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PORT SURVEYS FOR INTRODUCED MARINE SPECIES – BACKGROUND CONSIDERATIONS AND SAMPLING PROTOCOLS

CHAD L. HEWITT AND RICHARD B. MARTIN

CENTRE FOR RESEARCH ON INTRODUCED MARINE PESTS
CSIRO DIVISION OF FISHERIES
HOBART, TAS

Hewitt, Chad L. (Chad Leroi), 1960- .

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SUMMARY

There are over 100 exotic marine species reported from Australian waters. Almost all these species are unintentional introductions associated with mariculture and shipping activities. The importance of the different introduction methods has changed over various phases of Australian maritime history; hull fouling and ballast water discharge by shipping remain important vectors for the entry of exotic species into Australian ports.

A prerequisite for any attempt to control the spread by shipping of introduced marine pest species in Australian waters is a knowledge of the current distribution and abundance of exotic species in Australian ports. This information base is lacking for nearly all Australian ports. Recognition of the need for baseline studies led the CSIRO Centre for Research on Introduced Marine Species (CRIMP) and Australian Association of Ports and Marine Authorities (AAPMA) to initiate an introduced species port survey program. As port surveys designed to identify all exotic species will inevitably be subject to scientific, logistic and cost constraints, CRIMP and AAPMA have adopted a targeted approach which concentrates on a known group of species and provides a cost effective approach to the collection of baseline data for all ports.

As a number of agencies and research organisations are likely to participate in the national port survey exercise, the development of a uniform approach to surveys and a set of standard survey methods is a key requirement for the program. This technical report outlines the approach to survey design and the sampling protocols adopted by CRIMP for introduced species port surveys under the CRIMP/AAPMA agreement, and provides recommended archiving (data and specimens) and reporting requirements for an effective national survey program.

1. INTRODUCTION

Australia has over 70 trading ports which receive international and/or coastal shipping. In total these ports receive over 10,000 ship visits and around 160 million tonnes of ballast water each year (Kerr 1994). 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 exotic 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 exotic species in Australian ports. This information is currently lacking for all but a few Australian ports.

In 1995, the Australian Association of Port and Marine Authorities (AAPMA) and the CSIRO Centre for Research on Introduced Marine Pests (CRIMP) developed terms of reference for a national port survey program. The aim of this program is to collect baseline data on the occurrence of exotic species in Australian ports and provide a consistent basis on which the introduced species status of individual ports can be assessed. This information is central to the development of a national marine pest management strategy and a key component in domestic ballast water and hull fouling port-to-port risk assessments. As no single agency is likely to undertake all surveys, key requirements for a national ports survey program are a uniformity of approach, based on a standard set of survey protocols, and the central archiving of survey data, reference and voucher specimens and associated information.

Surveys designed to identify all exotic 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 AAPMA and CRIMP to adopt a targeted approach which concentrates on a known group of species and provides a cost effective approach to the collection of baseline data for all ports. These targeted surveys are designed to determine the distribution and abundance of a limited number of species in each port and to identify species of uncertain status (endemic or introduced) that are abundant in a port and/or are likely to become major pest species.

This report reviews background information on potential introduction vectors and summarises port specific characteristics that are likely to affect colonisation by exotic species and influence survey design. The report details the sampling protocols adopted by CRIMP for port surveys for introduced species and outlines specimen handling, and data and specimen archiving methods that should be adopted for a consistent national sampling program.

2. BACKGROUND CONSIDERATIONS

2.1 MECHANISMS FOR THE IMPORT AND TRANSLOCATION OF EXOTIC MARINE SPECIES

While the movement of indigenous peoples in the Australasia region may have provided a transport vector for the movement of some marine species within the Indo-Pacific bioregion, the advent of trans-bioregional introductions in the marine environment largely coincides with European expansion. There are over 100 known exotic species in Australian waters (Appendix 1) but only 4 of these have been deliberately introduced. The remainder are the result of unintentional introductions associated with shipping or mariculture activities.

These unintentional introductions of exotic species into Australian waters have come about as a results of four distinct mechanisms:

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

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

Ship hull fouling and boring can be divided into two distinct vectors: wooden hull vessels and steel hull vessels. Wooden hulled vessels (beginning with European expansion) plied the seas for centuries in exploration, conquest, trade and colonisation. The activities of commerce and colonisation led to the development of active trade routes between Europe, southern Africa, India, Southeast Asia, Japan, and Australasia. This vector initiated a period faunal mixing as a result of a continual movement of both hull fouling and wood boring organisms. The globalisation of the wood boring fauna (e.g., limnorid isopods and teredinid bivalves) is evidenced by the cosmopolitan nature of many species. While wooden hulled vessels continue to operate in some coastal situations, since the 1950s 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 anti-fouling paints and the increased speeds of modern vessels had also largely eliminated hull fouling as a potential import vector. Recent preliminary studies however have demonstrated that hull fouling may still be a significant vector for the transport encrusting species (Skerman 1960; Rainer 1995). 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 by hull fouling. Fouling on the hulls of coastal vessels and pleasure craft is likely to be an

important mechanism for the translocation of sedentary and encrusting species away from a port of first entry.

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 which is now well established in Tasmanian waters. 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. 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 wooden hulled vessel. 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 quite 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.

In steel hulled vessels, water replaced dry and semi-dry ballast. Ballast water is an ongoing and increasing vector for the introduction of exotic species to ports throughout the world (Carlton & Geller 1993). 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 cycle in the plankton such as larvae) and tychoplankton (species accidentally swept from the benthos into the plankton). While discharge of ballast water by international vessels is a potential source of new introductions, ballasting by coastal vessels is a likely vector for the domestic spread of introduced species away from the port of first entry. Ballast water is often discharged near harbour entrances, while vessels are entering harbours and in many cases, at the berth. Loading of ballast usually takes place as 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.

The last mechanism consists of intentional or deliberate introductions, either for stock enhancement or mariculture. While intentional introductions are usually associated with the release of a single species even these may result in unpredicted ecological impacts (e.g. extinction of native species, alteration of habitat).

2.2. PORT SPECIFIC CHARACTERISTICS

2.2.1 SHIPPING ACTIVITY

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

(i) directly from overseas ports by international shipping;

(ii) indirectly as a result of translocation by shipping from a port of first entry;

(iii) indirectly as a result of translocation by some non-shipping activity from a port of first entry or secondarily infected area; and

(iv) by natural range extension from a first entry port or secondarily infected area.

From an individual port perspective the importance these pathways will vary depending on shipping patterns in the port (e.g. the presence of domestic or international shipping), the commodities traded through the port (export vs import, bulk vs general cargo), the frequency of services, the level of non-shipping activity in the port and adjacent areas (e.g., mariculture, fishing), and the relative isolation of the port.

The export of bulk commodities through a port 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 nett 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 in a port of ballast discharge may have a significant impact on its importance 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; availability of suitable habitats which 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 exotic 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 an important vector for coastal translocation

particularly in situations where vessels are located in a number of adjacent ports for extended periods (Skerman 1960).

2.3.2 PORT DEVELOPMENT AND PORT OPERATIONS

Despite the volume of international shipping entering Australian ports and the load of exotic species that these vessels may carry (Williams *et al.* 1988), the relatively small number of known exotic species in Australian waters suggests that successful introductions are generally rare. Clearly it does not follow that inoculation of a port with one or more exotic species by any of the mechanisms outlined above will necessarily result in those species becoming established 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 resistance of local communities to invasion by new species are all likely to influence successful establishment of exotic species in a port.

