity of approach based on consistency in survey design, (ii) a standardised set of survey protocols (suitable for implementation by groups with varied skill-levels and equipment availabilities), and (iii) central archiving of survey data, reference and voucher specimens, and associated information. This latter information is central to the development of a

[1] Now known as the Australian Introduced Marine Pest Advisory Council (AIMPAC)

Table 1. Recent regional and local surveys for introduced marine and brackish water species.

Location	No. of Introduced species	Reference
United States	298[a, e]	Ruiz et al. 2000
Coos Bay, Oregon	93	Hewitt 1993; Carlton et al. unpubl data
Pearl Harbor, Hawaii	96	Coles et al. 1997; Coles et al. 1999a
Ala Wai Yacht Harbor, Hawaii	57	Coles et al. 1999b
Barbers Point, Hawaii	45	Coles et al. 1999b
Honolulu, Hawaii	73	Coles et al. 1999b
Keahi Lagoon, Hawaii	52	Coles et al. 1999b
Kewalo Basin, Hawaii	49	Coles et al. 1999b
San Francisco Bay, California	212[a]	Cohen and Carlton 1995
Chesapeake Bay, Maryland	116[a]	Ruiz et al. 1997
Puget Sound, Washington	52	Cohen et al. 1998
Baltic Sea	96[a]	Gollasch & Leppakoski 1999
New Zealand	167[a]	Cranfield et al. 1998
United Kingdom	50[b]	Eno et al. 1997
Black Sea	35[a, b]	Zaitsev & Mamaev 1997
Mediterranean Sea	240[a, c, d]	Ruiz et al. 1997
Australia (1990)	62	Pollard and Hutchings 1990a, b
Australia (2000)	210	Hewitt *in prep*
Port Phillip Bay, Victoria	99	Hewitt et al. 1999
Darwin, Northern Territory	5	Russel & Hewitt 2000; Hewitt *submitted*
Pt Hedland, Western Australia	16	Hewitt *submitted*
Fremantle, Western Australia	33	Hewitt *submitted*
Bunbury, Western Australia	12	Hewitt *submitted*
Mackay, Queensland	12	Hewitt *submitted*
Hay Point, Queensland	10	Hewitt et al. 1998; Hewitt *submitted*
Newcastle, New South Wales	25	Hewitt *submitted*
Eden, New South Wales	24	Hewitt *submitted*

[a] includes marine, brackish, freshwater and salt marsh species
[b] partial evaluation of species
[c] includes all species records, not limited to establishment
[d] includes Lessepsian migration as well as human mediated introductions
[e] limited to continental United States

national marine pest management strategy and a key component in both international and domestic ballast water, and hull fouling port-to-port risk assessments (Hayes and Hewitt 1998).

The baseline port survey program established by CRIMP in 1995 was intended to begin the process of determining the scope and scale of marine biological invasions in Australian coastal waters and at the same time provide a basis for assessing the efficacy of the recommended sampling protocols. This report provides an update to the survey protocols published in 1996 (Hewitt and Martin 1996) after five years of practical implementation. We reiterate the background information on potential introduction vectors, identify the port specific characteristics that influence survey design and provide a preliminary evaluation of the protocols based on information from the port surveys that have been conducted to date. Variations to the accepted minimum sampling protocols suggested by other participating agencies and organisations are discussed. Protocols for specimen handling and requirements for data and specimen archiving that are essential to a consistent and generalised national sampling program are presented. The report also outlines approaches to post-survey monitoring

programs and reviews the limited information that is available on invasion rates to provide an indication of resurvey frequency.

This report updates the survey standards accepted and ratified by the Australian Ballast Water Management Advisory Committee (ABWMAC), and by the Research Advisory Group (RAG) for purposes of baseline introduced species surveys of Australian ports. They are also explicitly used as the guidelines for a minimum survey standard for the AQIS Ballast Water Decision Support System (DSS). These protocols are also being trialed by the GEF/UNDP/IMO Global Ballast Water Management Programme (GloBallast) for surveys of six demonstration ports in developing countries (S. Raaymakers pers. comm.)

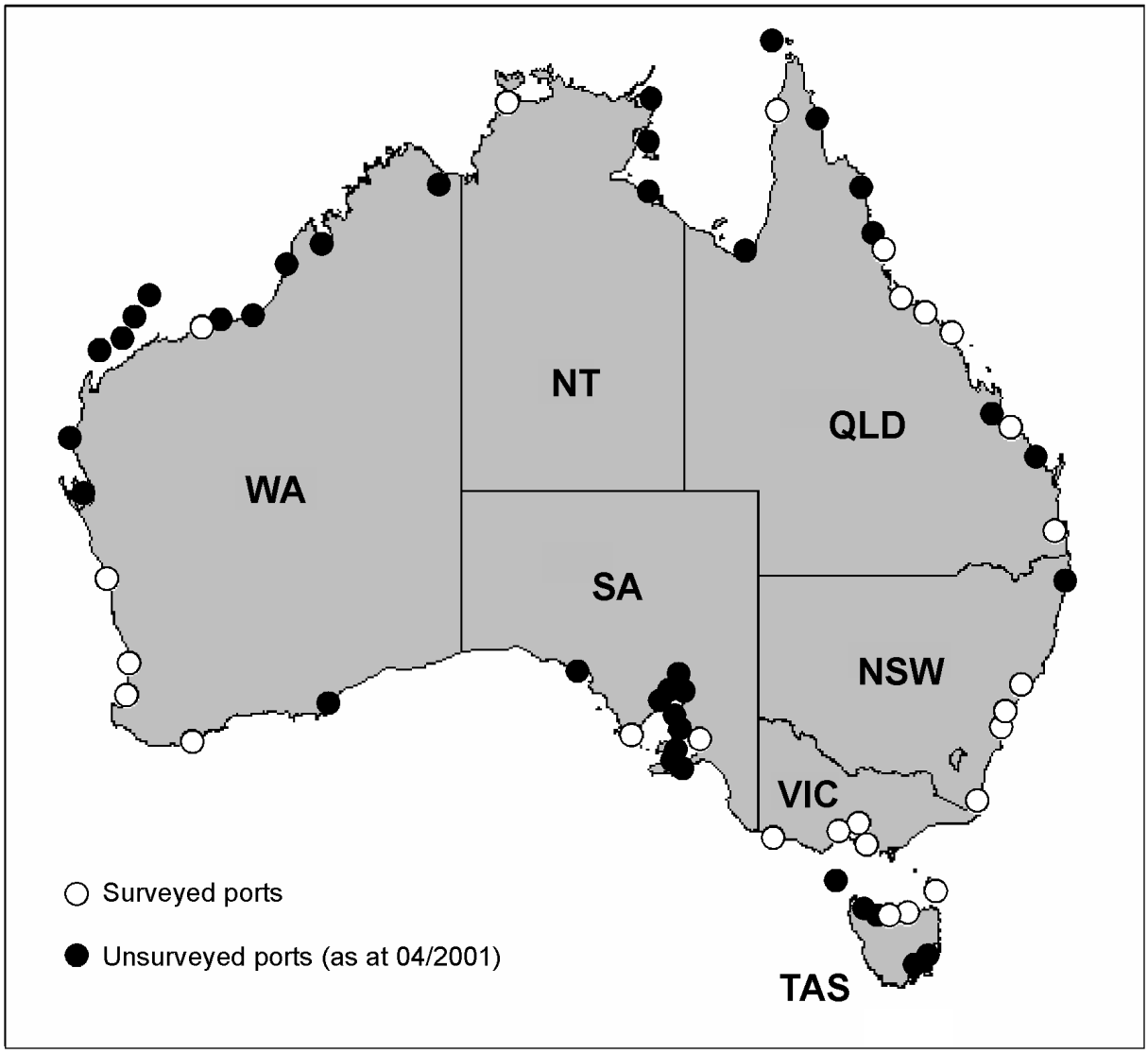


Figure 1. Australian ports receiving international and coastal shipping.

2 BACKGROUND CONSIDERATIONS

2.1 Mechanisms for the import and translocation of non-indigenous marine species

While the movement of indigenous peoples in the Australasian region may have provided a transport vector for some marine species within the Indo-Pacific bioregion, the advent of trans-bioregional introductions in the marine environment largely coincides with European expansion (see Crosby 1986; Campbell and Hewitt 1999). There are now over 200 known non-indigenous species in Australian waters (Hewitt in prep) but only four of these are known to have been deliberately introduced.

Intentional or deliberate introductions, either for stock enhancement or mariculture, are usually associated with the release of a single species. Historically, intentional releases of mariculture species (and to a lesser extent, stocked fish) may have translocated additional species as discussed below. Intentional releases of species may also result in unpredicted spread and unanticipated ecological impacts (eg. extinction of native species, alteration of habitat).

The remaining non-indigenous species in Australian waters are the result of unintentional introductions associated with shipping or mariculture activities. These unintentional introductions have come about as a result of four distinct mechanisms:

- ship hull fouling and boring;
- unintentional introductions associated with mariculture;
- dry and semi-dry ballast; and
- water ballast.

Together these mechanisms constitute multiple vectors that have acted during various phases of Australian maritime history.

Ship hull fouling and boring can be divided into two distinct vectors: wooden hulled vessels and steel hulled vessels. Wooden hulled vessels have plied the seas for centuries in exploration, conquest, trade and colonisation (Crosby 1986). The activities of commerce and colonisation led to the development of active trade routes between Europe, southern Africa, India, South East Asia, Japan, and Australasia. This vector initiated a period of faunal mixing as a result of a continual movement of both hull fouling and wood boring organisms. The globalisation of the wood-boring fauna (eg, limnorid isopods and teredinid bivalves) is evident in the cosmopolitan nature of many species. While wooden hulled vessels continue to operate in some coastal situations, since the 1950's the majority of vessels involved in international and coastal trade are steel hulled.

While the introduction of steel hulled vessels largely precluded the transport of wood boring organisms, it has often been assumed that the widespread use of toxic anti-fouling paints and the increased speeds of modern vessels has largely eliminated hull fouling as a potential import vector (Carlton 1985; Williams et al. 1988). Recent studies however have demonstrated that hull fouling may still be a significant vector for the transport encrusting species (Skerman 1960; Rainer 1995; Coutts 1999; Hewitt and Campbell in press; Hewitt submitted). An evaluation of the introduced fauna of Port Phillip Bay (Hewitt et al. 1999) has demonstrated that approximately 60% of non-indigenous species known from the bay are associated with fouling communities. Hull fouling may play an even more significant role in the tropics, with over 80% of the recognised introduced species detected in port baseline studies associated with the fouling community (Hewitt submitted). National and international moves to phase out the use of effective but highly toxic tin-based antifouling paints are likely to increase risk of transportation of non-indigenous species by hull fouling.

The propensity for smaller recreational and fishing vessels continue to act as a vector for the translocation of hull fouling organisms was demonstrated recently by the introduction of the black striped 'mussel', *Mytilopsis sallei*, into the Cullen Bay Marina in Darwin, Northern Territory (Bax 1999, Willan et al. 2000; Campbell and Hewitt in prep). These vessels may have long residence times

in donor and recipient ports, providing opportunity for settlement and spawning of attached species. In addition, such vessels are typically under the minimum length limit for the continued use of tin based antifouling paints and consequently have the potential to develop higher levels of fouling. Fouling on the hulls of coastal vessels and recreational craft is likely to be an important mechanism for the translocation of sedentary and encrusting species away from a port of first entry (Hay 1990; Clapin and Evans 1995; Hewitt et al. 1999; Hewitt and Campbell in press).

In the past, the introduction of species for mariculture often contributed to the movement of a diverse fauna, including encrusting, epifaunal and infaunal species. For example the movement of oysters between New Zealand and Tasmania in the late 1800's is believed to have also moved an associated fauna that is now well established in Tasmanian waters (Dartnall 1967; Hewitt et al. 1999). Similar introductions of groups of species, including predators and parasites, have been reported as a result of the attempts to establish oysters in various parts of North America (Carlton 1979; Cohen and Carlton 1995; Ruiz et al. 1997). While modern mariculture practices, particularly the movement of culture species in the larval stage, may have greatly diminished the incidence of accidental introductions, secondary translocation of associated fauna may continue to be a problem within Australian waters through the relocation of gear and equipment.

The use of dry and semi-dry ballasting was largely associated with wooden hulled vessels. Dry ballast, generally consisting of sand, gravel, cobbles, or rocks, was the main material used to maintain the trim and stability of wooden vessels. Hulls were typically leaky, creating a damp or semi-dry environment conducive to the survival of the meiofauna and infauna of sandy and cobble habitats. Dry ballast was typically off loaded in or near the harbour (hence the frequent occurrence a site named ‘Ballast Point’ adjacent to many ports). Dry ballasting alone may be responsible for the globalisation of a wide array of sand and cobble meiofauna prior to taxonomic collection in most areas of the world (Carlton 1996).

In steel-hulled vessels, water replaced dry and semi-dry ballast beginning around 1900. Ballast water is an ongoing and increasing vector for the introduction of non-indigenous species to ports throughout the world (Carlton 1985; Williams et al. 1988; Carlton and Geller 1993; Wonham et al. 2000). Ballast water is implicated in the spread of a diverse group of organisms including holoplankton (species which are entirely planktonic), meroplankton (species which spend a portion of the life in the plankton) and tychoplankton (species accidentally swept from the benthos into the plankton). Just as discharge of ballast water by international vessels is a significant potential source of new introductions, the transport of ballast by coastal vessels is a likely vector for the spread of introduced species away from ports of first entry. Ballast water is often discharged near harbour entrances, while vessels are entering harbours and in most cases, at the berth. Loading of ballast usually occurs while a vessel is unloaded at the wharf. This mechanism has the potential to facilitate the dispersal of species with a range of life history characteristics and from a wide variety of habitats (Carlton 1996b; Ruiz et al. 1997; Hayes and Hewitt 1998; Ruiz et al 2000).

2.2. Port specific characteristics

2.2.1 Shipping activity

Non-indigenous species may enter and become established in a port via a number of pathways. These include:

- directly from overseas ports by international shipping, recreational, fishing, and replica vessels;
- indirectly as a result of translocation by shipping from a port of first entry;
- indirectly as a result of translocation by some non-shipping activity from a port of first entry or secondarily infected area; and
- natural range extension from a port of first entry or secondarily infected area.

From the perspective of an individual port, the importance of these various pathways will vary depending on shipping patterns in that port. Considerations include the presence of domestic or international shipping, the commodities traded through the port (export or import, bulk or general cargo), the frequency of services, the presence of hull cleaning services (both in-water and in dry-dock), the level of non-shipping activity in the port and adjacent areas (eg. mariculture, recreational and fishing vessel berths and moorings, replica vessel visits), and the relative isolation of the port. In many ports these considerations must be placed in historical context, how long has the port been operating and what is the current level of invasion? Baseline evaluations will establish this benchmark so that monitoring of new introductions can determine the importance of various vectors.

The export of bulk commodities will inevitably involve ballast water discharge in the port whereas bulk imports will result in ballast water being loaded by visiting vessels. General cargo, either exported or imported, may result in little net ballast discharge or uptake in the port if the tonnage of cargo unloaded approximates that loaded by the same vessel. Roll on–roll off (RO-RO) vessels seldom take on or discharge large volumes of ballast but small volumes (100–200 tonnes) may be loaded or discharged in port to achieve a desired vessel trim.

The location of ballast discharge areas in a port may have a significant impact on the importance of ballast water as a transport vector. Ballast discharge at a berth or in relatively narrow shipping channels where the opportunities for dispersal are more limited is likely to provide a higher risk of colonisation than ballast discharged in open areas outside the port. Wharf areas contain a number of habitats suitable for colonisation by mobile, encrusting, benthic and infaunal species within relatively confined areas. The availability of suitable habitats that experience frequent disturbance at, or adjacent to, points of discharge may be key factors in colonisation success.

Ports with little or no international shipping may still be vulnerable to colonisation by non-indigenous species if they are linked to first entry ports by frequent coastal services. The type of organisms that might be translocated by these services will depend on whether ballast water is discharged during any of these ship visits. Hull fouling may be the most important vector for coastal translocation particularly in situations where vessels remain in ports for extended periods (Skerman 1960; Hewitt and Campbell in press).

2.2.2 Port development and port operations

Despite the volume of international shipping entering Australian ports and the potential load of non-indigenous species that these vessels may carry (Williams et al. 1988; Carlton and Geller 1993), the relatively small number of known introduced species in Australian waters suggests that successful invasions are generally rare. Clearly it does not follow that inoculation of a port with one or more non-indigenous species by any of the mechanisms outlined above will necessarily result in those species becoming established (ie, a reproductive, self-sustaining population) in the port. Factors such as the physical environment in the port, species specific requirements including minimum size of breeding populations, availability of suitable habitats and biotic resistance of local communities to invasion by new species, likely related to the ‘health’ of the native system, are all likely to influence successful establishment of non-indigenous species in a port.

While environmental mismatching between donor and recipient ports may eliminate many non-indigenous species, developments and activities associated with port operations may enhance the invasibility of a port by providing new habitats for colonisation, disturbing natural community processes or by altering the environmental characteristics of the port. The construction of new wharf areas, breakwaters and groynes, and the removal of fouling communities from existing structures will provide colonisation opportunities for sedentary and encrusting species. Similarly, dredging will expose new 'unoccupied' substrate for colonisation. Changes to drainage basins may significantly alter hydrographic regimes resulting in a reduction of the naturally occurring native communities and therefore making space available for invaders. These activities may facilitate colonisation of the port

by non-indigenous species by providing ‘windows of opportunity’ when competition with, or predation by, native species may be less effective in preventing invasions. The discharge of cooling water (eg. from power stations) into port areas can create local temperature regimes that may open these areas to invasions by species that would not survive in the normal port environment.

3 SURVEY REQUIREMENTS

3.1 Survey design

Survey design for the appropriate collection of biological material has been discussed by numerous authors (eg, O'Hara 1986; Maher et al. 1994; New 1996; Hayek and Buzas 1997; Yen and Butcher 1997). Maher et al. (1994) suggest a general framework that begins with identifying the question or problem being addressed. Once these have been articulated, the specific objectives of the sampling program need to be identified, preferably in a staged fashion. At this point the underlying conceptual models, key indicators and specific hypotheses can be identified. The survey design and sampling schedule (including site selection) result from these preliminary steps. Selection of sampling sites is explicitly driven by the objectives of the sampling program and the hypotheses to be tested.

The statistical implications of various sampling strategies are discussed by Hayek and Buzas (1997) who reiterate that the preliminary identification of objectives and hypothesis generation is fundamental to the selection of an appropriate sample design. They however, explicitly support stratified designs for a number of reasons. First, consistency between sampling regions is guaranteed. Second, sampling from each stratum eliminates a primary source of variation because the largest component of variability is between groups (rather than within them). Third, sampling intensity can be increased. They generally recommend either stratified sampling with replicates or replicated systematic sampling.

In order to develop effective inventories, baseline evaluations should incorporate a minimum suite of requirements that will facilitate future comparisons (New 1996; Yen and Butcher 1997). These include:

- an accepted minimum standard for sampling design, sampling methods and information archiving;
- identification of material to species level (or to Least Taxonomic Unit based on morpho-species) where possible;
- species identifications verified by taxonomic experts where possible; and
- survey material vouchered and placed in an appropriate collection (eg., national or state museum).

Surveys designed to specifically identify all non-indigenous species in a port will necessarily provide baseline information for native biodiversity. These congruities should be explicitly recognised so that data can be appropriately analysed for both purposes.

3.2 Survey strategy

While a complete survey of all available habitats is the only approach that is likely to detect all non-indigenous species in a port, the resources required to undertake such surveys on a national scale (or even within a single port) are not currently available. Surveys designed to identify all non-indigenous species in a port will inevitably be subject to scientific, logistic and cost constraints that will limit both their taxonomic and spatial scope. Recognition of these constraints has lead CRIMP to adopt a targeted survey philosophy that identifies strata on the basis of recognised points of introduction and provides a cost effective approach to the collection of baseline data for all temperate and tropical ports (Hewitt and Martin 1996).

For each port, these targeted surveys are designed to determine: (i) the distribution and relative abundance of a limited number of target species; (ii) a baseline assessment of introduced and cryptogenic species; and (iii) a baseline assessment of native species. In Australia, the current target species (i and ii) are made up of:

- those species listed on the Australian Ballast Water Management Advisory Council' s (ABWMAC) schedule of introduced pest species;

- a group of species that are major pests in overseas ports and that, on the basis of their invasive history and projected shipping movements, might be expected to colonise Australian ports; and
- those known introduced and cryptogenic species in Australian waters that currently are not assigned pest status.

The target species, their probable origin and likely introduction/translocation vectors are listed in Appendix 1. Introduced species of greatest concern, that is those species that are designated as 'pests' or have been listed by ABWMAC (Appendix 1), are likely to be widespread in the port environment and in high abundance if well established. This is not likely to be the case in the early stages of an invasion, or for many other non-indigenous species. Consequently, the sampling regime should be designed to detect 'rare' species with both limited distribution and limited abundance.

The baseline surveys should be designed to maximise the likelihood that target species in the port will be detected by concentrating sampling on habitats and sites in the port and adjacent areas that are most likely to have been colonised by the these species. It is assumed that non-indigenous species with limited distributions are most likely to be detected near the point of inoculation.

Knowledge of local conditions, activities and port-specific shipping patterns will strongly influence the sampling effort that is applied to any area of a port. Information of this type along with any 'intelligence' on the occurrence of non-indigenous species in the port will enable fine-tuning of the sampling program by identifying locations that are most likely to be colonised and the species that are likely to occur there. Having access to this information prior to the survey will greatly facilitate the design of a cost-effective sampling program for the port. An outline of the type of information that should, if possible, be provided by the port authority prior to the survey is given in Appendix 2.

Hewitt and Martin (1996) prioritised potential sampling sites according to their predicted inoculation pressure. A comparison of the number of introduced species detected at each site type (strata) during eight CRIMP or CRIMP-associated port surveys between 1995 and 2000 is given in Table 2. This comparison suggests that the stratified sampling design should be adjusted to allow increased effort in those locations where invasions indicate increased inoculation pressures (Table 2).

The second major concern in the design of a sampling program is the detection of rare species. Introduced species (other than well established pests) are likely to be rare or found in low abundance. A power analysis to determine the appropriate sampling effort, using the methods of Green and Young (1993) for rare species with a Poisson distribution, suggests that a sample size of approximately 13 samples will be necessary to detect a species with a mean Poisson density of 0.1 individuals per sample unit at a 95% probability (Figure 2). For example, in order to sample at this level at active berths, at least 7 sites would need to be evaluated (3 quadrats per depth or 3 cores x 5 sites = 15 quadrats/cores). Analyses of invasions at active berths in various ports (representing ~60% of all collected non-indigenous species; Table 2) indicate that an asymptote in the species/sample unit curve is attained after 5 to 9 berths (Figure 3).

3.3 Selection of sampling methods

Sampling methods must be selected to ensure comprehensive coverage of habitats and should provide presence/absence information and/or semi-quantitative indices of abundance. Sampling methods appropriate for port environments and the habitats sampled are summarised in Table 3. This selection is not comprehensive, however the methods chosen represent those that will provide the base level evaluation appropriate for an introduced species port survey. The most time consuming and costly component of any survey is not the field component itself but the post-survey sorting of samples and the identification of species. Sampling techniques that produce large volumes of material and require long sorting times should only be used when (i) there is a high probability that introduced species are present in a specific habitat, (ii) the species are cryptic or not readily recognised, or (iii) there is no other sampling technique that will effectively sample that habitat.

Table 2. Port areas to be sampled for introduced species with initial and revised priority rankings (see text) and average percentage (each port ± S.D.) of detected introduced species based on CRIMP or CRIMP-associated surveys (n=8).