While environmental mismatching between donor and receiver ports may eliminate many exotic species, developments and activities associated with port operations may enhance invasibility by providing new habitats for colonisation, disturbing natural community processes or altering port environmental characteristics. 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. Port activities that lead to habitat alteration or degradation may create opportunities for the establishment and spread of introduced species. Activities such as dredging and changes to drainage basins may significantly alter hydrographic regimes in a ports. These activities may facilitate exotic species colonisation of the port by providing "windows of opportunity" when competition with, or predation by native species may be less effective in preventing invasions.

3. SURVEY REQUIREMENTS

3.1 SAMPLING STRATEGY

While a systematic survey of all available habitats is the only approach that is likely to detect all exotic species in a port, the resources required to undertake such surveys on a national scale are not currently available. Surveys designed to identify all exotic 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 AAPMA and CRIMP to adopt a targeted approach which concentrates on a known group of species and provides a cost effective approach to the collection of baseline data for all ports. The surveys are designed to determine the distribution and abundance of a limited number of target species in each port. These species 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 which are major pests in overseas ports and which, on the basis of their invasive history and projected shipping movements, might be expected to colonise Australian ports; and
- those known exotic 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.

The surveys are 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 were most likely to have been colonised by the these species (see Appendix 2). The areas to be sampled, in priority order, are listed in Table 1 and, in the absence of port-specific information that might suggest otherwise, the distribution of sampling effort in a port should be determined on the basis of these area rankings.

Table 1. Port areas to be sampled for introduced species with priority ranking.

Port area		Priority
1.	Commercial shipping facilities in port	
	• active berths	1
	• slipways	1
	• channel markers	2
	• inactive/disused wharves	2
	• breakwaters, groynes, etc.	3
2.	Non-commercial facilities/areas in port	
	• mariculture facilities	1
	• fishing vessel berths/moorings	1
	• recreational vessel berths/moorings	2
	• marinas	2
	• wrecks	3
	• rock jetties, breakwaters, groynes	3
	• estuarine/brackish/lagoonal areas	3
3.	Adjacent areas outside port	
	• non-commercial shipping facilities	3
	• offshore expose areas	4
	• estuarine/brackish/lagoonal areas	4

Knowledge of local port conditions, port 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 exotic species in the port will enable fine tuning of the sampling program by identifying those habitats 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. A proforma that outlines the type of information that should, if possible, be provided by the port authority prior to the survey is given in Appendix 3.

3.2 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. As many of the target species are likely to be rare, sampling should concentrate on maximising coverage within a site with minimal sample replication.

Replicate sampling should only be undertaken in situations where small scale heterogeneity is likely to influence detection of target species (e.g. coring for dinoflagellate cysts). Sampling methods appropriate for ports environments and the habitat sampled are summarised in Table 2.

The most time consuming and costly component of any survey is not the survey 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 there is a high probability that introduced species are present in a habitat, they are cryptic or not readily recognised and there is no other sampling technique that will effectively sample that habitat.

3.3 PUBLIC AWARENESS PROGRAM

Overseas experience indicates that conspicuous introduced pest species are rarely first detected by scientists, but more often by fishermen, 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.

The public awareness program involves press releases and, where appropriate, interviews with local media groups prior to the survey to inform the pubic of the aims of the survey and to request for information that might assist with the survey. A contact telephone number is provided and responses followed up

Table 2. Appropriate sampling techniques for the detection of introduced species in different port habitats.

Sampling technique (#)	Habitat (#)				
	Soft substrate (1)	Hard substrate (2)	Seagrass/ macroalgal (3)	Plankton/ nekton (4)	Beach wrack (5)
Small cores (1)	X				
Large cores (2)	X		X		
20 µm mesh plankton net (3)				X	
100 µm mesh drop net (4)				X	
Traps - crab/shrimp (5)	X	X	X	X	
Qualitative visual surveys (6)	X	X	X		X
Quadrat scraping (7)		X			
Beam trawl/benthic sled (8)	X		X		
Poison stations (9)	X	X	X		
Beach seines (10)	X		X	X	

immediately. Press releases and interviews during the survey period should also be used to reinforce the message.

A 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 CRIMP SAMPLING PROTOCOLS

4.1 ABWMAC TARGET SPECIES

Sampling for several of the ABWMAC target species relies heavily on visual surveys. It is essential that divers and other personnel involved in the surveys are familiar with these species and can readily identify them in the field. Furlani (1996) provides identification material for ABWMAC target species and many other known exotic species in Australian waters (see Appendix 6 for details).

4.1.1 DINOFLAGELLATES

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 suitable sites for constructing the sedimentary history of the port environment. Cores taken from these areas will

provide information on the formation of dinoflagellate blooms. Coarse grained habitats may provide gross information (presence/absence) for a port environment. Recently dredged areas should be avoided.

Coring is carried out by divers using 200 mm long plastic tubes with a 25 mm internal diameter (see section 4.4.1). 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 air-tight seal. Triplicate cores are taken at each site. Cores are placed upright in the insulated box and stored in the dark at 4°C prior to size fractionation and examination for cysts. Cores must not be frozen and remain sealed until analysed.

SEDIMENT PREPARATION AND CYST IDENTIFICATION

The top 60 mm of 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 min to dislodge detritus particles. The sample is then screened through a 90 µm sieve and 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 counted and identified. Where possible, a total of at least 100 cysts are counted in each sample. Cysts of suspected toxic species are 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 µE $m^{-2}s^{-1}$ (12 h light: 12 h dark) and are 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.

PLANKTON SAMPLING AND CULTURE METHODS

Plankton samples are collected by vertical and horizontal tows of a small 20 µm plankton net (see section 4.4..3). For vertical tows the net is allowed to 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 are 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 must be returned promptly to the laboratory for incubation and care 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 mg l^{-1}) is added to inhibit overgrowth by diatom species and these enrichment cultures incubated as described for cysts above. Incubations should be examined regularly by light microscopy, and single cells of suspected toxic species isolated by micro pipette 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 (saxitoxin) production by High Performance Liquid Chromotography (HPLC) (Oshima *et al.* 1989).

4.1.2 CARCINUS MAENAS

TRAPPING

The European shore crab, *Carcinus maenas*, and other crab species are sampled using light-weight mesh-covered crab traps (see section 4.4.4). Locally available fish species such as Australian salmon, pilchards or pink ling are suitable bait. Traps are weighted with chain or divers weights and deployed with surface buoys or tethered to wharves or dolphins. Around wharf piles and dolphins, crab traps are deployed with shrimp traps (see section 4.4.5). The latter are attached to the crab trap tether and suspended from the wharf so that they are positioned just off the bottom. Whenever possible, traps are deployed in the late afternoon and recovered early the next morning. In areas where sea lice are abundant, shorter soak times may be appropriate.

VISUAL SEARCHES

Visual searches for crabs and other target species (see section 4.1.3) are made at selected wharves and breakwaters in the port areas. Divers swim the length of the wharf or breakwater at several depths (e.g. 0, 3, 7 m and the bottom, [a]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 are also included in the survey. Surveys of beach wrack are made on beaches and intertidal areas both inside and immediately adjacent to port entrances to collect crab exuviae.