Port area	1996 priority	Revised priority	Average % introduced species detected (±SD)
1. Commercial shipping facilities in port			
• active berths	1	1	60 ± 25
• inactive/disused wharves	2	1	47 ± 11
• channel markers	2	1	32 ± 30
• tug and pilot vessel berths*	1	1	20 ± 17
• slipways	1	1	13 ± 14
• dredge disposal and spoil grounds*	1	2	5 ± 8
• breakwaters, groynes, etc	3	3	3 ± 4
2. Non-commercial facilities/areas in port			
• recreational vessel berths/moorings, marinas	2	1	14 ± 23
• fishing vessel berths/moorings	1	1	14 ± 25
• beaches*	3	2	8 ± 15
• rock jetties, breakwaters, groynes	3	2	8 ± 12
• boat ramps*	2	2	6 ± 10
• mariculture facilities	1	2	6 ± 10
• wrecks and hulks	3	3	3 ± 4
• estuarine/brackish/lagoon areas	3	3	2 ± 4
3. Adjacent areas outside port			
• non-commercial shipping facilities	3	2	9 ± 20
• offshore exposed areas	4	2	8 ± 12
• beaches*	4	2	5 ± 8
• estuarine/brackish/lagoon areas	4	4	0

* not explicitly identified in Hewitt and Martin (1996)

Table 3. Appropriate sampling techniques for detecting introduced species in different port habitats.

Sampling technique	Taxa sampled	Habitat				
		Soft substrate	Hard substrate	Seagrass/ algal bed	Plankton/ nekton	Beach wrack
Small core	dinoflagellate cysts	X				
Large core	benthic infauna	X		X		
20 µm plankton net	dinoflagellates				X	
100 µm drop net	zoo/phytoplankton				X	
Traps	crab/shrimp	X	X	X	X	
Qualitative visual survey	macro biota	X	X	X		X
Quadrat scraping	sedentary/encrusting		X			
Video/ photo transect	sedentary/encrusting	X	X	X		
Beam trawl/benthic sled	mobile epifauna	X		X		
Poison station	fish	X	X	X	X	
Beach seine	fish/mobile epifauna	X		X	X	

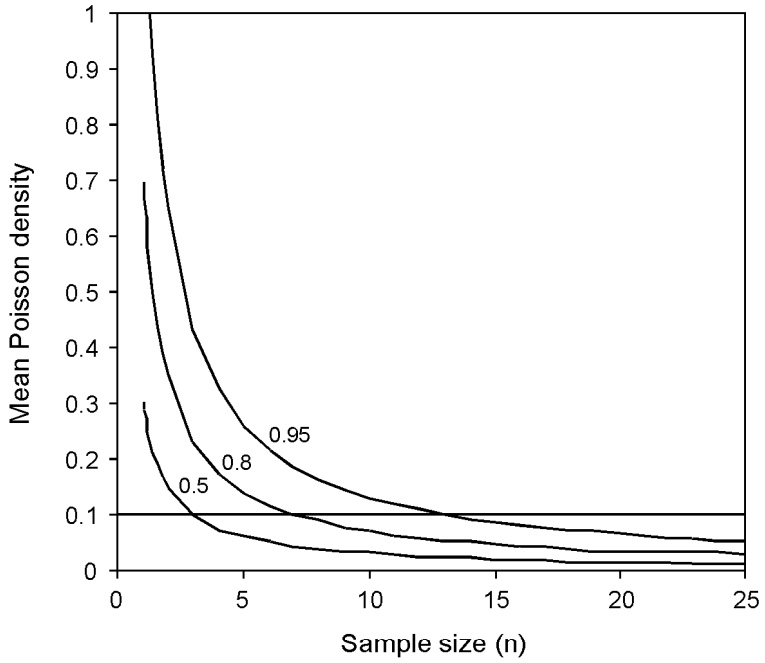


Figure 2. Power analysis of the number of samples (quadrats) required to detect Poisson distributed rare species of varying mean densities (number per sample area) with three degrees of power (1-ß): 0.95, 0.8 and 0.5. The solid horizontal line indicates an arbitrary threshold rarity (mean Poisson density of 0.1).

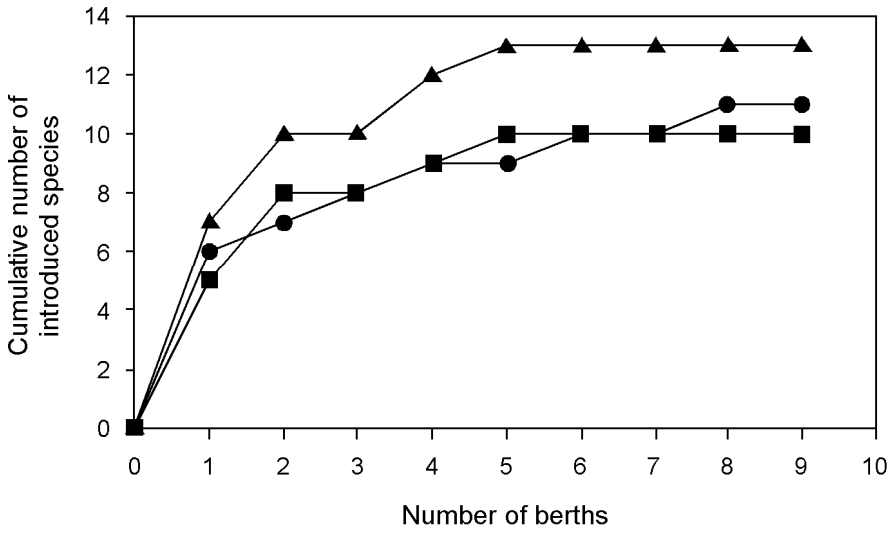


Figure 3. Species accumulation curves for quadrats taken at different depths from active berths in the Port of Newcastle, NSW. Symbols indicate quadrats at depths of -0.5 m (●), -3 m (▲), and -7 m (■).

Alterations to the protocols that exceed the minimum sampling requirements have been proposed by Parry et al. (1997) and Hoedt et al. (in press). These alterations include substituting a Smith McIntyre grab for small and large benthic cores and the use of an Ocklemann sled-dredge to replace diver searches.

The Smith McIntyre grab reduces dependence on divers, which in some regions is preferable because of the potential presence of hazards such as crocodiles, box jellies and sharks. In addition, the grab collects a larger sample volume and may also facilitate the collection of a larger number of samples. This sampling device (and other similar devices such as Eckman and Peterson grabs) does however require the use of a vessel capable of deploying and retrieving the grab. Because of vessel requirements and the inability to accurately place the device, it may be difficult to effectively sample the near and under wharf benthos. It should also be noted that sampling devices such as grabs that may expose the surface of sediment samples to winnowing or erosion during retrieval will preclude accurate sediment particle analyses and may compromise assessments of dinoflagellate cyst distribution.

The second proposed alteration is the use of an Ocklemann sled-dredge instead of diver searches. Diver searches represent a minimum level evaluation under the original protocols. In situations were sediment loads are high, or in areas where diver hazards are prevalent, the substitution of a higher-quality sampling device is both acceptable and advised. The choice of sled or beam trawl was not explicitly indicated in the original protocols. As was noted above, sampling methods that collect significant volumes of material with limited likelihood of detecting non-indigenous species should be avoided to minimise post sampling sorting costs. In addition, the use of dredges or sleds may require follow up sampling to accurately locate and determine the abundance of any introduced species that are collected.

3.4 Public awareness program

Local and overseas experience indicates that conspicuous introduced pest species are rarely first detected by scientists, but more often by fishermen, marine farmers, school groups and dive clubs. That is, community groups who visit the coast frequently and who are familiar the normal suite of species present in their local area. By reporting their observations, the public can play an important role in port surveys by providing information that might indicate the presence of introduced species in the port, the approximate date of their introduction and their impacts on marine communities in the area. The initiation of a public awareness program prior to the commencement of a port survey provides the opportunity for this information to input into the design of the survey.

A public awareness program involves press releases and, where appropriate, interviews with local media groups prior to and during the survey to inform the pubic of the aims of the survey and to request information that might assist in achieving these aims. A contact telephone number should be provided and responses followed up immediately. Public awareness programs will inevitably develop some expectations on the part of the media and the public that the results of the survey will become public knowledge in the future. The time frames associated with reporting and subsequent disclosure of survey results should be indicated in any interviews.

4 DESCRIPTION OF SAMPLING PROTOCOLS

4.1 Plankton

4.1.1 Phytoplankton

Dinoflagellates and other phytoplankton are collected by vertical and horizontal tows of a small 20 μm plankton net (see section 4.5.3). For vertical tows the net should sample during both descent and retrieval, and several drops (up to 3) may be required to ensure that an adequate concentration of cells is obtained in each sample. Horizontal tows should be carried out at a depth of approximately 2 m below the surface. Net haul and tow rates must not exceed 0.25–0.30 m s^{-1} and must take account of any current at the sampling site. Following careful washing of the net, retained samples are sealed in plankton jars and placed in an insulated container. Samples should be returned promptly to the laboratory for incubation and care must be taken to ensure that sample are not subjected to thermal shock during transport.

In the laboratory, net samples are diluted 1:1 with growth medium. Germanium dioxide (10 mgl^{-1}) is added to inhibit overgrowth by diatom species and these enrichment cultures incubated as described for cysts below (see section 4.3.3). Incubations should be examined regularly by light microscopy, and single cells of suspected toxic species isolated by micropipette for further culture and toxicity testing.

Toxicity testing

Suspected toxic species are grown in laboratory culture, under the conditions described above, and tested for toxin production by High Performance Liquid Chromatography (HPLC) (Oshima et al. 1989).

4.1.2 Zooplankton

Zooplankton species are sampled with a standard 100 μm mesh free-fall drop net (see section 4.5.4). The net is weighted to achieve a fall rate of approximately 1 m s^{-1} and the depth reached monitored using a maximum indicating (diver's) depth gauge attached to the frame of the net. Each drop is timed with a stopwatch and the net is allowed to fall from the surface to a depth 0.5–1 m from the bottom before being stopped and closed by the choking bridle. Timing of the drop commences when the cod end of the net sinks below the surface. One drop should be conducted at each site. On recovery the net is washed down on the outside only to avoid contamination of the sample. Retained plankton is preserved in 5% formalin.

4.2 Hard substrates

4.2.1 General (qualitative) methods

Trapping

Mobile epifauna, such as the European shore crab, *Carcinus maenas*, and other crab species, are sampled using light weight mesh-covered crab traps (see section 4.5.5). Locally available fish species such as Australian salmon, pilchards or pink ling appear to be suitable baits (there has been no rigorous evaluation of the relative success with different baits). Traps should be weighted with chain or lead weights and deployed with surface buoys or tethered to wharves or dolphins. Around wharf piles and dolphins, crab traps should be deployed with shrimp traps (see section 4.5.6). The latter are attached to the crab trap tether and suspended from the wharf so that they are positioned just off the bottom. It is preferable to deploy traps in the late afternoon and recover them early the next morning (a catch period of approximately 12 hours). In areas where sea lice are abundant, shorter soak times may be appropriate. At least three traps should be deployed per site. Efforts should be made to deploy in shallow water (0–2 m). Additionally, *Carcinus maenas* prefers the mouths of small streams as well as intertidal areas under rocks.

Visual searches

Visual searches for crabs and other target species should be made at selected wharves and breakwaters in port areas. Divers should swim the length of the wharf or breakwater at several depths (e.g. -0.5, -3, -7 m and the bottom, ~10–12 m) to provide complete visual coverage of the structure and adjacent bottom. In situations where a wharf is supported by several rows of piles, the inner piles should also be included in the visual survey. Visual searches for the northern Pacific seastar, *Asterias amurensis*, the Pacific oyster, *Crassostrea gigas*, the Asian date mussel, *Musculista senhousia*, the black striped mussel, *Mytilopsis sallei*, the European fan worm, *Sabella spallanzanii* and the Japanese kelp *Undaria pinnatifida*, are to be carried out by divers in rocky reef, wharf areas, and over soft bottoms. Divers are either free swimming or towed using a manta board. When using the manta board, divers should be towed along 100 m transects at depths of -2, -5 and -10 m (depending on water depth) at speeds of less than 2 knots (not useful for *Musculista* or *Mytilopsis*).

Surveys of beach wrack should be made on beaches and intertidal areas both inside and immediately adjacent to port entrances to collect crab exuviae and mollusc shells.

Poison stations

Rotenone or other suitable material (quinaldine, clove oil, etc) should be used to sample small gobies, blennies and other benthic fish around piles, breakwaters or groynes, or around hulks and wrecks. The rotenone is mixed with an approximately equal volume of seawater (containing a small quantity of detergent) immediately prior to use and dispensed from plastic bags by divers. Stations should be sampled at slack water to minimise rotenone dispersion and to assist in the retention of poisoned fish in the area. Poisoned fish are collected by divers underwater and snorklers at the surface using hand nets. Sampled fish and bycatch species should be recorded, and specimens preserved in 10% seawater buffered formalin (Note: permits are required to use rotenone in state waters).

4.2.2 Site specific methods

Wharf piles and dolphins

Piles or projecting steel facings and dolphins associated with wharves are to be accorded a high priority in sampling. In the absence of shipping information that might indicate otherwise, all wharves and berths in the port are to be regarded as equally likely points of introduction and should be targeted for sampling. For each berth three piles or facings are selected to provide a series of vertical samples. The first pile/facing selected should be located about 10 m from the end of the berth to reduce 'edge effects', and subsequent piles/facings at a spacing of 10–15 m. In the case of dolphins that may be separated by more than 10–15 m, samples should conform to the available spacing. Where a wharf or berth has inner and outer rows of piles, the inner piles should be surveyed visually.

Prior to sampling, the selected piles, facings, or dolphins are marked with paint above high water mark, their positions recorded and the overall site photographed. For each pile/facing the following protocols should be followed:

1. Three 0.10 m^2 quadrats are fixed to the outer surface of the pile at -0.5 m, -3.0 m, and -7.0 m (MLW) from the surface using bungee cord or some other suitable material. Quadrats cannot easily be fixed to facings and will need to be held by divers and the outline scraped into the biota.
2. A video transect of the outer surface of each pile/facing is made from approximately high water down to the deepest exposed part of the pile/facing using a video camera/recorder in an underwater housing (see section 4.5.7). The camera is maintained at a constant distance (approx. 0.5 m) from the surface of the pile using a distance measuring rod. A scale and depth meter attached to the rod are positioned so that they fall within the field of view of the camera. Care should be taken to ensure that reflected light does not obscure the readout on the depth meter. The vertical transect is to include the three 0.10 m^2 quadrats and when possible the video camera

should be used to record close-ups of the 0.10 m^2 quadrats by using the zoom capabilities of the camera and scanning the surface of the quadrat for increased resolution.

3. Still photographs, using a standard 35 mm underwater camera with a 1:6 close-up frame (see section 4.5.8), are taken to provide high resolution records of the fouling communities. Still photographs of the 0.10 m^2 quadrats should be made prior to destructive sampling. Additional photographs using a close-up lens should be made in conjunction with qualitative sampling of fouling communities.
4. Quantitative destructive sampling of the fouling/encrusting communities should be made by carefully scraping the fauna and flora inside each 0.10 m^2 quadrat into a large plastic collection bag. These samples are used to provide a detailed analysis of the fouling/encrusting community and associated fauna at specific depths.

Samples should be collected and kept chilled on ice or transported immediately back to the laboratory for processing. All faunal samples (quantitative and qualitative) should be rough sorted into subsamples of representative fauna and either preserved directly in 90% alcohol or narcotised with isotonic magnesium chloride or menthol for at least one hour prior to formalin preservation, as appropriate (refer to Section 5) within 8 hrs of collection. Representative flora should be pressed between sheets of herbarium paper. The remaining floral component of each sample is to be preserved in 7% seawater buffered formalin.

Breakwaters, groynes, rockwall facings and natural rocky reefs

Breakwaters, groynes, rockwall facings ('riprap') and natural rocky reefs will vary in both their proximity to wharf areas and their propensity for colonisation by non-indigenous species. These habitats should be targeted for visual surveys (see section 4.2.1) and, in areas where the rocky areas extend to depths greater than 7 m, more detailed vertical transects should be carried out. Vertical transects follow similar protocols to those described for wharf pile sampling and involve the placing of three vertical transect lines, 10–15 m apart, from high water to the base of the rocky area. 0.10 m^2 quadrats are placed at -0.5, -3, and -7 m (MLW) and both video and 35 mm still photographs of the transects and the quadrats are taken as described above. In instances where ledges, overhangs and crevices occur, divers should attempt to sample a 0.10 m^2 'projected' quadrat and take still photographs and record information on relief where appropriate.

In situations where the breakwater, groyne, riprap, or natural rocky reef area is relatively shallow (<5 m), a 50 m transect line should be run along the rock face, videoed and paired 0.10 m^2 quadrats (-0.5 m and 'bottom') sampled at 5 randomly selected locations along the line. Still photographs are taken of the quadrats prior to sampling and qualitative sampling is carried out within the visual survey area.

Intertidal areas (optional)

Rigorous sampling of intertidal areas using transects and quadrats may be included in the survey design if deemed appropriate. While intertidal habitats tend not to be heavily inoculated with non-indigenous species and are therefore of lower priority, sampling these areas may be useful to provide a baseline for determining the subsequent spread of species, both in and outside the port. It is recommended that 0.10 m^2 quadrats be used to maintain consistency with other aspects of the protocols (eg, hard substrates). Samples should be collected from -0.5 m (MLW) if possible to enable comparisons to be made with the highest level wharf pile scrapings.

Wrecks and hulks

Qualitative visual surveys of wrecks and hulks within port areas should be carried out in a similar manner to that described for wharf piles (see section 4.2.1) and supplemented with still photographs and sample collection as appropriate. Wrecks and hulks provide ample opportunity for encrusting and fouling community development. Often hulks were transported from other locations where they had

remained in the water (collecting extensive communities) prior to transport and 'disposal'. These communities often contain non-indigenous species.

Recreational and fishing vessel moorings and hulls

Qualitative visual surveys of moorings and recreational and fishing vessel hulls within port areas should be undertaken when possible and supplemented with still photographs and sample collection as appropriate. Fouling communities on these vessels are typically invaded and may provide early detection of new introductions via hull fouling. Sampling of the berths and wharf piles should follow that outlined above.

Mariculture facilities

Many introduced species have been translocated via mariculture and in particular epifaunal and parasitic organisms associated with oyster and mussel farming. Mussel grow-out lines, oyster racks and other facilities such as cages, jetties and pipelines should be examined using qualitative survey and collection methods. This will involve qualitative video and 35 mm still photographs and qualitative specimen collection as appropriate. Specimen collection should include samples of the oyster or mussel "culch" (settlement materials), and the oysters or mussels themselves.

4.3 Soft substrates

4.3.1 Epibenthos

Visual searches

Visual searches by divers to locate and collect non-target, soft-bottom, epibenthic species are undertaken at wharves where pile surveys were carried out (see section 4.2.2). Divers lay out a 50 m transect line perpendicular to the wharf, starting at the base of one of the sample piles (generally that closest to the end of the berth). The transect line should be marked at 1 m intervals, with the 5 m and 10 m points marked uniquely. If visibility is adequate for filming, the transect is videoed and epibenthos within the transect area photographed and collected as appropriate. While in position, the transect line is also used to locate sites for core sampling of benthic infaunal (see section 4.3.4).

A similar approach is used for sampling epibenthos in offshore areas such as dredge spoil dump sites. A video transect is recorded along a 50 m marked transect line laid cross the bottom and this is supplemented with 35 mm still photographs and sample collection as appropriate.

In regions where visibility is less than 1 m, visual searches and video recordings of transects are unlikely to be a practical option. Alternative sampling methods are discussed in section 3.2.

4.3.2 Mobile epibenthos

Trapping

As discussed previously (section 4.2.1), mobile epifauna can be sampled using lightweight mesh-covered, baited crab traps. Sampling in soft substrate areas is conducted in similar fashion to that described for hard substrates. It is preferable to deploy traps in the late afternoon and recover them early the next morning (a catch period of approximately 12 hours). In areas where sea lice are abundant, shorter soak times may be appropriate. At least three traps should be deployed per site. Efforts should be made to deploy in shallow water (0 m–2 m). Bycatch in both crab and shrimp traps should also be recorded as a qualitative measure of mobile epibenthos.

Trawls and dredges (optional)

A lightweight roller-beam trawl (see section 4.5.9) can used to sample mobile epibenthos over soft-sediment areas and seagrass beds. Depending on the water depth at each site, trawls should be made at depths of 2 and 10 m. Towing speed should not exceed 0.4–0.5 m s^{-1}. Tows should either be of known length (100 m) or known duration (3–5 mins) but should be reduced in areas where algae, seagrass or other benthic material causes rapid filling of the trawl (see discussion on optional methods in section 3.2 above).

Nets

Beach seines are used to sample nearshore fishes over sandy or muddy substrates. A 25 m seine with 15 mm mesh is suitable for deployment and recovery by two people and can be used in areas where the availability of clear bottom is limited. Sampled fish and bycatch species should be recorded, and representative specimens preserved in 10% seawater buffered formalin.

4.3.3 Cyst-forming species

Sediment sampling for cyst-forming species

Sediment cores are taken from locations within the port where the deposition and undisturbed accumulation of sediment and cysts is likely to occur. Selection of sites is based on depth, local hydrography and sediment characteristics of the area. As a general guide, sites where there is an accumulation of uncompacted fine sediment to a depth of 20–30 cm are most suitable sites for constructing the sedimentary history of the port environment. These cores will provide information on the formation of dinoflagellate blooms. Coarse-grained habitats may provide only gross information (presence/absence) for a port environment. Recently dredged areas should be avoided. In addition to these sites, collection of cores within 1 m of the pile base near the berth and 50 m off the berth will guarantee comparisons with other port evaluations. Samples should also be collected at offshore dredge spoil disposal sites and anchorages.

Coring is carried out by divers using 200 mm long plastic tubes with a 25 mm internal diameter (see section 4.5.1). Divers force coring tubes into the undisturbed sediment to a depth that leaves the top 20–50 mm of the tube unfilled. It is important not to allow sediment to overflow the top of the tube. The top of the tube is capped with a bung before the core is withdrawn from the sediment. The lower end is capped after withdrawal to provide an ait tight seal. Triplicate cores are taken at each site. Cores are placed upright in the insulated box and stored in the dark <15°C prior to size fractionation and examination for cysts. Cores must not be frozen and should remain sealed until analysed. Cores do not require immediate transport to a culturing facility, but should be kept at <5°C if retained for extended periods.

Sediment preparation and cyst identification

The top 60 mm of a sediment core is carefully extruded from the coring tube and stored at 4°C in a sealed container prior to examination. Subsamples (approx. 1–2 cm^3) of each core sample are mixed with filtered seawater to obtain a watery slurry. Subsamples (5–10 ml) of the slurry are then sonicated for 2 mins to dislodge detritus particles. The sample is then screened through a 90 μm sieve, collected onto a 20 μm sieve, and panned to remove denser sand grains and larger detritus particles. Subsamples (1 ml) are then examined on wet-mount slides, using a compound light microscope, and cysts are counted and identified. Where possible, a total of at least 100 cysts should be counted in each sample. Cysts of suspected toxic species should be photographed with a light microscope using bright field or differential interference contrast illumination.

Cyst germination

Following sonication and size-fractionation of sediment subsamples, cysts of suspected toxic species are located and isolated by micro pipette under a compound microscope and then washed twice in filtered seawater. Individual cysts are then placed into tissue culture wells containing 2 ml of 75% filtered seawater with nutrients added according to medium GPM of Loeblich (1975). Additional sediment incubations using subsamples from the 20–90 μm size fraction are carried out in Parafilm® sealed, sterile polystyrene petri-dishes containing 20 ml of growth medium. All incubations are carried out at 20°C at a light intensity of 80 $\mu E\ m^{-2}s^{-1}$ (12 h light: 12 h dark) and should be examined regularly for germinated cells. Actively swimming dinoflagellate cells from incubations are isolated by micro-pipette and washed in sterile growth medium prior to identification.

4.3.4 Benthic infauna

Benthic infauna is sampled by divers using a tubular 0.025 m^2 hand corer (see section 4.5.2). Divers force the corer into the sediment to a depth of 200–250 mm and seal the hole in the top with rubber bung before the corer is withdrawn from the sediment. Each sediment core is transferred from the corer to a 1 mm mesh bag with a drawstring mouth and agitated underwater, either in situ or after the diver has returned to the surface, to remove fine sediment from the sample. The retained material is then washed into a plastic bag and preserved in 7% seawater buffered formalin.