4.1.3 ASTERIAS AMURENSIS, UNDARIA PINNATIFIDA, AND SABELLA SPALLANZANII

VISUAL SEARCHES

Visual searches for the northern Pacific seastar, *Asterias amurensis*, the macroalga *Undaria pinnatifida*, and the European fan worm, *Sabella spallanzanii* are 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 are towed along 100m transects at depths of 2, 5 and 10 m (depending on water depth) at speeds of less than 2 knots. Diver searches in wharf areas and beach wrack surveys (for *Asterias* and *Undaria*) follow procedures described for *Carcinus* (see section 4.2.1).

4.2 NON TARGET SPECIES

4.2.1 ZOOPLANKTON

Zooplankton species are sampled with a standard 100 µm mesh free-fall drop net (see section 4.4.4). The net is weighted to achieve a fall rate of approximately 1 m per second 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 is 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.

4.2.2 HARD SUBSTRATE INVERTEBRATES

WHARF PILES AND DOLPHINS

Piles or projecting steel facings and dolphins associated with wharves are accorded a high priority in sampling. In the absence of shipping information that might indicate otherwise, all wharves and berths in the port are regarded as equally likely points of introduction and are targeted for sampling. For each berth three piles or facings are selected to provide a series of vertical samples. The first pile/facing selected is 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 which may be separated by more than 10–15 m, samples conform to the available spacing. Where a wharf or berth has inner and outer rows of piles, the inner piles are surveyed visually (see section 4.2.1).

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 are followed:

(i) 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 from the surface using bungee cord or some other suitable material. Quadrats cannot easily be fixed to facings and are held by divers.

(ii) 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 Hi8 video camera recorder in an underwater housing (see section 4.4.7). 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 is taken to ensure that reflected light does not obscure the digital readout on the depth meter. The vertical transect includes the three 0.1.0 m^2 quadrats and when possible the video is used to record close-ups of the 0.10 m^2 quadrats using the zoom capabilities of the camera and scanning the surface of the quadrat for increased resolution.

(iii) Still photographs, using a standard 35 mm underwater camera (see section 4.4.8), are taken to provide high resolution records of the fouling communities. Still photographs of the 0.10 m^2 quadrats are made prior to destructive sampling. Additional photographs using a close-up lens are taken in conjunction with qualitative sampling of fouling communities.

(iv) Quantitative destructive sampling of the fouling/encrusting communities are 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.

On shore all samples (quantitative and qualitative) are rough sorted into subsamples of representative fauna and either preserved directly in 90% alcohol or narcotised with isotonic $MgCl_2$ or menthol for at least one hour prior to formalin preservation, as appropriate (refer to Appendix 4). Representative flora are pressed between sheets of herbarium paper. The remaining component of each sample is preserved in 7% seawater buffered formalin.

BREAKWATERS, GROYNES, ROCKWALL FACINGS AND NATURAL ROCKY REEFS

Breakwaters, groynes, rocky rockwall facings ("riprap") and natural rocky reefs vary in both their proximity to wharf areas and their propensity for colonisation by exotic species. These habitats are targeted for visual surveys (see section 4.1.3) and, in areas where the rocky areas extend to depths greater than 7 m, more detailed vertical transects are 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 and both video and 35 mm still photographs of the transects and the quadrats are taken as described above (see section 4.2.2). In instances where ledges, overhangs and crevices occur, divers 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 is run along the rockwall and 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. Preservation of samples follows procedures outlined for wharf piles (see section 4.2.2.).

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 4.2.2) and supplemented with still photographs and sample collection as appropriate.

MARICULTURE FACILITIES

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

4.2.3 SOFT SUBSTRATE INVERTEBRATES

EPIBENTHOS

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 is marked at 1m 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 core sites for sampling benthic infaunal (see below).

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.

MOBILE EPIBENTHOS

A lightweight roller-beam trawl (see section 4.4.9) is used to sample mobile epibenthos over soft-sediment areas and seagrass beds. Depending on the water depth at each site, trawls are made at depths of 1, 2, 5 and 10 m at a towing speed of no more than 25–30 m per minute. Tows are either be of known length (100 m) or known duration (3–5 mins) but are reduced in areas where algae, seagrass or other benthic material causes rapid filling of the trawl. Bycatch in both crab and shrimp traps are recorded as a qualitative measure of mobile epibenthos.

BENTHIC INFAUNA

Benthic infauna is sampled by divers using a tubular 0.025 m^2 hand corer (see section 4.4.2). 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 is taken within 1 m of the base of each sampled pile/facing (see section 4.2.2). Care is 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 a minimum of 3 inner and 3 outer cores will be taken. Where a wharf has a known history of ballast water discharge, additional cores may be taken along the 50 m transect line.

When sampling benthic infauna adjacent to single piles or channel markers or underneath mariculture facilities, three 0.025 m^2 cores are taken within 2 m of the pile and at least 2 m away from each other. An alternative method used 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 the transects, paired cores, 1 m on either side of the line, are taken from the centre of a 0.10 m^2 quadrats. Each quadrat is photographed prior to coring This method is employed when the channel is commonly used for deballasting or is adjacent to seagrass beds.

4.2.4 FISH

POISON STATIONS

Rotenone is 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 are 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 are recorded, and specimens preserved in 10% seawater buffered formalin.

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 are recorded, and specimens preserved in 10% seawater buffered formalin.

4.3 ENVIRONMENTAL DATA

4.3.1 TEMPERATURE, SALINITY AND 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 such as air temperature, cloud cover (in eighths), sea state (Beaufort scale) and wind speed/direction are recorded. Data on seasonal meteorological patterns (e.g. wind strength and direction, rainfall) are obtained from meteorological records. Historic information on seasonal changes in temperature and salinity for various parts of the port are accessed where available.

4.3.2 SEDIMENT ANALYSIS

SEDIMENT COLLECTION

Sediment samples (minimum 100g wet weight) are to be taken for analysis of grain size and organic content, from areas immediately adjacent to large benthic infauna sampling cores. Sediment samples 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 subsample 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 F 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

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.

4.4 SAMPLING EQUIPMENT

4.4.1 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 is sharpened by bevelling around the inner surface of the tube and the top of the tube is 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) are available to meet the total sampling requirements for the port. The tubes are kept in a partitioned insulated box that allows cores to be stored upright.

4.4.2 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 appropriate 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.

4.4.3 DINOFLAGELLATE 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 codend 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 codend is closed with a plastic screw top sample jar secured in the codend with a circular clamp.

4.4.4 ZOOPLANKTON 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 codend 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 codend is terminated with plastic or stainless steel cup which can either be drained through a tap in the base of the cap or unscrewed from the net to recover the plankton sample.

4.4.5 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.

4.4.6 SHRIMP TRAPS

Commercially available bait traps or any small mesh trap of a similar configuration to the crab traps are used 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 longline clips and are positioned just clear of the bottom. Locally available fish are appropriate bait.

4.4.7 VIDEO CAMERA

A Hi8 video camera recorder (Sony CCD-TR3000E or similar) in an underwater housing (Sony MPK-TRB Handycam Marine Pack or similar) is suitable for video transects on piles and for recording epibenthos on soft and hard bottoms. The housing is fitted with twin 20W underwater lights and these are positioned to minimise back scatter from suspended particles in the water. The housing is 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.

4.4.8 35 MM STILL CAMERA

A standard 35 mm underwater still camera (Nikonos V or similar) with two flash units is used to provide high resolution photographic records of epibenthic

communities. The flash units are positioned to minimise back scatter from suspended particles in the water.

4.4.9 BEAM TRAWL

A lightweight roller-beam trawl is used to sample mobile epibenthos over soft-sediment and seagrass beds. A suitable trawl is described by Young (1973) and is 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.

5. INFORMATION ARCHIVING

5.1 SURVEY DATA

In order to facilitate general availability from a single source it is preferable that data from all surveys (AAPMA/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 co-ordinated archiving of introduced species survey information. Survey data will also be used to update and add to the CRIMP Introduced Species Guide (Furlani 1996).

5.2 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.

A reference collection will be maintained at the CSIRO Division of Fisheries site in Hobart and will include examples of all species and forms collected. This reference collection will be registered to the Tasmanian Museum but placed on long term loan to the Centre for Research on Introduced Marine Pests (CRIMP). Additional voucher collections for each Port Survey will also be placed in the Tasmanian Museum registry. This ensures that all reference and 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 distributed 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 so that a central archive of reference materials can be maintained.

5.3 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 which hang in file cabinets. 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.

6. REPORTING

While a standard reporting format is not mandatory it is desirable that port 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/AAPMA has adopted a set of minimum reporting requirements; these are outlined in Appendix 6.

7. REFERENCES

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(1) ABWMAC target introduced pest species, (2) marine pest species that pose a threat to Australia, and (3) known or likely exotic species in Australian waters: probable origin, likely introduction vector and reported distribution.

Phylum	Class/Order	Genus	Species	Probable origin	Likely introduction vector *						Reported distribution (by state)						
					1	2	3	4	5	?	WA	SA	Vic	Tas	NSW	Qld	NT
1. ABWMAC target introduced pest species																	
Annelida	Polychaeta	*Sabella*	*spallanzanti*	Mediterranean, Europe	X			X			X	X	X	X			
Arthropoda	Decapoda	*Carcinus*	*maenas*	N Europe	X	X	X	X			X	X	X	X	X		
Echinodermata	Asteroidea	*Asterias*	*amurensis*	NW Pacific				X					?	X			
Phycophyta	Dinophyceae	*Alexandrium*	*catenella*	Global temperate			X	X			X	X	X		X		
		Alexandrium	*minutum*	Mediterranean, Atlantic Europe			X	X			X	X	X		X		
		Alexandrium	*tamarense*	Global temperate			X	X				X	X	X			
		Gymnodinium	*catenatum*	E Pacific, N Europe			X	X					X	X			
	Phaeophyceae	*Undaria*	*pinnatifida*	Japan, Korea, China	X			X						X			
2. Marine pest species that pose a threat to Australia																	
Ctenophora	Lobata	*Mnemiopsis*	*leidyi*	NW Atlantic				X									
Mollusca	Bivalvia	*Mytilus*	*galloprovinctalis*	Mediterranean, Europe	X	X		X	X								
		Potamocorbula	*amurensis*	Japan, Korea, China				X									
	Gastropoda	*Philine*	*auriformis*	New Zealand	X	X		X									
Phycophyta	Chlorophyceae	*Codium*	*fragile tomentosoides*	NW Pacific	X												
3. Known or likely exotic marine species in Australian waters																	
Annelida	Polychaeta	*Boccardia*	*proboscidea*	N Pacific	X		X	X					X				
		Euchone	*sp.*	unknown			X	X					X				
		Hydroides	*elegans*	Europe	X	X		X			X		X	X	X		
		Mercierella	*enigmatica*	Mediterranean, Europe	X	X		X							X		
		Polydora	*ciliata*	Europe		X	X	X			X				X		
		Pseudopolydora	*pauctbranchiata*	N Pacific		X	X	X					X				
Arthropoda	Amphipoda	*Caprella*	*acanthogaster*	Japan	X	X	X										
	Cirripedia	*Balanus*	*improvisus*	N Atlantic	X	X		X				X					
		Megabalanus	*rosa*	Japan, China, Taiwan	X						X						
		Megabalanus	*tintinnabulum*	uncertain	X						X						
		Notomegabalanus	*algicola*	South Africa	X										X		

* Vectors: (1) hull fouling/boring; (2) mariculture (e.g., with oyster, mussel, etc.); (3) dry and semi-dry ballast; (4) water ballast; (5) intentional introduction; (?) unknown

Known or likely exotic marine species in Australian waters (continued).

					Likely introduction vector *						Reported distribution (by state)						
Phylum	Class/Order	Genus	Species	Probable origin	1	2	3	4	5	?	WA	SA	Vic	Tas	NSW	Qld	NT
Arthropoda	Copepoda	*Mytilicola*	*orientalis*	NE Pacific		X											
	Decapoda	*Cancer*	*novaezelandiae*	New Zealand		X							X	X			
		Halicarcinus	*innominatus*	New Zealand	X	X								X			
		Palaemon	*macrodactylus*	Japan, China, Korea	X			X							X		
		Petrolisthes	*elongatus*	New Zealand		X								X			
		Pyromaia	*tuberculata*	NE Pacific			X	X			X						
		Rhithropanopeus	*harisii*	NW Atlantic	X	X	X	X									
	Isopoda	*Cirolana*	*harfordi*	NE Pacific	X	X	X				X		X		X		
		Eurylana	*arcuata*	New Zealand	X	X	X					X			X		
		Ligia	*oceanica*	N Atlantic	X	X	X										
		Limnoria	*lignorum*	unknown	X												
		Limnoria	*quadripunctata*	unknown	X												
		Limnoria	*tripunctata*	unknown	X												
		Paracerceis	*sculpta*	NE Pacific	X											X	
		Paradella	*dianae*	NE Pacific	X											X	
		Sphaeroma	*serratum*	NE Atlantic	X	X					X						
		Sphaeroma	*walkeri*	N Indian Ocean	X										X	X	
		Synidotea	*laevidorsalis*	E Asia	X	X											
	Mysidacea	*Neomysis*	*japonica*	Japan	X			X							X		
	Tanaidacea	*Leptochelia*	*dubia*	SE Atlantic	X	X	X										
		Tanais	*dulongi*	Europe	X	X	X					X					
Chordata	Ascidiacea	*Ascidiella*	*aspersa*	N Europe	X	X		X			X	X	X	X			
		Botrylloides	*leachi*	N Europe	X	X											
		Botrylloides	*violaceus*	NW Pacific	X	X											
		Botryllus	*schlosseri*	NE Atlantic	X	X											
		Ciona	*intestinalis*	N Atlantic	X	X					X	X	X	X	X	X	
		Molgula	*manhattensis*	N Atlantic	X	X							X		X		
		Styela	*clava*	NW Pacific	X	X							X				
		Styela	*plicata*	Philippines	X	X					X	X			X	X	
	Pisces	*Acanthogobius*	*flavimanus*	Japan, Korea, China				X					X		X		
		Forsterygion	*varium*	New Zealand				X						X			

* Vectors: (1) hull fouling/boring; (2) mariculture (e.g., with oyster, mussel, etc.); (3) dry and semi-dry ballast; (4) water ballast; (5) intentional introduction; (?) unknown

Known or likely exotic marine species in Australian waters (continued).