When sampling benthic infauna adjacent to wharves, a single core should be taken within 1 m of the base of each sampled pile/facing. Care should be taken to ensure that material that may have fallen to the bottom during sampling of encrusting communities on the pile is not included in the core. A second core is taken at the end of a 50 m transect run out perpendicular to the wharf from each of the sampled piles (see above). Thus for each wharf sampled 3 inner (0 m) and 3 outer (50 m) cores will be taken. Where a wharf has a known history of ballast water discharge, taking additional cores (12.5 m, 25.0 m, 37.5 m) along the 50 m transect line should be considered.

When sampling benthic infauna adjacent to single piles or channel markers or underneath mariculture facilities, three 0.025 m^2 cores should be taken within 2 m of the pile and at least 2 m away from each other. An alternative method for channel markers is to run 50 m transect lines perpendicularly away from the navigation channel. A video transect is then run along each line and at 5 random distances along each transect, paired cores, 1 m on either side of the line, are taken from the center of 0.10 m^2 quadrats. Each quadrat is photographed prior to coring. This method should be employed when the channel is commonly used for deballasting or is adjacent to seagrass beds.

As discussed above (section 3.2), the option exists to replace diver coring by a vessel deployed grab, particularly in situations where diving may be hazardous.

4.4 Environmental data

4.4.1 Temperature, salinity, turbidity

A submersible data logger (SDL) equipped with pressure, conductivity, and temperature sensors or a salinity/temperature meter is used to record depth profiles of salinity and water temperature in 1 m increments from the surface to near the bottom. Turbidity is measured using a 150 mm diameter Secchi disk and reported as the Secchi depth. In conjunction with these readings, additional environmental conditions should be recorded such as air temperature, cloud cover (in eighths), sea state (Beaufort scale) and wind speed/direction. Data on seasonal meteorological patterns (e.g. wind strength and direction, rainfall) can be obtained from meteorological records. Historic information on seasonal changes in temperature and salinity for various parts of the port are desirable.

4.4.2 Sediment analysis

Sediment collection

Sediment samples (minimum 100 g wet weight) are to be taken for analysis of grain size and organic content, from areas immediately adjacent to large benthic infauna sampling cores (section 4.3.4). Sediment samples will allow characterisation of the habitats associated with any introduced epifaunal or infaunal species found. Samples are collected in sealable plastic vials or bags and immediately frozen to stabilise organic content levels.

Particle size analysis

After samples are thawed in the laboratory, a subsample of approximately 25 g (dry weight) is removed for organic content analysis. The remaining sediment is wet sieved through a 2 mm mesh sieve and separated into <2 mm and >2 mm size fractions. Both fractions and the organic content subsamples are oven dried at 80°C for 48 to 96 hrs. The two fractions for particle analysis are analysed as follows:

>2 mm fraction – The total fraction is dry sieved through a nest of sieves and the fraction retained on each sieve (2.0, 2.8, 4.0, 5.6, and 8.0 mm meshes: 0.5 ϕ intervals) weighed. Sediment retained on the largest sieve includes all particles with a size >8 mm. The individual sieved weights are then added to the dry weight of the <2 mm fraction to give a total dry weight for the entire sediment sample. The proportion of each component in the >2 mm fraction is calculated as a percentage of the total dry sample.

<2 mm fraction – The dry weight of the total <2 mm fraction is measured to 0.01 g and the whole sample or, depending on the amount available, a subsample (taken by 'coning and quartering'), analysed using a Laser Particle Size Analyser to comply with the standards of the Marine Geophysical Laboratory, James Cook University, Queensland. Particle size data from this analysis is then combined with data from the analysis of the >2 mm fraction.

Organic content (optional)

Approximately 25 g of dry, unsieved sediment is weighed in a crucible to the nearest 0.00001 g and then ashed in a muffle furnace at 480°C for 4 hrs. The crucible is then transferred to a desiccating chamber and allowed to cool for 1 hr prior to being reweighed. The difference between nett dry and nett ash-free dry weights is then calculated. This difference or weight loss is expressed as a percentage of initial dry weight and represents the organic content (combustible carbon) in the sediment sample.

4.5 Sampling equipment

4.5.1 Small sediment corers (dinoflagellate cysts)

Sediment cores for detecting dinoflagellate cysts are taken with 200 mm long tubes with a 25 mm internal diameter. Corers made from 25 mm plastic plumbing pipe are suitable but clear plastic pipe is preferred as this allows the vertical structure in the core to be observed without disturbing the core. Clear tubes, however, have the disadvantage that light may affect changes in the peripheral sediment of the core if the tubes are not stored in the dark. The lower end of each corer should be sharpened by bevelling around the inner surface of the tube and the top of the tube must be clearly marked and numbered. Rubber bungs are used to seal the ends of the tube and must fit tightly to ensure an air tight seal. Sufficient corers and bungs (2 per tube) should be available to meet the total sampling requirements for the port. The tubes should be kept in a partitioned insulated box that allows cores to be stored upright.

4.5.2 Large sediment corer (benthic infauna)

Sediment cores for sampling benthic infauna are taken with a tubular corer 400 mm long and 179 mm internal diameter. The corer has a pair of handles close to its upper end and is marked externally with grooves at 200 mm and 250 mm from the bottom to indicate the depth to which the corer is to be forced into the sediment. The upper end of the corer is closed except for a mesh-covered 80 mm diameter hole. This is sealed with a rubber bung to aid retention of the sediment core when the corer is withdrawn from the sediment.

4.5.3 20 µm mesh plankton net

A small hand-hauled plankton net is used for sampling dinoflagellates in the water column. The net is 450 mm high with a 250 mm diameter mouth and a 50 mm diameter cod-end opening. The net is made from 20 μm HD Nytal mesh (Swiss Screens (Aust) P/L) throughout. The net and bridle are attached to a 250 mm diameter ring made from 5 mm diameter stainless steel rod. The cod-end is closed with a plastic screw top sample jar secured in the cod-end with a circular clamp.

4.5.4 100 µm mesh drop net

A standard 100 μm mesh free-fall drop net is used for taking zooplankton samples. The net is 3–4 m long with a 700 mm diameter mouth and a 100 mm cod-end opening. The net and bridle are attached to a 700 mm ring made from 20–25 mm galvanised steel pipe. A choking bridle is fitted which closes

the net when the hauling line is tensioned. Divers weights are added to the ring to achieve a desired net fall rate. The cod-end is terminated with plastic or stainless steel cup that can either be drained through a tap in the base of the cap or unscrewed from the net to recover the plankton sample.

4.5.5 Crab traps

Collapsible Japanese crab traps are recommended for sampling *Carcinus* and other crabs. These have a light-weight plastic-coated wire frame (600 mm long, 450 mm wide and 20 mm high) covered with 12.7 mm square mesh netting. Crabs enter the trap through slits at the apex of inwardly directed V-shaped panels at each end of the trap. An internal mesh bait bag is secured to the upper frame. The trap is fixed in the erected position by two clips along the upper mid-line of the trap. Releasing these clips collapses the trap and also allows access to the inside of the trap for baiting and removal of crabs.

4.5.6 Shrimp traps

Commercially available bait traps or any small mesh trap of a similar configuration to the crab traps are suitable for sampling small crustaceans and other small mobile organisms. Each trap consists of a box or cylinder 150–200 mm high and 400–500 mm long made from 2 mm plastic mesh supported by wire hoops. Each end of the trap has a tapered inwardly directed entry cone. Access to the trap for baiting and removal of any catch is via zippered closures or the separation of the trap into halves at its midline. The shrimp traps are attached to crab trap tethers using long line clips and are positioned just clear of the bottom. Locally available fish are appropriate bait.

4.5.7 Video camera

An analogue or digital video recorder (Sony CCD-TR3000E[2] or similar) in an underwater housing (Sony MPK-TRB Handycam Marine Pack[2] or similar) is suitable for video transects on piles and for recording epibenthos on soft and hard bottoms. The housing is fitted with twin 20 W underwater lights and these should be positioned to minimise back-scatter from suspended particles in the water. The housing should also be fitted with a distance-measuring rod with a scale and digital depth meter fitted to its distal end. The rod ensures that the camera is a constant distance from the pile or sea floor (approx. 500 mm). The scale and depth meter are positioned so that they fall within the field of view of the camera at the wide-angle setting to provide real-time depth information on the video recording.

4.5.8 35 mm still camera

A standard 35 mm underwater still camera (Nikonos V[2] or similar) with two flash units is used to provide high-resolution photographic records of epibenthic communities. The flash units should be positioned to minimise back-scatter from suspended particles in the water. For quantitative photography of 0.10m^2 quadrats, the camera should be fitted with a 1:6 overlens close-up frame.

4.5.9 Beam trawl

A lightweight roller-beam trawl can be used to sample mobile epibenthos over soft-sediment and seagrass beds. A suitable trawl is described by Young (1973) but should be modified by adding a tickler chain to the runners near the mouth. The mouth opening is 1 m wide and 0.50 m high and the trawl fitted with a tapered, 2–5 mm square mesh net.

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[2] Reference to brand names does not imply endorsement of these products by CSIRO.

5 SPECIMEN HANDLING

The following section provides both general and taxa specific specimen handling techniques. Table 4 provides a summary of the preferred and optional narcotising and fixing agents.

5.1 General techniques

Site and sample coded labels should be placed inside collection bags immediately, preferably when collected. In the majority of climates, biological and sediment samples should be placed on ice or transported to a laboratory for sorting and preservation. In all instances material should be narcotised and preserved within 8 hours of collection. Narcotising and preservation agents are frequently carcinogenic, consequently Material Safety Data Sheets should be made available to all individuals participating in specimen narcotising and preservation. The following list provides general information for specimen handling.

1. All references to formalin below mean formalin stock diluted 1:9 with seawater (stock is formalin with propylene glycol (propane-1-2-diol), mixed 1:1).
2. Mix alcohol with de-ionised water to avoid precipitates.
3. The volume of the specimen MUST be included as part of, not additional to, the water volume when making up solutions. This is particularly important for large specimens or those with a large water content (eg., ascidians, cnidarians, and sponges). Failure to include specimen volume will result in the solution being too weak.
4. Always completely submerge specimens in preservative, and make sure the specimen is not too big for the jar. If squashed into jars, specimens will distort and, more importantly, will probably not fix properly and may start to decompose.
5. Preserving solutions (both formalin and alcohol) used to fix material rapidly become very acidic. If material cannot be processed promptly upon return from the field, it is advisable to change the preserving solutions to avoid acidity problems. No material should remain in its initial fixing solution for more than one month.
6. Sort specimens and group according to fixing requirements. Do not mix hard and soft animals; some fragile specimens may be damaged or destroyed.
7. Soft bodied animals or unique specimens should be sorted directly into individual specimen jars.
8. Put labels inside a small plastic bag INSIDE the sample bag or jar. The small plastic bag protects the label from chafing, discolouration or other physical damage from specimens during transport and storage. If an outside label is needed, it must be additional to that inside the jar. With very large specimens, attach the label directly to the specimen as well as one on the outside of the bag.
9. When labelling specimens during field collecting, be aware that some live animals will eat or otherwise destroy paper labels.
10. Any material that may be required for DNA analysis must be either frozen or fixed in 100% ethanol. Subsample if necessary.
11. When freezing to relax or store specimens, do not thaw and re-freeze them. Defrost once, photograph if necessary, and then fix in preservative.
12. It is important to cross-reference any photographs to the actual specimen photographed. Make sure that field labels record this. It is usually best for the person who took the photos to collect the specimens and to do the sorting, both in the field and in the laboratory.
13. Material that has been fixed properly in formalin can be transported damp without liquid if it is in sealed containers. This can greatly reduce weight for transport. Preservative should be replaced as soon as practicable. Delicate specimens and alcohol specimens must have some liquid around

them when transported, but the volume can be reduced. Alcohol specimens must have some liquid with them, otherwise they will dry out very quickly, even in a sealed container.