					Likely introduction vector *						Reported distribution (by state)						
Phylum	Class/Order	Genus	Species	Probable origin	1	2	3	4	5	?	WA	SA	Vic	Tas	NSW	Qld	NT
Chordata	Pisces	*Lateolabrax*	*japonicus*	Japan, Korea, China, Taiwan				X							X		
		Oreochromis	*mossambicus*	SE Asia					X		X					X	
		Salmo	*salar*	N America					X					X			
		Salmo	*trutta*	Britain					X					X			
		Sparidentex	*hasta*	Arabian Gulf				X			X						
		Tridentiger	*trigonocephalus*	Japan, China, Korea				X			X		X		X		
		Triso	*dermopterus*	W Pacific-equatorial				X								X	
		Sparidentex	*hasta*	Arabian Gulf				X			X						
		Tridentiger	*trigonocephalus*	Japan, China, Korea				X			X		X		X		
		Triso	*dermopterus*	W Pacific-equatorial				X								X	
Cnidaria	Anthozoa	*Diadumene*	*lineata*	Japan	X	X											
	Hydroidea	*Bougainvillea*	*ramosa*	Europe	X	X									X		
		Cordylophora	*lacustris*	Europe	X	X											
		Ectopleura	*crocea*	Europe	X	X											
		Sarsia	*tubulosa*	Europe	X	X											
Echinodermata	Asteroidea	*Astrostole*	*scabra*	New Zealand	X	X		X						X			
		Pateriella	*regularis*	New Zealand	X	X								X			
Ectoprocta	Cheilostomata	*Anguinella*	*palmata*	N Atlantic	X	X									X		
		Bugula	*flabellata*	Europe	X	X						X			X		
		Bugula	*neritina*	Europe	X	X											
		Conopeum	*tenuissimum*	NE Atlantic	X	X										X	
		Conopeum	*tubigerum*	Europe	X	X											
		Cryptosula	*pallasiana*	N Atlantic, cosmopolitan	X	X								X			
		Membranipora	*membranacea*	N Atlantic, cosmopolitan	X	X		X				X	X	X			
Ectoprocta	Cheilostomata	*Schizoporella*	*unicornis*	Japan, cosmopolitan	X	X					X	X			X	X	
		Watersipora	*arcuata*	E Pacific, Mexico	X	X					X	X			X	X	
Entoprocta	Barentsiidae	*Barentsia*	*benedeni*	N Europe	X	X											
Mollusca	Bivalvia	*Corbula*	*gibba*	SE Asia			X	X					X	X			
		Crassostrea	*gigas*	Japan	X			X	X		X	X	X	X	X		
		Ischadium	*demissum*	NW Atlantic		X											
		Musculista	*senhousia*	NW Atlantic	X			X			X		X	X			

* Vectors: (1) hull fouling/boring; (2) mariculture (e.g., with oyster, mussel, etc.); (3) dry and semi-dry ballast; (4) water ballast; (5) intentional introduction; (?) unknown

Known or likely exotic marine species in Australian waters (continued).

					Likely introduction vector *						Reported distribution (by state)						
Phylum	Class/Order	Genus	Species	Probable origin	1	2	3	4	5	?	WA	SA	Vic	Tas	NSW	Qld	NT
		Perna	*canaliculatis*	New Zealand	X			X						X			
		Soletellina	*donacioides*	New Zealand?		X	X	X						X			
		Tapes	*japonica*	Japan, Philippines		X	X	X									
		Teredo	*navalis*	Europe	X												
		Theora	*lubrica*	NW Pacific		X	X	X			X		X	X			
		Venerupis	*largillierti*	New Zealand		X		X						X			
	Gastropoda	*Aeolidiella*	*indica*	uncertain	X	X									X		
		Batillaria	*attramentaria*	Japan		X											
		Bullia	*annulata*	South Africa			X				X						
Mollusca	Gastropoda	*Ceratostoma*	*inornatum*	Japan		X											
		Corambe	*pacifica*	Japan	X	X											
		Crepidula	*fornicata*	N Atlantic		X											
		Cymatium	*cutaceum africanum*	South Africa			X				X						
		Doridella	*steinbergae*	NE Pacific	X	X											
		Godiva	*quadricolor*	South Africa	X						X						
		Haliotis	*sanguinea*	South Africa	X						X						
		Illyanassa	*obsoleta*	NW Atlantic	X	X											
		Janolus	*hyalinus*	Europe, Mediterranean	X								X				
		Maoricolpus	*roseus*	New Zealand			X							X	X		
		Nassarius	*kraussianus*	South Africa			X				X						
		Okenia	*plana*	Japan	X								X		X		
		Polycera	*capensis*	South Africa	X										X		
		Polycera	*hedgpethi*	NE Pacific	X						X		X		X		
		Sakuraeolis	*enosimensis*	Japan	X												
		Tenellia	*adspersa*	Japan	X												
		Thecacera	*pennigera*	Europe	X										X		
		Urosalpinx	*cinerea*	NW Atlantic		X											
		Zeacumantus	*subcarinatus*	New Zealand			X								X		
	Polyplacophora	*Chiton*	*glaucus*	New Zealand	X	X								X			
Phycophyta	Chlorophyceae	*Caulerpa*	*filiformis*	South Africa	X										X		
		Caulerpa	*germinata*	Queensland, Australia	X												

* Vectors: (1) hull fouling/boring; (2) mariculture (e.g., with oyster, mussel, etc.); (3) dry and semi-dry ballast; (4) water ballast; (5) intentional introduction; (?) unknown

Known or likely exotic marine species in Australian waters (continued).

					Likely introduction vector *						Reported distribution (by state)						
Phylum	Class/Order	Genus	Species	Probable origin	1	2	3	4	5	?	WA	SA	Vic	Tas	NSW	Qld	NT
Phycophyta	Chlorophyceae	*Caulerpa*	*racemosa*	Queensland, Australia	X												
		Caulerpa	*taxifolia*	pan-tropical species	X						X						
	Phaeophyceae	*Discosporangium*	*mesarthrocarpum*	Mediterranean, Europe						X		X					
		Sargassum	*muticum*	Japan, China, Korea	X	X											
		Spacella	*subtilissima*	Mediterranean, Europe						X		X					
		Zosterocarpus	*sp.*	Mediterranean, Europe						X		X					
	Rhodophyceae	*Antithamnionella*	*spirographidis*	N Hemisphere						X							
		Arthrocladia	*villosa*	N. Hemisphere						X							
		Polysiphonia	*brodiaei*	N Europe						X							
		Polysiphonia	*pungens*	unknown						X							
		Sperococcus	*compressus*	N. Hemisphere						X							

* Vectors: (1) hull fouling/boring; (2) mariculture (e.g., with oyster, mussel, etc.); (3) dry and semi-dry ballast; (4) water ballast; (5) intentional introduction; (?) unknown

Likely occurrence in different habitats and appropriate sampling methods for (1) ABWMAC target species, (2) threat species and (3) other known or likely exotic species in Australian waters.

		Habitats*						Sampling techniques**									
Genus	Species	1	2	3	4	5	?	1	2	3	4	5	6	7	8	9	10
1. IABWMAC target introduced pest species																	
Sabella	*spallanzanii*	X	X	X		X			X				X	X	X		
Carcinus	*maenas*	X	X			X						X	X	X	X		
Asterias	*amurensis*	X	X			X						X	X	X	X		
Alexandrium	*catenella*	X			X			X		X							
Alexandrium	*minutum*	X			X			X		X							
Alexandrium	*tamarense*	X			X			X		X							
Gymnodinium	*catenatum*	X			X			X		X							
Undaria	*pinnatifida*		X			X							X	X	X		
2. Marine pest species that pose a threat to Australia																	
Mnemiopsis	*leidyi*				X						X		X				X
Mytilus	*galloprovincialis*		X			X							X	X			
Potamocorbula	*amurensis*	X		X					X						X		
Philine	*auriformis*	X											X		X		
Codium	*fragile tomentosoides*		X			X							X	X	X		X
3. Known or likely exotic marine species in Australian waters																	
Boccardia	*proboscidea*	X	X			X			X					X	X		
Euchone	*sp.*	X		X					X						X		
Hydroides	*elegans*		X			X							X	X			
Mercierella	*enigmatica*		X			X							X	X			
Polydora	*ciliata*	X		X					X					X	X		
Pseudopolydora	*paucibranchiata*	X	X	X					X						X		
Caprella	*acanthogaster*		X											X	X		
Balanus	*improvisus*		X										X	X			
Megabalanus	*rosa*		X										X	X			
Megabalanus	*tintinnabulum*		X										X	X			
Notomegabalanus	*algicola*		X										X	X			
Mytilicola	*orientalis*		X											X			
Cancer	*novaezelandiae*	X	X									X	X	X	X		
Halicarcinus	*innominatus*		X									X	X	X	X		
Palaemon	*macrodactylus*	X	X	X								X	X		X		
Petrolisthes	*elongatus*		X			X						X	X	X			
Pyromaia	*tuberculata*	X	X			X						X	X	X	X		
Rhithropanopeus	*harisii*	X	X									X	X	X			
Cirolana	*harfordi*	X	X						X						X		
Eurylana	*arcuata*	X							X						X		
Ligia	*oceanica*	X	X			X							X	X	X		
Limnoria	*lignorum*		X			X								X			
Limnoria	*quadripunctata*		X			X								X			
Limnoria	*tripunctata*		X			X								X			
Paracerceis	*sculpta*		X			X			X						X		
Paradella	*dianae*		X			X			X					X	X		
Sphaeroma	*serratum*		X						X						X		
Sphaeroma	*walkeri*		X											X			

* Habitats: (1) soft substrate; (2) hard substrate; (3) seagrass/macroalgae; (4) plankton/nekton; (5) beach wrack.

** Sampling techniques: (1) small cores; (2) large cores; (3) 20 µm plankton net; (4) 100 µm drop net; (5) traps; (6) visual surveys; (7) quadrat scraping; (8) beam trawl/sled; (9) poison stations; (10) beach seins.

Habitats and sampling techniques continued:

		Habitats*						Sampling techniques**									
Genus	Species	1	2	3	4	5	?	1	2	3	4	5	6	7	8	9	10
Synidotea	*laevidorsalis*		X											X			
Neomysis	*japonica*	X	X									X			X		
Leptochelia	*dubia*	X	X											X			
Tanais	*dulongi*	X	X						X					X	X		
Ascidiella	*aspersa*		X										X	X			
Botrylloides	*leachi*		X	X									X	X	X		
Botrylloides	*violaceus*		X	X									X	X	X		
Botryllus	*schlosseri*		X	X									X	X	X		
Ciona	*intestinalis*		X										X	X			
Molgula	*manhattensis*		X	X									X	X			
Styela	*clava*		X	X									X	X			
Styela	*plicata*		X	X									X	X			
Acanthogobius	*flavimanus*	X														X	X
Forsterygion	*varium*		X													X	X
Lateolabrax	*japonicus*	X														X	X
Oncorhyncus	*mykiss*				X											X	X
Oreochromis	*mossambicus*				X											X	X
Salmo	*salar*				X											X	X
Salmo	*trutta*				X											X	X
Sparidentex	*hasta*		X													X	X
Tridentiger	*trigonocephalus*		X													X	X
Triso	*dermopterus*		X													X	X
Diadumene	*lineata*		X	X									X	X			
Bougainvillea	*ramosa*		X	X										X			
Cordylophora	*lacustris*	X	X											X			
Ectopleura	*crocea*		X										X	X			
Sarsia	*tubulosa*		X											X			
Astrostole	*scabra*	X			X							X	X	X	X		
Paterella	*regularis*	X			X							X	X	X	X		
Anguinella	*palmata*	X	X											X			
Bugula	*flabellata*		X										X	X			
Bugula	*neritina*		X										X	X			
Conopeum	*tenuissimum*		X											X			
Conopeum	*tubigerum*		X											X			
Cryptosula	*pallasiana*		X											X			
Membranipora	*membranacea*		X	X		X							X	X			
Schizoporella	*unicornis*		X										X	X			
Watersipora	*arcuata*		X										X	X			
Barentsia	*benedeni*		X											X			
Corbula	*gibba*	X		X					X						X		
Crassostrea	*gigas*		X			X			X				X	X	X		
Ischadium	*demissum*	X							X						X		
Musculista	*senhousia*	X	X										X	X			
Mya	*arenaria*	X							X						X		
Neilo	*australis*		X										X	X			
Ostrea	*lutaria*	X	X										X	X			
Perna	*canaliculatis*		X										X	X			
Soletellina	*donacioides*	X	X						X						X		
Tapes	*japonica*	X							X						X		
Teredo	*navalis*	X												X			

* Habitats: (1) soft substrate; (2) hard substrate; (3) seagrass/macroalgae; (4) plankton/nekton; (5) beach wrack.

** Sampling techniques: (1) small cores; (2) large cores; (3) 20 µm plankton net; (4) 100 µm drop net; (5) traps; (6) visual surveys; (7) quadrat scraping; (8) beam trawl/sled; (9) poison stations; (10) beach seins.

Habitats and sampling techniques continued:

Theora	*lubrica*	X							X						X				
Venerupis	*largillierti*	X							X						X				
Aeolidiella	*indica*	X											X	X					
Batillaria	*attramentaria*		X						X						X				
Bullia	*annulata*		X						X				X		X				
Ceratostoma	*inornatum*		X											X	X				
Corambe	*pacifica*		X										X	X					
Crepidula	*fornicata*		X										X	X					
Cymatium	*cutaceum africanum*	X							X						X				
Doridella	*steinbergae*		X										X	X					
Godiva	*quadricolor*		X										X	X					
Haliotis	*sanguinea*		X										X	X					
Illyanassa	*obsoleta*	X	X						X						X				
Janolus	*hyalinus*		X										X	X					
Maoricolpus	*roseus*	X	X						X				X	X	X				
Nassarius	*kraussianus*	X							X						X				
Okenia	*plana*		X										X	X					
Polycera	*capensis*		X										X	X					
Polycera	*hedgpethi*		X										X	X					
Sakuraeolis	*enosimensis*		X																
Tenellia	*adspersa*		X										X	X	X				
Thecacera	*pennigera*		X										X	X					
Urosalpinx	*cinerea*	X											X	X	X				
Zeacumantus	*subcarinatus*	X	X						X						X				
Chiton	*glaucus*		X										X	X					
Caulerpa	*filiformis*		X										X	X	X				
Caulerpa	*racemosa*		X										X	X					
Caulerpa	*taxifolia*		X										X	X	X				
Caulerpa	*germinata*		X																
Discosporangium	*mesarthrocarpum*						X												
Sargassum	*muticum*						X						X	X					
Spacella	*subtilissima*		X																
Zosterocarpus	*sp.*						X												
Antithamnionella	*spirographidis*						X												
Arthrocladia	*villosa*						X												
Polysiphonia	*brodiaei*						X						X	X					
Polysiphonia	*pungens*		X										X	X					
Sperococcus	*compressus*						X												

* Habitats: (1) soft substrate; (2) hard substrate; (3) seagrass/macroalgae; (4) plankton/nekton; (5) beach wrack.