5.2 Preservation methods for specific taxa

Sponges

If at all possible, photograph live specimens in situ to record colours and form. Some species will disintegrate when handled. In the field, freeze specimens (if possible), then fix in the laboratory. If this is not possible, preserve as below, but DO NOT leave material in formalin for more than 24 hrs (8–12 hrs is best). Fix in either 100% alcohol, or in well-buffered formalin overnight. Formalin is a better fixative but sponges must be thoroughly rinsed in water to remove the formalin before being stored in 70% alcohol. If any formalin remains, or the sponge is left in formalin too long, the spicules will start to dissolve and the specimen becomes almost impossible to identify. For small or very delicate sponges, fix in 100% alcohol if possible, but if formalin is used, do not leave them in formalin for more than 2–3 hrs and rinse in water very thoroughly. Store in 70% alcohol.

Anemones

If at all possible, photograph and relax live specimen before fixing. Put in jar with enough seawater to allow the specimen to fully expand, then freeze or add menthol or magnesium chloride and leave overnight. Fix in formalin by adding the correct amount of stock formalin to the frozen specimen making sure it mixes as it defrosts. Store in formalin.

Hydroids and hard corals

Photographs of live animals can be useful. Narcotise large hydroids (particularly athecates) in menthol or magnesium chloride overnight prior to fixation. Fix in formalin. Store in formalin or 70% alcohol.

For hard corals, if possible, part of the sample should not be fixed, but just dried. The animal can then be removed to reveal details of the corallite or corallium (for corals), which is essential for identification. Store in formalin or 70% alcohol, or dry for hard corals.

Soft corals (octocorals)

If at all possible, photograph live and relax specimens before fixing. Put in a jar with enough seawater to allow the specimen to expand fully, then freeze or add menthol or magnesium chloride. Leave until relaxed, and then fix in formalin for a maximum of 12 hrs (2–4 hrs best). Rinse thoroughly in water, then store in 70% alcohol. If any formalin remains, or the animal is left in formalin too long, the spicules will start to dissolve, and the specimen becomes almost impossible to identify. Fix delicate species directly in 100% alcohol. Store in 70% alcohol.

Cnidarian medusae

If at all possible, photograph live and relax specimens before fixing. Put in a jar with enough seawater to allow the specimen to expand fully, then freeze or add menthol or magnesium chloride and leave overnight Fix in formalin. Do not freeze. Store in formalin.

Ctenophores (comb jellies)

Most species are virtually impossible to preserve. It is ESSENTIAL that good, detailed photographs and video (if possible) is taken of all specimens. A few of the more solid species, e.g. *Beroe* spp., and all benthic ctenophores, can be fixed in formalin, and stored in formalin or 70% alcohol. To fix benthic ctenophores flat, the methods used for platyhelminths can be successful. Most species of ctenophores, however, simply disintegrate within minutes of being preserved, no matter what fixative or narcotising agent is used.

Platyhelminths

If at all possible, specimens should be photographed alive. It is important that they are preserved as flat as possible. They can be relaxed using menthol or magnesium chloride overnight, but this is not always successful, and specimens often disintegrate. The best method is to freeze a small amount of

Table 4. Summary of recommended narcotizing and fixation techniques for different taxa (XX = preferred: X = alternative technique).

Phylum	Taxa	Photos	Narcotizing agents						Fixatives			Notes
			None	Freshwater	Chill or freeze	Menthol	Napthelene	MgCl2	70% EtOH	7–10% formalin	Formalin to EtOH	
Annelida	Leaches			XX	X	X	X	X		XX		
	Polychaetes & Oligochaetes	yes				X	X	XX			XX	
Arthropoda	All		XX							XX		Do not freeze
	Barnacles			XX				X		X	XX	
	Pycnogonids		XX						XX	X		
Brachiopoda	All		XX	X	X					X	XX	Air dry valves, or wedge valves open to allow formalin entry
Chordata	Pisces	yes	X							XX		Inject fixative into body cavity of larger individuals
	Urochordates	yes	X			X		X		X	XX	Inject fixative into body cavity of larger individuals
Cnidaria	Alcyonaria				XX			X	XX			Must be narcotised; do not use formalin
	Anthozoa - Corals				XX	X	X	X		X	XX	Air dry a portion of skeleton
	Anthozoa - Anemones				XX	X	X	X		XX		
	Hydroida		XX		X			X		XX	X	
	Scyphozoa & Hydromedusae	yes	XX			X		X		XX		Large volumes of fixative
Ctenophores	All	yes	XX					X		XX		Large volumes of fixative; most are ineffective
Echinodermata	Asteroidea & Echinoids		XX	X		X	X	X		XX	X	Fix in formalin then air dry – ensure seastars are flat
	Crinoids					XX		XX			XX	
	Holothuroids			X		XX	X	X	XX			Do not use formalin
	Ophiuroids				XX	X		X			XX	
Echiura	All			XX		X		X		XX		Must be narcotised prior to fixation
Ectoprocts	Cheilostomes & Cyclostomes		XX								XX	Short time in formalin; can also air dry
	Ctenostomes		XX					X		XX	X	
Entoprocts	All		XX			X		X		XX	X	
Mollusca	Bivalves		XX	X	X					XX	X	Air dry valves, or wedge valves open to allow formalin entry
	Aplocophora	yes			X	XX		XX			XX	
	Cephalopods	yes			XX			X			XX	
	Gastropods - Opisthobranchs	yes			XX	X		X			XX	
	Gastropods & Scaphopods		X		X	X	XX	X			XX	Air dry after microwaving
	Polyplacophora		XX							XX	X	Tie flat
Nemertea	All					XX		X		XX		Must be narcotized (see detailed methods)
Phoronids	All		X		X	XX		X		XX		
Platyhelminthes	All	yes			X	X		XX		XX		
Porifera	All	yes	XX						XX			
Sipuncula	All					XX		X		XX		

formalin stock in a jar, then place the specimen on top. It will freeze onto the surface of the formalin, die flat and be fixed at the same time. Add the appropriate amount of seawater to make up the solution. If there is no other option, fix directly in formalin on ice.

Sipunculan worms

If possible, specimens should be relaxed prior to fixing so that the proboscis is everted. This is best done with menthol or magnesium chloride in seawater overnight. Freezing does not work particularly well for sipunculans. Fix in formalin and store in formalin. Note: dead gastropod shells often contain sipunculans; check contents before discarding any shells.

Echiuran worms

Relax and preserve as for sipunculans. Do not freeze as specimens will disintegrate. In some species, the proboscis is deciduous; make sure it is retained. It can often be an advantage to leave echiurans alive in clean seawater for some hours prior to fixing, to allow them to void sand in the gut. This facilitates later dissection for identification. Echiurans exude a chemical that is toxic to most other animals; beware of this if putting them in containers with other invertebrates when collecting. Fix in formalin and store in formalin.

Nemertean worms

If possible photograph alive, as the colour patterns are distinctive, then relax and preserve as for sipunculans. Freezing does not work particularly well with these worms. Nemerteans will often break up into pieces when fixed but can still be identified, so all fragments should be kept. Like echiurans, some species of nemerteans exude a toxic chemical and are best kept separate during collecting. Fix in formalin and store in formalin.

Oligochaete worms

Relax and preserve as for sipunculans. Photographs of live specimens can be useful for subsequent identification. Fix in formalin and store in formalin or 70% alcohol.

Polychaete worms

Can usually be fixed directly in formalin. Some larger species may need to be relaxed using menthol or magnesium chloride prior to fixing. Try to remove tube dwelling species from their tube to allow proper fixation, but always retain the tubes. This is particularly important with serpulids. Many species will fragment when fixed; all fragments should be retained. Fix in formalin and store in formalin or 70% alcohol. In the case of species with calcareous tubes, transfer from formalin to 70% alcohol within 24 hrs of fixing.

Leeches

These must be relaxed before fixing. Use either menthol in seawater or iced seawater overnight, but do not freeze. Shark and ray leeches can be relaxed by submerging specimens in freshwater for a few hours. Transfer to fixative as soon as they stop moving or they will start to rot. Fix in formalin and store in formalin.

Ectoprocts

If possible, photograph alive as living colours can be useful identification features. Hard species: fix in formalin if possible (not essential) then dry. Store dried. Soft and lightly calcified species: fix in formalin but do not leave for more than a few days (4–12 hrs is best). Store in 70% alcohol. In the field either fix specimens in formalin overnight and transfer to alcohol in the morning, or fix directly in alcohol.

Brachiopods

Fix in formalin. Store in formalin. It helps to wedge open the valves with a match stick or similar, to allow better penetration of the preservative. Many species will clamp shut so tightly that this is impossible to do.

Molluscs (general)

Most molluscs can simply be put straight into formalin to fix, and are usually also stored in formalin (except for very small specimens that are stored in 70% alcohol). The exceptions are detailed below. It can be helpful to relax gastropod specimens, but this is rarely practical.

Aplacophora

Best if relaxed first, usually with menthol, magnesium chloride or iced water, then fix in formalin. Rinse in water and store in 70% alcohol. Do not leave in formalin for more than a few days, or the spicules will start to dissolve.

Polyplacophora

These curl up when removed from their substrate. Specimens should be put onto a flat surface (eg. a glass slide or wooden board) and tied flat using cotton tape. Fix in formalin, then untie and store in formalin. Store very small and deep sea species in 70% alcohol.

Opisthobranchs (and other reduced-shell gastropods)

These MUST be photographed alive as form and colour pattern are very important diagnostic features. Record food if possible as well. Specimens must be relaxed before fixing. The best method for relaxing is to put specimen in a jar with enough seawater for it to crawl around with rhinophores, gills, etc. fully extended, then freeze overnight. Add enough stock formalin to frozen jar to make up solution of appropriate strength, and make sure it is mixed as the seawater thaws. If freezing is impractical, use menthol, magnesium chloride in seawater or iced seawater, overnight, to relax specimens. However, none of these methods are usually as good as freezing. Fix in formalin. Do not leave specimens in formalin for more than 1–2 weeks at most, and if possible, only for about 12 hrs. Prolonged exposure to formalin will dissolve the mantle spicules or vestigial shell. Store in 70% alcohol.

Bivalves

For species with valves that seal tightly, place a match stick or similar object between valves prior to fixation to ensure that fixative can reach internal tissues. To get bivalves to gape, either warm until they relax enough, or freeze them. Fix in formalin and store in formalin, except for species with very thin shells, which should be stored in 70% alcohol.

Cephalopods

Photograph alive, showing different colour patterns if possible. Kill specimens by freezing, chilling or suffocation. Thaw frozen specimens before fixing. When using chilling (eg. on ice or in fresh ice water) or suffocation (e.g. in sealed jar or bag with little water), leave the specimen for several hours before fixing with formalin. Fix in formalin in a dish, arranging the arms and tentacles so they are straight and the specimen is not distorted. It may be necessary to use weights or pins to hold the specimen in place. Fix in formalin for at least 6 hours. Store in formalin or 70% alcohol. Note: To prevent fixed specimens of cuttlefish floating in the jar, the cuttlebone should be carefully removed by cutting straight down the back. The cuttlebone should be kept with the specimen in the jar if stored in 70% alcohol, or in a bag attached to the jar if stored in formalin. The cuttlebone is critical for subsequent identification.

Crustaceans

Photograph specimens alive if possible, particularly shrimps. For commensal species, it is very important to also record and, if possible, collect the host. Do not freeze crustaceans unless there is no other option, as they do not fix as well after they have been frozen. Specimens are best fixed alive. Remove hermit crabs from their shells and tube-dwelling species from their tubes prior to fixing (keep any tubes or shells, etc.). Commensals are often associated with hermit crabs and tube dwelling species; these may need to be fixed differently to their hosts. If hermit crabs have anemones on their shells, remove the crabs then treat the anemones as detailed above. A pair of multigrips is very useful for breaking open shells to remove hermit crabs. Avoid putting specimens with chelipeds in with other

animals as they may grab and damage more fragile species. It is sometimes preferable to kill large crabs individually and then put them into a communal container to fix. Very large specimens may need to be injected with formalin to ensure sufficient fixative reaches internal tissues. Fix in formalin and store in formalin (preferable for all except very small specimens) or in 70% alcohol.