** Sampling techniques: (1) small cores; (2) large cores; (3) 20 µm plankton net; (4) 100 µm drop net; (5) traps; (6) visual surveys; (7) quadrat scraping; (8) beam trawl/sled; (9) poison stations; (10) beach seins.

PROFORMA FOR PORT INFORMATION REQUIREMENTS

A. 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
5. Location (berth) in port
4. Turn round time
   - 4.1 average turn round time
   - 4.2 maximum time in port

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

1. Type/name of vessel
2. Previous location
   - 2.1 name of port
   - 2.2 duration of stay in that port

3. Duration of stay in port
4. Location (berth or area of operation) in port
5. Destination (if departed)
6. Hull condition
   - 6.1 at arrival
     - 6.1.1 recently cleaned
     - 6.1.2 not cleaned
   - 6.2 on departure
     - 6.2.1 recently cleaned
     - 6.2.2 not cleaned

C. PORT OPERATIONS

1. Dredging activities
   - 1.1 frequency of dredging
     - 1.1.1 previously
     - 1.1.2 currently
   - 1.2 planned future dredging operations
2. Port maintenance programs (other than dredging)
3. Port development
   - 3.1 brief chronological history of the port
     - 3.1.1 shipping
     - 3.1.2 development of wharves, breakwaters, groynes, etc
     - 3.1.3 alterations to estuarine/tidal flow characteristics
   - 3.2 current berths
     - 3.2.1 age of current berths (and of any disused or derelict wharves)
     - 3.2.2 date and nature of any in-water modifications or upgrading
   - 3.4 planned future developments

Summary of recommended narcotising and fixation techniques for different taxa (X = preferred, x = alternative technique). Refer to information in Appendix 4 for details on specific taxa.

			Narcotizing agents						Fixatives			
Phylum	Taxa	Photo.	None	Freshwater	Chill or freeze	Menthol	Napthelene	MgCl2	70% Alcohol	7–10% Formalin	Formalin to alchol	Notes
Annelida	Leaches			X	x	x	x	x		x		
	Polychaetes & oligochaetes	yes				x	x	X			x	
Arthropoda	All		X							x		Do not freeze
	Barnacles			X				x		x	x	
	Pycnogonids		X						x	x		
Brachiopoda	All		X	x	x					x	x	Air dry valves, or stick match in valve to allow formalin entry
Chordata	Pisces	yes	x							X		Inject fixative into body cavity
	Ascidians	yes	x			x		x		x	X	Inject large solitary ascidians with formalin
Cnidaria	Alcyonaria				X			x	X			Must be narcotized; do not use formalin
	Anthozoa - corals				X	x	x	x		x	X	Air dried portion
	Anthozoa - sea anemones				X	x	x	x		X		
	Hydroids		X		x			x		X	x	
	Scyphozoa & hydromedusae	yes	X			x		x		X		Use large volumes of fixative
Ctenophora	All	yes	X					x		X		Use large volumes of fixative; fixation usually unsuccessfull
Echinodermata	Asteroids & echinoids		X	x		x	x	x		x	x	Fix in formalin then air dry; ensure seastars are flat
	Crinoids					X		X			x	
	Holothurians			x		X	x	x	x			Do not use formalin
	Ophiuroids				X	x		x			x	
Echiura	All			X		x		x		X		Must be narcotized
Ectoprocta	Cheilostomes & cyclostomes		X								X	Can short fix in formalin; specimens may be air dried
	Ctenostomes		X					x		X	x	
Mollusca	Bivalves		X	x	x					x	x	Air dry valves, or stick match in valve to allow formalin entry
	Cephalopods	yes			X			x		x		
	Gastropods - opisthobranchs	yes			X	x		x			x	
	Gastropods & scaphopods		x		x	x	X	x			x	Air dry after microwaving
	Chitons		X							X	x	Tie flat
Nemertea	All					X		x		X		Must be narcotized (see detail methods)
Phoronidea	All		x		x	X		x		X		
Platyhelminthes	All				x	x		X		X		
Porifera	All	yes	X						X			Do not use formalin
Sipuncula	All					X		x		X		Must be narcotized (see detail methods)

GENERAL FIXATION AND PRESERVATION TECHNIQUES

- 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).
- Mix alcohol with de-ionised water to avoid precipitates.
- Remember that the volume of the specimen MUST be included as part of the water volume when making up solutions, not additional. This is particularly important for large specimens or those with a large water content (e.g., ascidians, cnidarians, porifera) or the solution will end up too weak.
- Always completely submerge specimens in preservative, and make sure the specimen is not too big for the jar - if it is squashed in, it will distort and more importantly, probably not fix properly and start to rot.
- Preserving solutions (both formalin and alcohol) used to fix material become very acidic very quickly. If material cannot be processed promptly upon return from fieldwork, at least change the preserving solutions to avoid acidity problems. No material should remain in its initial fixing solution for more than one month.
- Sort specimens and group according to fixing requirements. Do not mix hard and soft animals - some specimens will get damaged or even destroyed.
- Put labels inside a small plastic bag INSIDE the sample bag or jar. If an outside label is needed, it is additional to the one inside. With very large specimens, attach the label directly to the specimen as well as one on the outside of the bag. The small plastic bag protects the label from chafing, discolouration or other physical damage from specimens during transport and storage.
- When labelling specimens during field collecting, be aware that some live animals will eat or otherwise destroy paper labels.
- Any material that may be required for DNA analysis must be either frozen or fixed in alcohol. Subsample if necessary.
- When using freezing to relax or store specimens, do not thaw and re-freeze them. Defrost once, photograph if necessary, and then fix in preservative.
- It is important to cross-reference any photographs to the actual specimen photographed (see section 3.6). 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.

- 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. However, replace preservative 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.

PRESERVATION METHODS FOR SPECIFIC TAXA

SPONGES

If at all possible, photograph live specimen in situ to record colours and form. Some species will disintegrate when handled. In field - freeze (if possible), then fix in laboratory. If this is not possible, preserve as below, but DO NOT leave in formalin for more than 24 hrs (8-12 hrs is best). Fix in either 100% alcohol, or in well buffered formalin overnight. Although formalin is a better fixative, the sponge 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, or spicule damage will occur. Store in 70% alcohol.

ANEMONES

If at all possible, photograph live specimen and relax before fixing. Put in jar with enough seawater to expand fully, then freeze or put menthol or $MgCl_2$ in overnight, and then fix in formalin by adding the correct amount of stock formalin to the frozen specimen, and making sure it mixes as it defrosts. Store in formalin.

HYDROIDS AND HARD CORALS

Photographs of live animals can be useful. Fix in formalin. Store in formalin. 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 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 specimen and relax before fixing. Put in jar with enough seawater to expand fully, then freeze or put menthol (or $MgCl_2$) in, 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. For delicate species, fix directly in 100% alcohol. Store in 70% alcohol.

CNIDARIAN JELLYFISH

If at all possible, photograph live specimen and relax before fixing. Put in jar with enough seawater to expand fully. put menthol or $MgCl_2$ in jar, leave overnight, and then 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 if possible, video, 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, they should be photographed alive. It is important that they are preserved as flat as possible. They can be relaxed using menthol or $MgCl_2$ overnight, but this is not always successful, and they often disintegrate. The best method is to freeze a small amount of formalin stock in a jar, then place the platyhelminth on top. It will freeze onto the surface of the formalin, die flat and be fixed all at the same time, then 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, they should be relaxed prior to fixing so that the proboscis is everted. This is best done with menthol or $MgCl_2$ in seawater overnight. Freezing does not work particularly well. Fix in formalin, store in formalin. Note:- many dead gastropod shells actually contain sipunculans in the shells - check before discarding any shells.

ECHIURAN WORMS

Relax and preserve like sipunculans. Do not freeze - they 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, as this allows them to void most of the sand in the gut, and makes later dissection for identification much easier. Echiurans exude a chemical that is toxic to most other animals - beware of this if putting them in the same jar as something else when collecting. Fix in formalin, store in formalin.

NEMERTEAN WORMS

If possible photograph alive, as the colour patterns are distinctive, then relax and preserve like sipunculans - freezing does not work particularly well. They will often break up into pieces when fixed, but can still be identified, so should be kept. Like echiurans, some species exude a toxic chemical, so they are best kept separate during collecting. Fix in formalin, store in formalin.

OLIGOCHAETE WORMS

Relax and preserve like sipunculans. Photographs of live specimens can be useful. Fix in formalin, 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 $MgCl_2$ 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 - try to keep all the bits. Fix in formalin, store in formalin or 70% alcohol. In the case of species with calcareous tubes transfer from formalin to 70% alcohol within 24 hrs after fixing.

LEECHES

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 in freshwater for a few hours, but fix as soon as they stop moving or they will start to rot. Fix in formalin, store in formalin.

BRYOZOANS

If possible, photograph alive - living colours can be useful. Hard species - fix in formalin if possible, but not essential, then dry. Stored 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.

BRACHIOPODS

Fix in formalin. Store in formalin. It can help to wedge open the valves with a match stick or something similar, to allow better penetration of the preservative, but 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, store 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, $MgCl_2$ or iced water, fix in formalin, but store in 70% alcohol after rinsing in water. 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. Should be put onto a flat surface, e.g. a glass slide or wooden board, and tied flat using cotton tape. Fix in formalin, then untie, usually store in formalin, but for very small and deep sea species, store in 70% alcohol.

OPISTHOBRANCHS (AND OTHER REDUCED-SHELL GASTROPODS)

These MUST be photographed alive, form and colour patterns are very important. Record food if possible as well. Must be relaxed before fixing. Best method for relaxing is to put in a jar with enough seawater for it to crawl around with rhinophores, gills, etc. fully extended, then freeze overnight. Best if in known volume in jar, then just add stock formalin to frozen jar to make up solution, and make sure it is mixed as the lot defrosts. If freezing is impractical, use menthol or $MgCl_2$ in seawater overnight, or iced seawater, to relax them. However, this is usually not as good as freezing. Fix in formalin. Do not leave them in formalin for more than 1-2 weeks at most, if possible, only for about 12 hrs, of the mantle spicules or vestigial shells will start to dissolve. Store in 70% alcohol.

BIVALVES

Always try to get formalin inside the shells in species that form a tight seal. The best way is to put a match stick or something similar in between the valves when they are open, then put them in preservative. To get bivalves to gape, either warm them until they relax enough, or freeze them. Fix in formalin, store in formalin, except for species with very thin shells - store in 70% alcohol.

CEPHALOPODS

Photograph alive, showing different colour patterns if possible. Freeze, chill or suffocate specimens until they are dead. When freezing, defrost before fixing. When chilling (e.g. on ice or in fresh ice water), or using suffocation (e.g. in sealed jar or bag with little water), leave the specimen for several hours before fixing, otherwise there will still be a reaction from the nerves and muscles, and the arms will curl up over the head, and the specimen will distort. 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:- after fixing, the cuttlebone should be carefully removed from cuttlefish by cutting straight down the back. This is so that the specimen does not float in the jar. 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 very important for identification.

CRUSTACEANS

Photograph alive if possible, particularly shrimps. For commensal species, it is very important to record, and collect if possible, the host. Do not freeze unless there is no other option, as they do not fix as well after they have been frozen. They 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.). Look out for commensals with hermit crabs and tube dwelling species - they may need to be fixed differently to the hermits. In particular, 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, particularly soft ones, as they will grab them and cause damage. 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 have formalin injected into them to ensure sufficient reaches internal tissues. Fix in formalin, 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 concentrated formalin (mix stock 1:5 with seawater) that covers the seastar, and leave overnight. Make sure the seastars are not distorted before they are put into the fixative. Remove and put on paper towel to dry in shade. Make sure they do not stick to the paper - move them around regularly, and keep their labels with them. When they start to change colour to pale cream/yellow/orange, put in a plastic bag with their label. In lab, dry in microwave oven - on high for 30 sec to 1 minute, cool for a while then repeat until no more moisture comes off. Beware of putting seastars with too much moisture in the microwave - 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 preferably in a microwave oven that is not used for food preparation. If the latter is unavoidable, clean and air the microwave oven after use. Store dry. Alternatively, fix in formalin for 24-48 hrs and transfer to 70% alcohol for long term storage. The latter method will preserve colour in most instances.

OPHIUROIDS

Photograph alive if possible. Large and solid specimens - treat as for asteroids. Others - fix in formalin, 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, but put in dish alive, and pour preservative over them until the spines stop moving - all spines should be erect. When they are removed to dry, puncture the membrane surrounding the teeth with a needle to allow liquid to drain. They are more prone to exploding than seastars when microwaved. Others - fix in formalin, store in 70% alcohol.

CRINOIDS

Photograph alive if possible. Fix in formalin, but not for more than 2-3 days. Store in 70% alcohol. Crinoids almost always fall apart when preserved, there are very few species which do not. Therefore, try to keep all the bits together, and be aware that crinoids usually have a lot of commensals associated with them.

HOLOTHURIANS

Photograph alive if possible. Always isolate large specimens when collecting as they often eject their guts as a defence, which tend to stick to anything they

touch. 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 plate are essential for identification.

UROCHORDATES

Compound, colonial and other gelatinous ascidians MUST be photographed alive, form and colour patterns are very important. 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.

SURVEY REPORTING

SUGGESTED OUTLINE FOR SURVEY REPORTS

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 exotic 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
    - 11.2 Prevention of new introductions
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

Furlani (1996), *A Guide to the Introduced Marine Species in Australian Waters*

The guide provides information on more than 70 species of marine organisms which have been introduced to Australian waters, including ABWMAC target pest species, and a number of species that pose a threat to Australia but have not to date been recorded from Australian waters. Diagrams and photographs are included to facilitate identification of exotic species and both native range and known Australian distribution are provided.

The guide is produced in a open format to allow for the inclusion of additional information and new species supplements. Copies can be obtained from CRIMP by contacting:

CRIMP Handbook Information
CSIRO Marine Laboratories
GPO Box 1538, Hobart, TAS 7000

Ph: +64 3 6332 5452
Fax: +64 3 6232 5485
Email: crimp@ml.csiro.au