Asteroids

Photograph alive if possible. Place live into a dish of sufficient concentrated formalin (mix stock 1:5 with seawater) to cover the seastar and leave overnight. Make sure that seastars are not distorted before they are put into the fixative. Remove specimens form fixative, place on paper towel and dry in shade. Ensure specimens do not stick to the paper by moving them around regularly (keep their labels with them). When specimens start to change to a pale cream/yellow/orange colour, put them in a plastic bag with their label. In the laboratory, dry specimens in a microwave oven on high for 30 sec to 1 minute; cool for a while then repeat until no more moisture is released. Beware of putting seastars with too much moisture in the microwave as they can explode. Note: 'cooking' seastars in the microwave will cause then to give off vaporised formalin. Only do this in a well ventilated area, and in a microwave oven that is not used for food preparation. Store dry. Alternatively, fix in formalin for 24–48 hrs and transfer to 70% alcohol for long term storage. The latter method will preserve the colour in most specimens.

Ophiuroids

Photograph alive if possible. Large and solid specimens should be treated as for asteroids above. Others, fix in formalin and store in 70% alcohol. Be aware that most species will drop arms. Specimens left in formalin for too long become very fragile.

Echinoids

Large specimens and species with large spines: treat as for asteroids above. Place live specimens in a dish and pour preservative over them until the spines stop moving (all spines should be erect). When specimens are removed for drying, puncture the membrane surrounding the teeth with a needle to allow liquid within the test to drain out. Echinoids are more prone to exploding than seastars when microwaved. Others: fix in formalin and store in 70% alcohol.

Crinoids

Photograph alive if possible. Fix in formalin, but not for more than 2–3 days. Store in 70% alcohol. There are very few species that do not fall apart when preserved. Try to keep all the fragments together and be aware that crinoids usually carry commensals.

Holothurians

Photograph alive if possible. Always isolate large specimens when collecting as they often eject their guts when disturbed; tubules tend to adhere to everything they come in contact with. Either fix in formalin overnight then rinse thoroughly in water, or fix in 100% alcohol. Store in 70% alcohol. It is very important that holothurians are not left in formalin too long and are thoroughly rinsed when removed from it, otherwise their skeletal plates will start to dissolve. These plates are essential for subsequent identification.

Urochordates

Compound, colonial and other gelatinous ascidians MUST be photographed alive as form and colour patterns are very important diagnostic features. Photograph any other ascidians alive if possible. Large solitary ascidians should be relaxed before fixing; menthol or magnesium chloride in seawater overnight is usually effective. Large solitary ascidians may also need to have preservative injected into them to ensure adequate fixation. Fix in formalin. Store in formalin or 70% alcohol.

6 INFORMATION/SPECIMEN ARCHIVING

6.1 Survey data

In order to facilitate general availability from a single source it is preferable that data from all surveys (CRIMP and others) be centrally archived to facilitate synthesis of Australia-wide ports information and input into developing ballast water management initiatives. This data will be generally available to all researchers, subject to confidentiality clauses that may be specific for particular ports.

CRIMP has developed a survey database and reference/voucher collection for the coordinated archiving of introduced species survey information. Templates for data collection are available from CRIMP to facilitate consistency in data collection.

Alpha-numeric site codes have been developed on the following basis:

[**XX**X]	[**XX**X]	[**X**X]	[XX]	[**XX**XX]	[**X**]	[X]	[-]	[XX]
1	2	3	4	5	6	7	8	9

1. Two (or three) characters refer to the country code

   [eg, AUS – Australia, USA – United States of America, NZ – New Zealand]

2. Two (or three) characters refer to the state or territory

   [eg, NSW, VIC, TAS, QLD, SA, NT, WA]

3. One (or two) characters refer to the port

   [eg, PH – Port Hedland; D – Darwin, N – Newcastle]

4. Two characters refer to the subregion of the port as stipulated in the survey design (in some locations this will be unnecessary)

   [eg, IH – Inner Harbour; OS – Off Shore; WB – Western Basin]

5. Two (to four) characters refer to the site (sample station) code

   [eg, BHP4 – BHP Wharf 4; CH10 – Channel Marker 10; W1 – Wharf 1]

6. One character refers to the sample type

   [P – Pile scraping; C – Core; T – Trap; S – Shrimp Trap; R – Rotenone; N – Net; Q – Qualitative]

7. One character refers to the replicate sample [eg 1, 2, or 3] and is not recorded at sites without replication (eg, channel markers)

8. A dash identifying the end of the preceding information (is not recorded for some samples)

9. A numeric character identifying the depth (for pile scrapings) or distance from the berth (for cores)

   [Pile Scrapings: 0, 3, 7; Cores: 0, 50]

Table 5 provides examples of several site codes.

6.2 Reference and voucher specimens

A reference collection of species collected during the surveys is essential to ensure consistency of identifications across ports, particularly where surveys are conducted by a number of agencies. Reference and voucher specimens also provide the opportunity for subsequent re-evaluation of both the identity and the introduced status of species collected during the surveys.

Voucher and reference collections will be maintained at the CSIRO Marine Research Laboratories and will include examples of all species and forms collected. The voucher collections for each Port Survey will be registered to the Tasmanian Museum but placed on long term loan to the Centre for Research on Introduced Marine Pests (CRIMP: curator Karen Gowlett-Holmes). Additional reference collections will also be placed in the Tasmanian Museum registry. This ensures that all reference and

Table 5. Examples of CRIMP site code formats.

Code	Country	State	Site	Region	Sample site	Sample type	Replicate	Dash	Depth/distance
AUSNSWNIHBHP4P1-0	Australia	New South Wales	Newcastle	Inner Harbour	BHP Berth 4	pile scraping	Pile 1	–	-0.5 m MLW
AUSQLDMAB3C1-50	Australia	Queensland	Mackay		Berth 3	core	base Pile 1	–	50 m
AUSQLDHPDTB1Q3	Australia	Queensland	Hay Point	Dalrymple	Berth 1 Terminal	qualitative collection	Pile 3		
AUSWAFIHQVB1R3	Australia	Western Australia	Fremantle	Inner Harbour	QVB1	rotenone	sample 3		
AUSWABOHIBSN2	Australia	Western Australia	Bunbury	Outer Harbour	Inner Basin	seine net	shot 2		
AUSNTDCBMPhotoQual	Australia	Northern Territory	Darwin		Cullen Bay Marina	photo/qualitative collection			
USACASFSBP44T3	USA	California	San Francisco	South Bay	Pier 44	crab trap	replicate 3		
SAFPEIHBCB1P3-7	South Africa		Port Elizabeth	Inner Harbour	Bulk Cargo Berth 1	pile scraping	Pile 3	–	-7 m MLW

voucher materials are accessible, part of a well-maintained permanent collection, and that examples of all species and all vouchers are available from a single source location. Additional collected material will be disbursed to other state museums and, particularly for the port surveys, a complete (if possible) duplicate voucher collection will be lodged with the state museum relevant to that port for local reference. In all cases where duplicate materials are provided to museums other than the Tasmanian Museum, the registration numbers shall be cross-referenced to the voucher and reference materials in the CSIRO-CRIMP collection database. This will enable researchers to source additional lots of material from other museums when necessary. This collection will be open and available to include collections from other agencies undertaking port surveys such that a central archive of reference materials can be maintained.

6.3 35 mm slide and video film archiving

As noted in the protocols, 35 mm still photography will be extensively used to archive visual information about collection sites, pre-destructive sampling quadrats, and qualitative sample collections. These slides are to be regarded as archival information and treated in a similar manner to voucher and reference specimen collections. A duplicate collection of slides shall be provided to the CSIRO-CRIMP collection database for archival storage. These slides should be prepared in the following manner. Slides should be labelled with permanent adhesive labels, preferably computer generated, with full data including: photographer; sample location; species identification; and Tasmanian Museum reference number when appropriate. The museum reference number should also be permanently marked on the slide frame itself.

Slides will be stored in archival quality slide sheets that hang in file cabinets. The storage room will be maintained under library archival conditions (controlled temperature and humidity). The slide references will be cross-indexed with specimen reference and voucher collections as well as the information database on introduced species. In addition, slide material will be digitised and archived on CD-ROM.

Video material has an effective life of around 5 years under general storage conditions. CD-ROM archiving of video material from surveys is currently being assessed.

7 REPORTING

7.1 Report format and content

While a standard reporting format is not mandatory it is desirable that survey reports are consistent in both the presentation of the survey results and in the assessment of risks associated with current shipping and port activities. CRIMP has adopted a set of minimum reporting requirements discussed below and outlined in Appendix 3 (section numbering below accords with that in Appendix 3).

1 Description of the port

1.1 General features – a basic description of the history, geology, hydrography, and biogeography of the port.

1.2 Shipping movements – a summary of the shipping movements and trading patterns with a discussion of the Last Port of Call (LPOC) and Next Port of Call (NPOC) activities for both international and domestic vessels. An analysis of ballast discharge patterns with specific information concerning primary discharge locations in the port. In addition, the activities and movement patterns of small vessels (fishing and recreational) and slow moving vessel (oil drilling platforms, barges, and itinerant dredges) should be discussed if possible.

1.3 Port development and port maintenance activities – as part of the historic understanding of invasions, the development of the port including the establishment of breakwaters, jetties and groins should be presented. Recent maintenance activities (10 yrs) may also provide information to explain introductions in specific areas of the port.

2 Review of existing biological information

A review of previous sampling activities in the port including the methodologies used and results for the port and adjacent coastal regions. This should provide some indication of the general knowledge of native biota in the region, and an indication of any recognised introduced species that have been detected previously.

3 Survey methods

A description of the survey design (provide a map using the specific site codes) and the sampling methods used at each site. Refer to the Appendices for specific information. Provide an indication of the port regions explicitly used for delineating survey sites.

4 Public awareness program (if applicable)

Provide a summary of public awareness activities initiated before or during the survey.

5 Survey results

5.1 Port environment – describe the environmental characteristics of the port based on temperature, salinity and secchi depth readings.

5.2 Introduced species in the port – identify the introduced and cryptogenic species detected in the port and describe their native regions and worldwide distributions.

5.3 Public awareness program - provide a summary of the outcomes of any public awareness program initiated before or during the survey (if applicable).

6 Distribution and potential impacts of introduced species found in the port

Describe the distribution of introduced species in the port providing a synthesis where possible of the linkages between sites (eg, ballast discharge, wood chip berth, etc) and identify the potential impacts of any introduced and cryptogenic species detected in the port based on Australian or overseas experience.

7 Origin and possible vectors for the introduction of non-indigenous species found in the port

Identify the possible transport vectors for each introduced and cryptogenic species detected in the port.

8 Influences of the port environment and port practices on colonisation and survival of introduced species

Identify any linkages between port environmental characteristics, trading practices or port operations that are correlated with higher levels of invasions. Examples might include: regions of increased invasions that are associated with new berth development; higher invasions associated with temperature anomalies from power plant cooling water discharge; higher incidence of north western Pacific species in port areas associated with north western Pacific ballast discharge or vessel berthing.

9 Assessment of the risk of new introductions to the port

Based on the current pattern of invasions, identify the risk of new invasions from both international and domestic source ports.

10 Assessment of the risk of translocation of introduced species found in the port

Evaluate the likelihood of the existing introductions detected in the port being translocated by domestic traffic (both commercial and recreational).

11 Recommendations

11.1 Management of existing introduced species in the port – based on management requirements specified in national and local (state) legislation, identify the need for development and implementation of management activities. If a rapid response has already occurred, describe the plans and activities as well as the success of the activity. If additional, fine temporal or spatial scale data is needed for further management, provide recommendations to undertake such activity. For example, the detection of toxic dinoflagellate cysts in port sediments does not indicate whether blooms occur, or the time of year at which vegetative cells enter the plankton.

11.2 Prevention of new introductions – identify the most prevalent vectors of invasion into the port and recommend management actions.

11.3 Monitoring and re-sampling – provide a recommendation on the frequency of monitoring and re-sampling for the port (see section 8.1 and 8.2).

12 References

13 Appendices

Appendix 1: Target species – a list of recognised introduced and cryptogenic species in the region. In addition any species which have been identified by relevant management agencies as having unwanted or 'pest' status should be identified.

Appendix 2: Details of port facilities – on a berth by berth basis provide information about current configurations, date of original placement (and preceding structures if any), and recent construction/renovation activities.

Appendix 3: Shipping movements in the port – provide a graphic summary of the international and domestic trading patterns for Last Port of Call (LPOC) and Next Port of Call (NPOC). Additional information should be provided concerning volumes (and origins) of ballast water discharges, and if possible the locations within the port of these activities.

Appendix 4: Summary of dredging or other operations – provide a graphic or tabular summary of dredging locations, dredge spoil volumes and locations of dredge spoil disposal sites (if applicable).

Appendix 5: Sampling procedures – a summary of the sampling procedures used during the survey. Any alterations to the protocols should be explicitly recorded with a comparative analysis of the differences between the accepted sampling methodology and the one implemented. Appropriate analyses include direct comparisons during the survey and published comparisons in the literature.

Appendix 6: Sampling site details – provide a table with the site, location (lat/long), date of sampling, site code used during the survey, and sampling procedures implemented at the site.

Appendix 7: Media release (if applicable)

Appendix 8: Survey results – a table of survey results should be presented in the form of a species by site (or sample) matrix to facilitate subsequent cross-comparisons between surveys of different ports or in different regions. Presence/absence information is required at the minimum.

8 CONSIDERATIONS FOR MONITORING AND RE-SURVEYING

8.1 Monitoring

The specific need for monitoring activities should be determined on the basis of:

- finding a targeted 'pest' species in the port at low densities or in limited distribution;
- identifying toxic dinoflagellate cysts in the sediments; or
- the survey does not detect a target 'pest' species that is common in an adjacent or frequent trading domestic port.

Monitoring activities should be determined on the basis of the specific need. For example, the detection of a target pest with limited distribution within a port will require a staged process in which (i) the true distribution of the species is determined by fine scale sampling; and (ii) monitoring of the population abundance and distribution or eradication of the populations and subsequent monitoring to determine eradication success.

In contrast, the detection of toxic dinoflagellate cysts may necessitate a fortnightly plankton-sampling regime to determine the phenology of vegetative cell presence in the water column. This monitoring activity will aid in determination of high-risk times for ballast water uptake and will input to the AQIS Ballast Water Decision Support System.

When a survey does not detect target 'pest' species that are common in the region or in a frequent domestic trading partner's port, a monitoring program should be established to provide early warning of incursion. This monitoring program can be limited in two ways: (i) taxonomic difficulties can be minimised by targeting 'pest' species, and (ii) sites should be selected on the basis of high frequency trading activities with high-risk ports (either international or domestic), and those berths or wharves that had high diversity and abundances of introduced species detected during the baseline evaluation.

Passive sampling devices such as fouling panel collectors for hard substrate species, traps for mobile epifauna and settlement trays for soft sediment infauna, can be deployed at selected sites and retrieved on a periodic basis. A minimum of fifteen collectors should be deployed at each site to adequately sample an area to detect rare species. Alternately, a limited scale re-sampling using the methods described for the baseline evaluation can be implemented at target locations.

8.2 Re-surveying

8.2.1 Frequency

The frequency of re-surveying needs to be empirically evaluated to determine the length of time that a survey adequately represents the species found at a location. At present, no port has been completely re-surveyed and no empirical determination of the frequency of re-surveying has been undertaken. We can use anecdotal evidence to provide some indication of likely re-survey frequency.

The Port of Darwin survey consisted of a complete evaluation of the port in August 1998 (dry season) and a partial re-survey seven months later in March 1999 (wet season) (Russell and Hewitt 2000). During the wet season survey, the black striped 'mussel', *Mytilopsis sallei*, was detected in Cullen Bay Marina at densities up to 26,000 individuals/m^2 (Bax 1999; Willan et al. 2000). *Mytilopsis* covered virtually all hard substrates in the marina, yet seven months earlier it was not detected during quantitative sampling. Consequently it can be assumed that inoculation and establishment occurred during the intervening seven months and a re-surveying frequency of three years would have resulted in a well-established population and little opportunity to affect an eradication.

An alternate and indirect method of determining the appropriate survey frequency is to estimate the background rate of invasions (establishment of non-indigenous species). Cohen and Carlton (1995, 1998) evaluated the invasions into the San Francisco Bay and Delta region (California, USA) using literature, museum collections and field sampling. They estimated that a new marine, brackish or

freshwater species arrived every 55 weeks between 1851 and 1960, but every 14 weeks between 1961 and 1995 (Cohen and Carlton 1998). If we limit the evaluation to marine and brackish species only, San Francisco Bay has an estimated introduction every 32 weeks (Thresher et al. 1999). A similar evaluation for Port Phillip Bay (Victoria, Australia) indicates that a new marine or brackish invader arrives every 41.5 weeks, however one new benthic species is estimated to arrive every 20 weeks since 1960 (Thresher et al. 1999). Coles et al. (1997, 1999b) reported that Pearl Harbor (Hawaii, USA) received approximately one new species every 66 weeks prior to 1940 and one species every 38 weeks since 1940.

The frequency of re-surveying needs to be based on a balance between survey costs and likelihood of detecting a new species (the likelihood of new information) versus the likelihood that a harmful species will have established and spread. Using the information above, we can estimate the number of introductions likely to occur with increasing time since the baseline survey given varying estimated invasion rates (Figure 4). The current recommendations of re-surveying every three to five years will result in between 16 and <2 introductions depending on the estimated invasion rate.

Clearly, the re-survey frequency should be optimised to balance the cost of surveys with likelihood of new invasions, based on information from individual ports. In the absence of specific information, the current recommendation that ports should be re-surveyed every three to five years provides a mechanism to refine estimates of invasion rates and the recommended resurvey frequency.

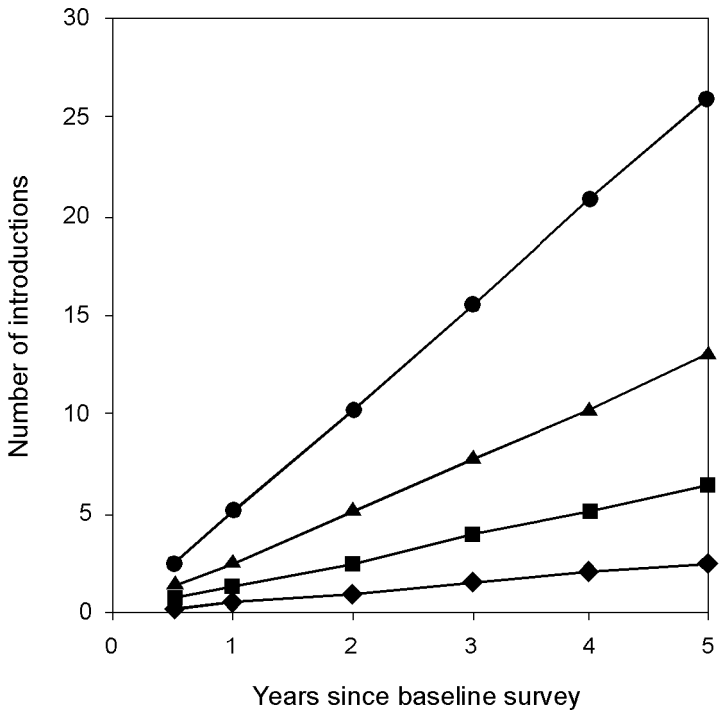


Figure 4. Number of introductions with increasing time since baseline survey with varying estimated invasion rates. Symbols indicate: one invasion per 10 weeks (●); 20 weeks (▲); 40 weeks (■); and 100 weeks (◆).

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APPENDIX 1. ABWMAC Target Pest Species

Phylum	Class/Order	Species	Probable origin	Likely transport vector •				
				1	2	3	4	5
Annelida	Polychaeta	*Sabella spallanzanii*	Mediterranean, Europe	X				
Arthropoda	Decapoda	*Carcinus maenas*	N Europe	X	X	X	X	
Echinodermata	Asteroidea	*Asterias amurensis*	NW Pacific				X	
Mollusca	Bivalvia	*Crassostrea gigas* (feral)	Japan	X			X	X
		Corbula gibba	SE Asia			X	X	
		Musculista senhousia	NW Atlantic	X			X	
Phycophyta	Dinophyceae	*Alexandrium catenella*	Global temperate			X	X	
		Alexandrium minutum	Mediterranean, Atlantic Europe			X	X	
		Alexandrium tamarense	Global temperate			X	X	
		Gymnodinium catenatum	E Pacific, N Europe			X	X	
	Phaeophyceae	*Undaria pinnatifida*	Japan, Korea, China	X			X	

• Vectors: (1) hull fouling/boring; (2) accidentally by mariculture; (3) dry and semi-dry ballast; (4) water ballast; (5) intentional.

APPENDIX 2: PORT INFORMATION REQUIREMENTS

VISITING VESSELS

1. Origin of vessel entering the port
    - 1.1 International
        - 1.1.1 Last international port
        - 1.1.2 Last port of call (if any) within Australia
    - 1.2 Domestic
        - 1.2.2 Last port of call
        - 1.2.2 Other ports visited
2. Frequency of visits
    - 2.1 Regular service
        - 2.1.1 Frequency
        - 2.1.2 Duration of service
    - 2.2 Occasional visits
        - 2.2.1 Frequency
        - 2.2.2 Over what period
3. Ballasting
    - 3.1 No ballast water discharged or loaded
    - 3.2 Reballasting in port
        - 3.2.1 Ballast water discharged
            - within port (estimated volume discharged)
            - outside port (estimated volume discharged)
        - 3.2.2 Ballast water loaded
            - within port (estimated volume loaded)
            - outside port (estimated volume loaded)
4. Location (berth) in port
5. Turn round time
    - 5.1 Average turn round time
    - 5.2 Maximum time in port

VESSELS IN PORT FOR EXTENDED PERIODS (DREDGES, BARGES ETC)

6. Type/name of vessel
7. Previous location
    - 7.1 Name of port
    - 7.2 Duration of stay in that port
8. Duration of stay in port
9. Location (berth or area of operation) in port
10. Destination (if departed)
11. Hull condition

11.1 At arrival

6.1.1 Recently cleaned

6.1.2 Not cleaned

11.2 On departure

6.2.1 Recently cleaned

6.2.2 Not cleaned

PORT OPERATIONS/DEVELOPMENTS

12. Dredging activities

12.1 Frequency of dredging

12.1.1 Previously

12.1.2 Currently

12.2 Planned future dredging operations

13. Port maintenance programs (other than dredging)

14. Port development

14.1 Brief chronological history of the port

14.1.1 Shipping

14.1.2 Development of wharves, breakwaters, groynes, etc

14.1.3 Alterations to estuarine/tidal flow characteristics

14.2 current berths

14.2.1 Age of current berths (and of any disused or derelict wharves)

14.2.2 Date and nature of any in-water modifications or upgrading

14.4 Planned future developments

APPENDIX 3: SURVEY REPORTING REQUIREMENTS

Preface

Executive summary

1. Description of the port
    - 1.1 General features
    - 1.2 Shipping movements
    - 1.3 Port development and port maintenance activities
2. Review of existing biological information
3. Survey methods
4. Public awareness program (if applicable)
5. Survey results
    - 5.1 Port environment
    - 5.2 Introduced species in the port
        - 5.2.1 ABWMAC target species
        - 5.2.2 Other introduced species
    - 5.3 Public awareness program (if applicable)
6. Distribution and potential impacts of introduced species found in the port
7. Origin and possible vectors for the introduction of non-indigenous species found in the port
8. Influences of the port environment and port practices on colonisation and survival of introduced species
9. Assessment of the risk of new introductions to the port
10. Assessment of the risk of translocation of introduced species found in the port
11. Recommendations
    - 11.1 Management of existing introduced species in the port
1. Prevention of new introductions
2. Monitoring and re-surveying
12. References

Appendices

Appendix 1: Target species

Appendix 2: Details of port facilities

Appendix 3: Shipping movements in the port

Appendix 4: Summary of dredging or other operations (if appropriate)

Appendix 5: Sampling procedures

Appendix 6: Sampling site details

Appendix 7: Media release (if appropriate)

Appendix 8: Survey results (preferably in the form of a species by site or sample matrix).